

Role of Treg Cells in B Cells: Implication of Treg therapy on Lupus Nephritis

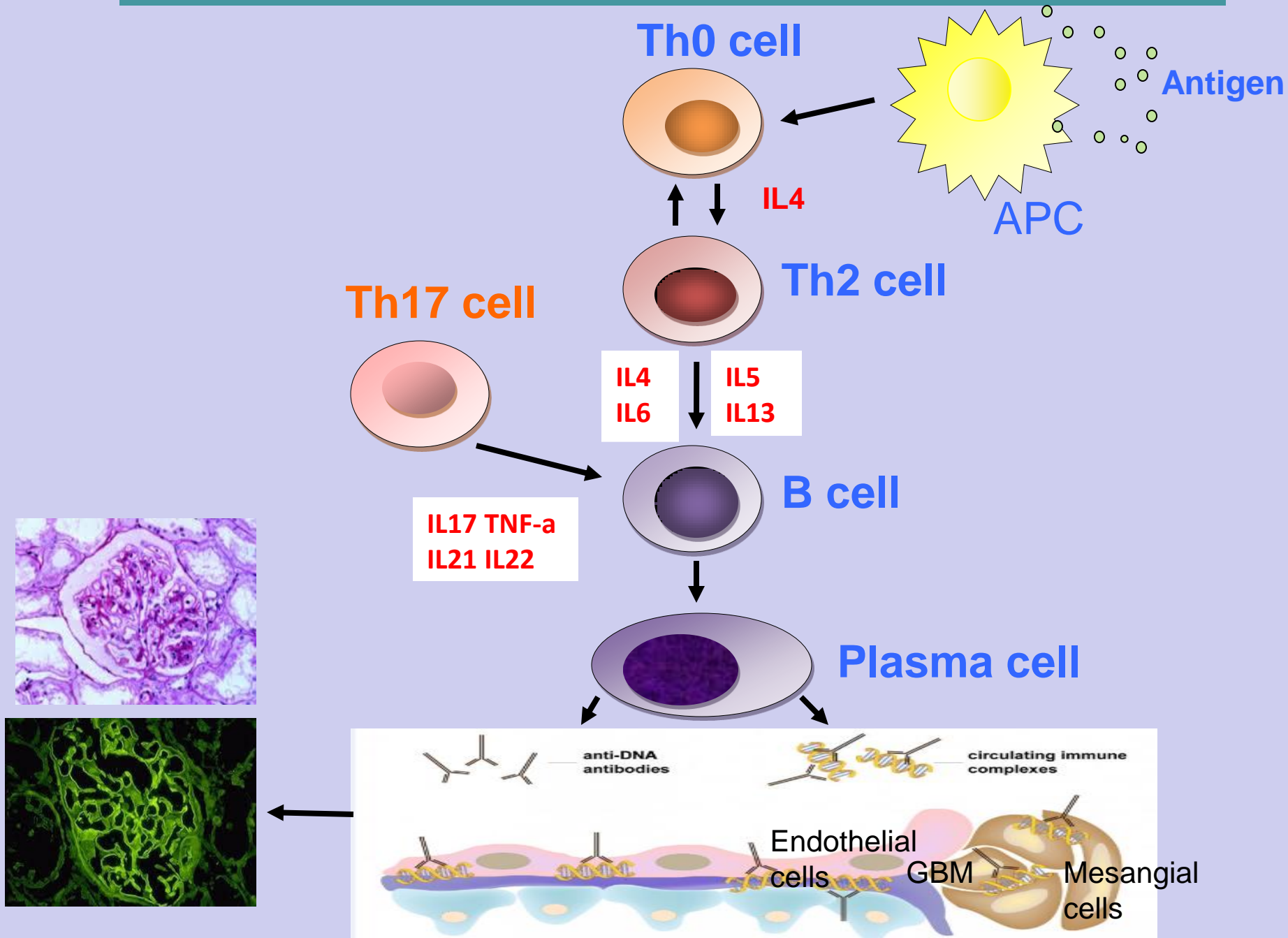
Song Guo Zheng (郑颂国), MD, PhD

Professor and Director

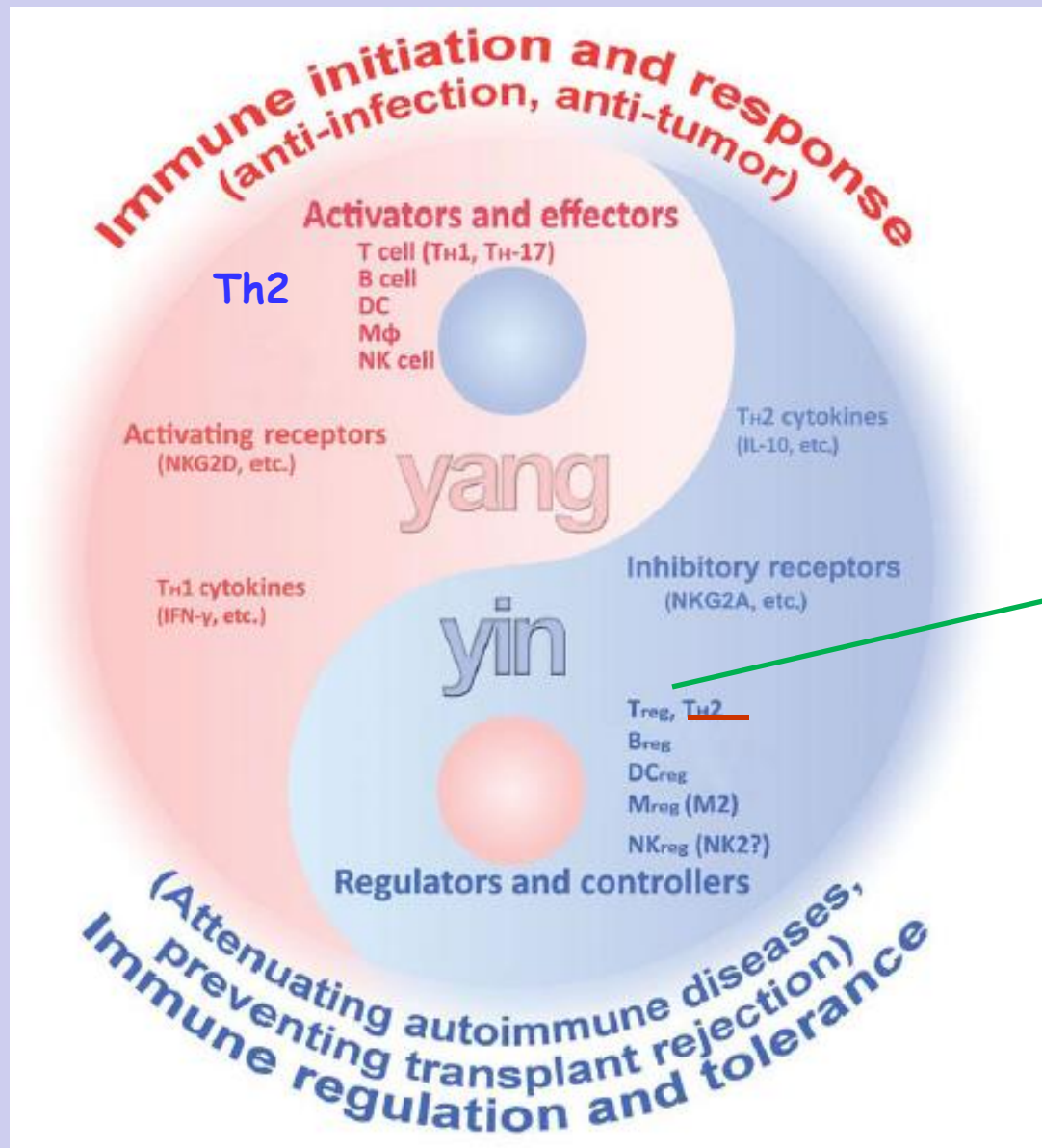
Rheumatology Research Center and Division of
Rheumatology & Immunology, Department of
Medicine, Penn State University

ICCN 2015, Kong Kong, Dec. 13, 2015

Mechanisms of tissue injury in lupus nephritis



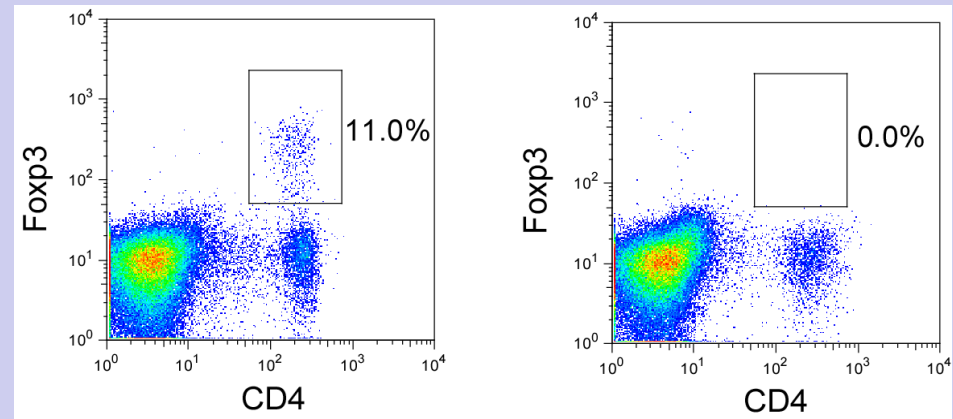
Balance between Yin and Yang



Treg cells

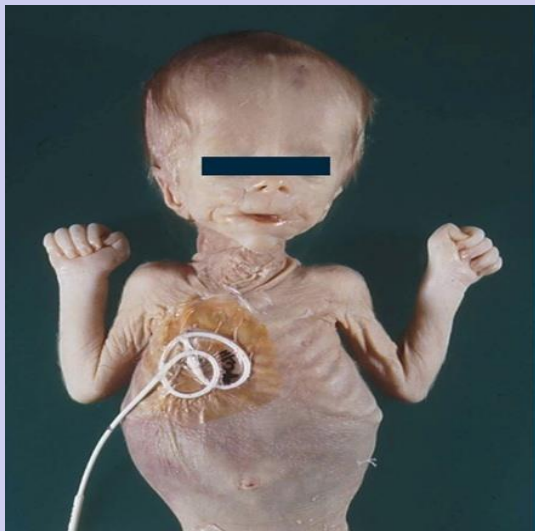
Importance of nTreg in the maintenance of homeostasis

- **Scurfy mice** lack Treg: mice died 3-4 weeks after birth



CD4+CD25+; Thymus; Foxp3; Suppressor Cells

- **IPEX in human**

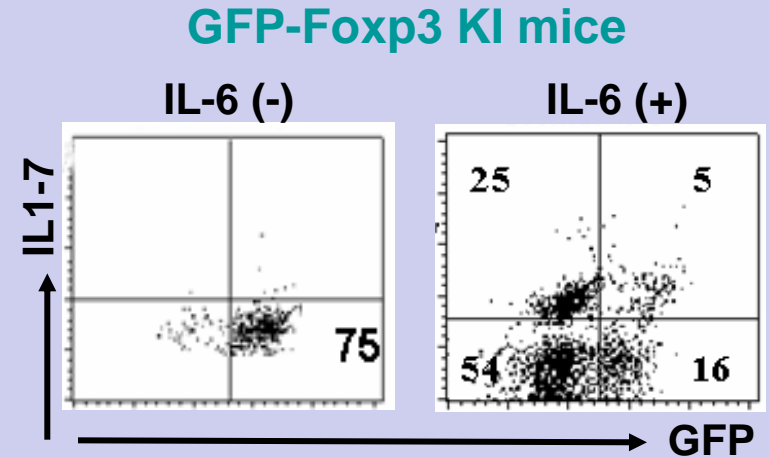
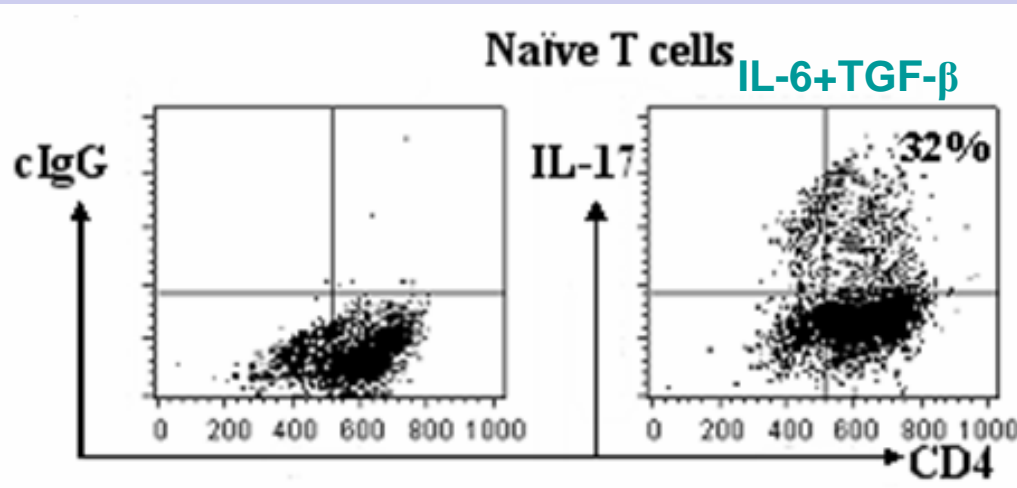


- Immune dysregulation
- Polyendocrinopathy
- Enteropathy
- X-linked syndrome

Therapeutic effects of nTregs on the established autoimmune diseases are unsatisfactory

- Injection of CD4⁺CD25⁺ cells to the established CIA failed to suppress CIA
Bardos et al. *Arthritis Res Ther.* 2003; 5:R106-R113
Morgan et al. *Arthritis Rheum.* 2005; 52:2212
Frey et al. *Arthritis Res Ther.* 2005; 7: R291-301
Zhou et al. *Cutting-Edge, J Immunol.* 2010; 185:2675
- Injection of expanded CD4⁺CD25⁺ cells to the established lupus had mild protective effect (Scalapino KJ et al. *J Immunol*, 2006;177:1451-1459)
- Injection of CD4⁺CD25⁺ cells failed to control Th17-mediated gastritis (Huter EN et al. *J Immunol*, 2008;181:8209-8213)

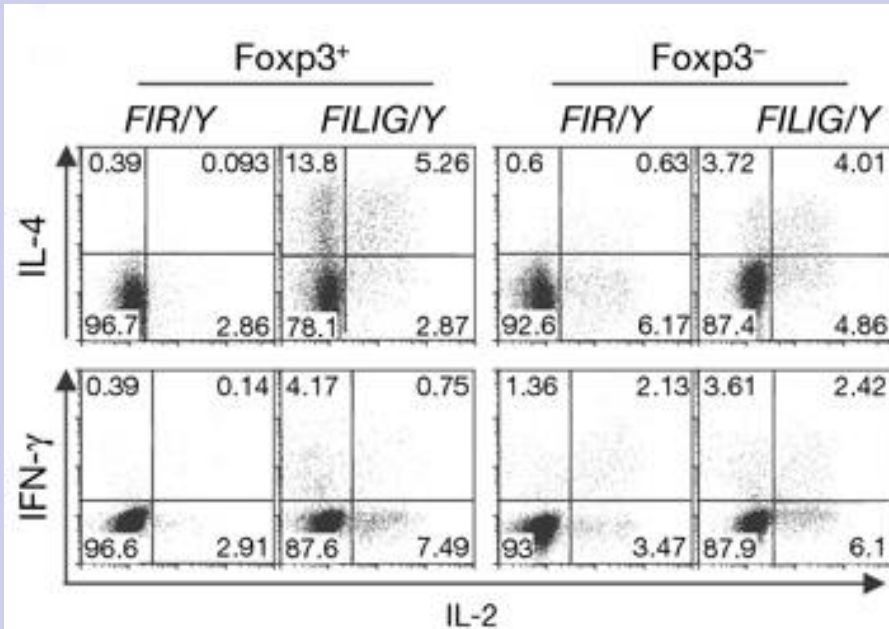
nTregs become Th17 and Th2 cells when stimulated with IL-6



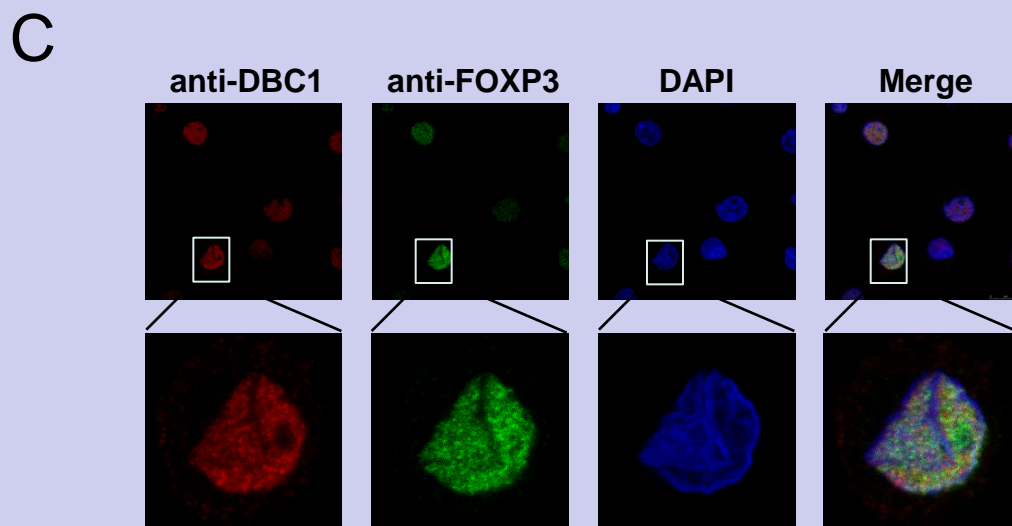
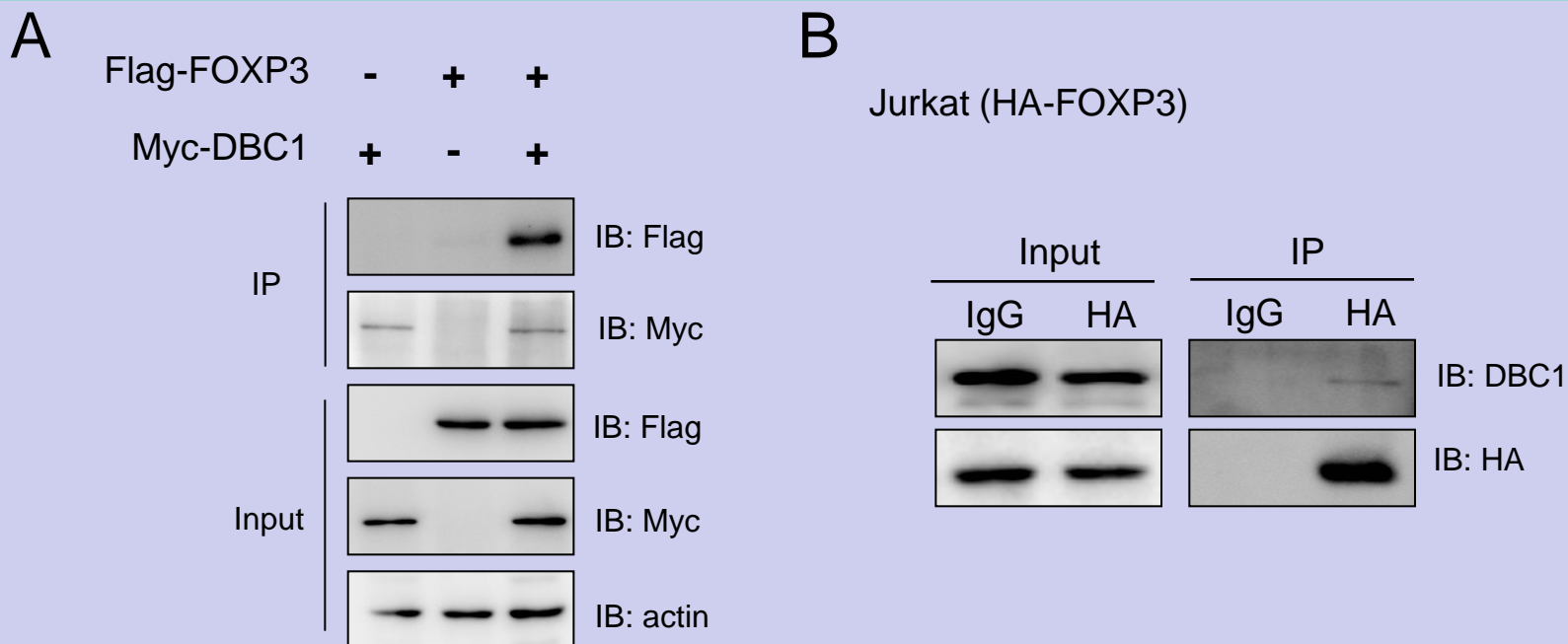
Xu et al, Cutting-Edge, J Immunol. 2007;178:6725-6729.

Zheng SG et al, Cutting-Edge J Immunol. 2008; 180:7112-7126

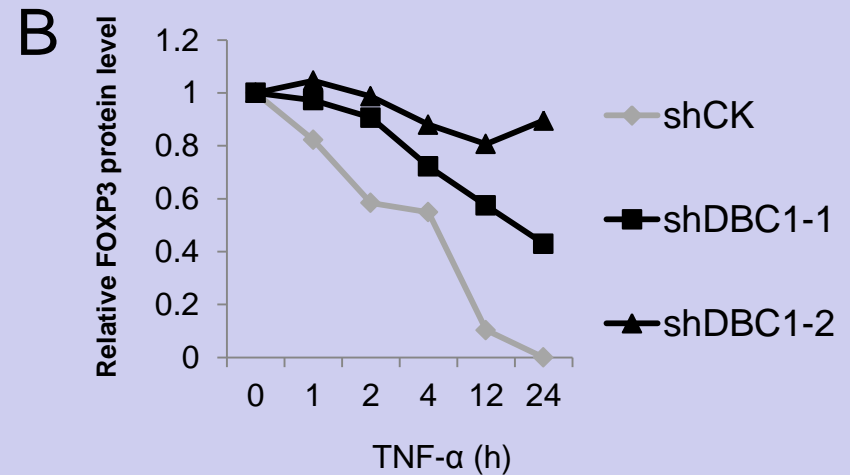
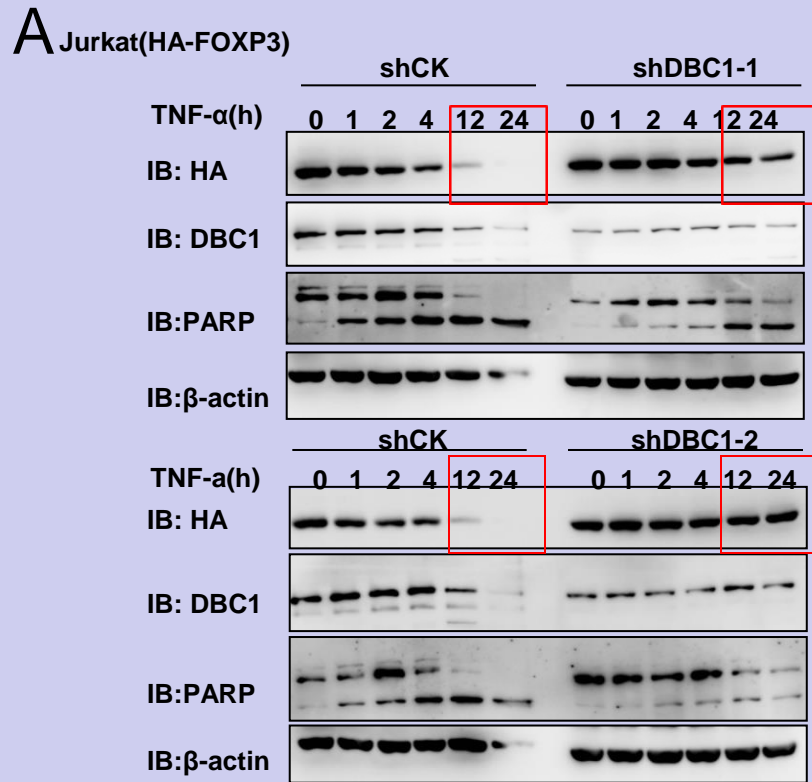
Flavell group: Nature 2007; 445|: 766-770



FOXP3 interacts with DBC1

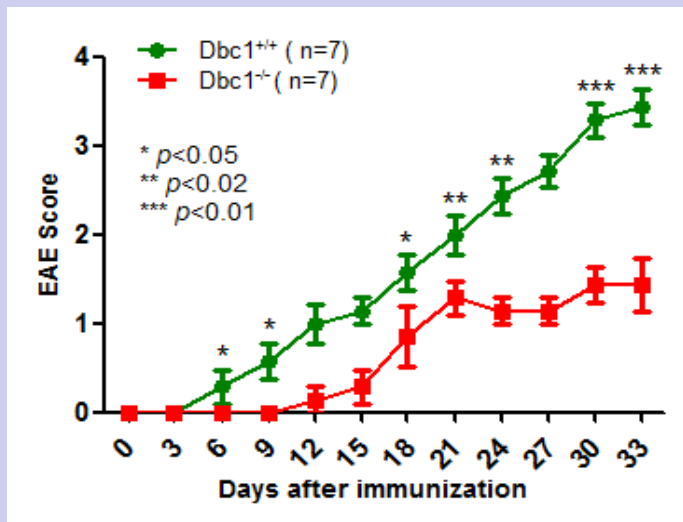


DBC1 knockdown cells are resistant to TNF- α mediated FOXP3 degradation

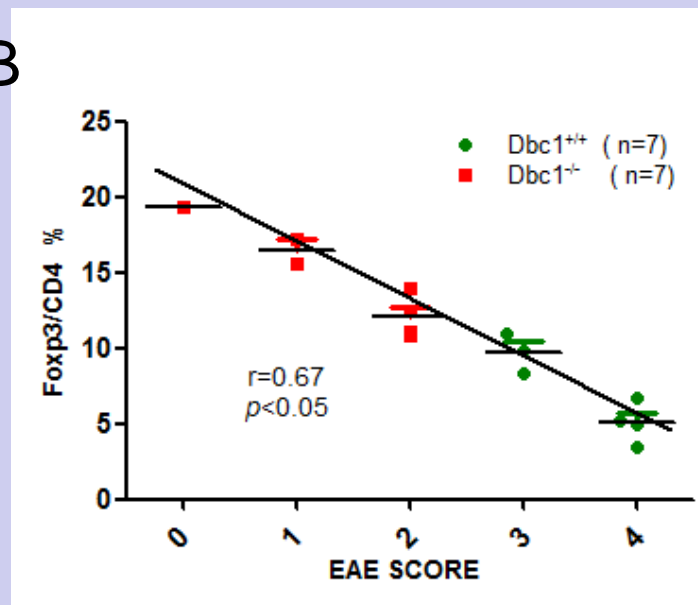


Dbc1^{-/-} mice develop less severe autoimmune disease symptom in EAE

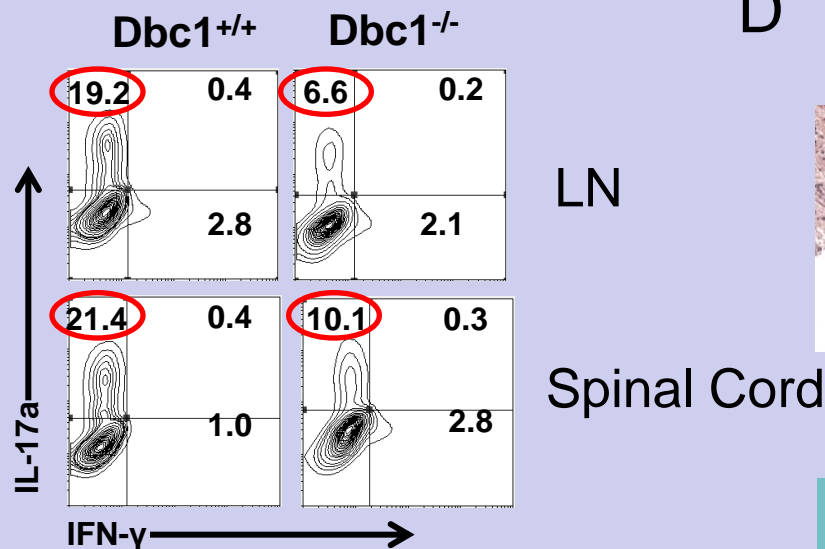
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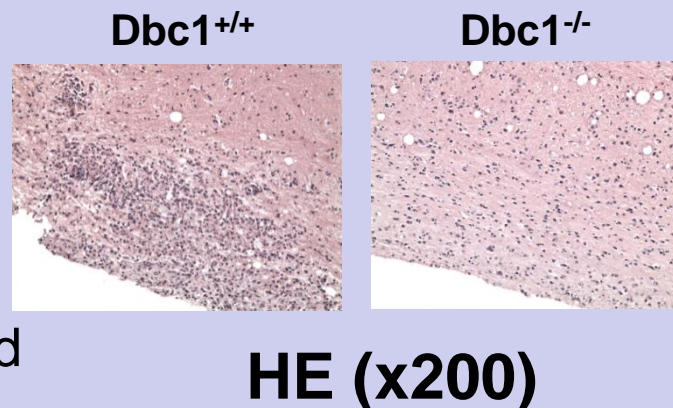
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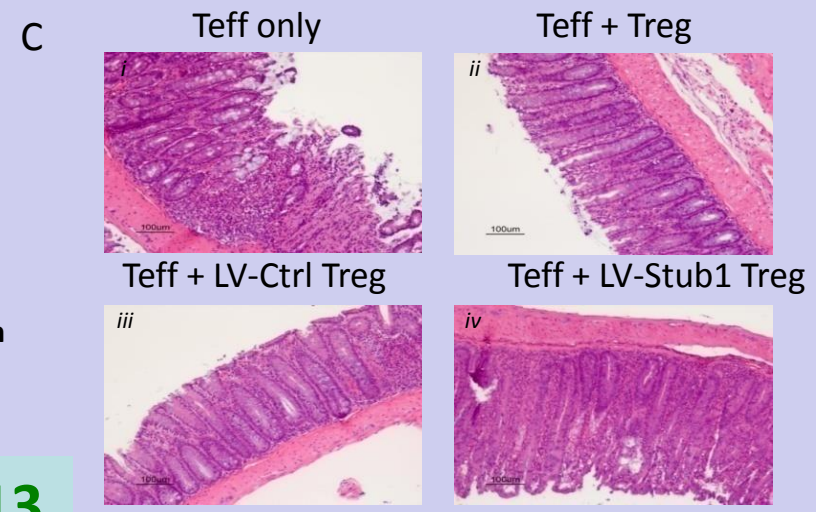
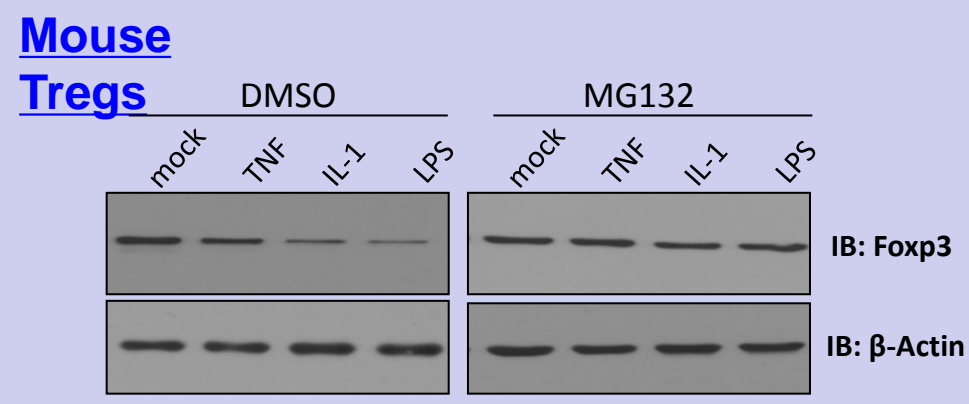
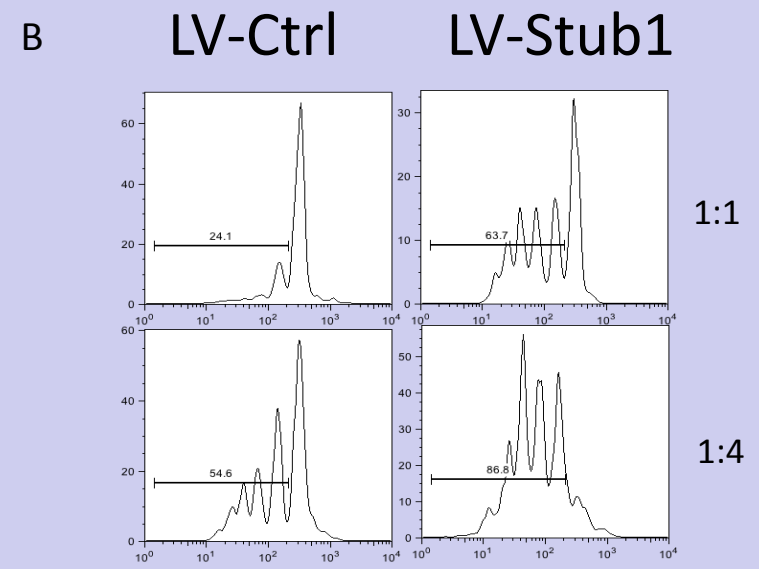
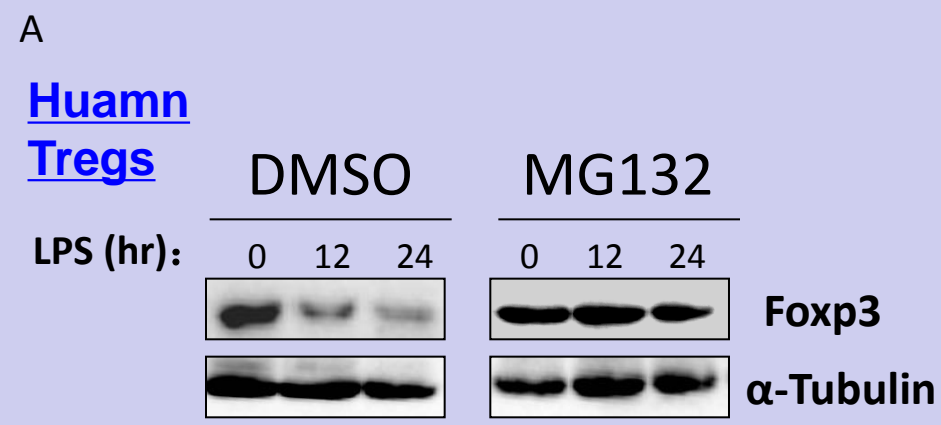
C



D



The ubiquitin ligase Stub1 negatively modulates Treg function by promoting degradation of Foxp3



Cutting Edge

THE JOURNAL OF
IMMUNOLOGY

Cutting Edge: All-*Trans* Retinoic Acid Sustains the Stability and Function of Natural Regulatory T Cells in an Inflammatory Milieu

Xiaohui Zhou,^{*,†} Ning Kong,^{*,‡} Julie Wang,^{*} Huiming Fan,[†] Hejian Zou,[‡] David Horwitz,^{*} David Brand,[§] Zhongmin Liu,[†] and Song Guo Zheng^{*}

Critical role of *all-trans* retinoic acid in stabilizing human natural regulatory T cells under inflammatory conditions

Ling Lu^{a,b,1,2}, Qin Lan^{b,c,1}, Zhiyuan Li^{d,1}, Xiaohui Zhou^{b,c}, Jian Gu^{a,b}, Qiang Li^b, Julie Wang^b, Maogen Chen^b, Ya Liu^b, Yi Shen^c, David D. Brand^e, Bernhard Ryffel^f, David A. Horwitz^b, Francisco P. Quismorio^b, Zhongmin Liu^c, Bin Li^d, Nancy J. Olsen^g, and Song Guo Zheng^{b,c,g,2}

Treg Product Patent



US008951796B2

(12) **United States Patent**
Zheng et al.

(10) **Patent No.:** **US 8,951,796 B2**
(45) **Date of Patent:** **Feb. 10, 2015**

(54) **METHODS AND COMPOSITIONS FOR
EXPANDING AND STABILIZING NATURAL
REGULATORY T CELLS**

(75) Inventors: **Song Guo Zheng**, Arcadia, CA (US);
David A. Horwitz, Santa Monica, CA
(US); **Juhua Wong**, Arcadia, CA (US)

(73) Assignee: **University of Southern California**, Los
Angeles, CA (US)

(*) Notice: Subject to any disclaimer, the term of this
patent is extended or adjusted under 35
U.S.C. 154(b) by 0 days.

(21) Appl. No.: **13/642,811**

(22) PCT Filed: **Apr. 21, 2011**

OTHER PUBLICATIONS

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Lal and Bromberg, Blood. Oct. 29, 2009;114(18):3727-3735.*

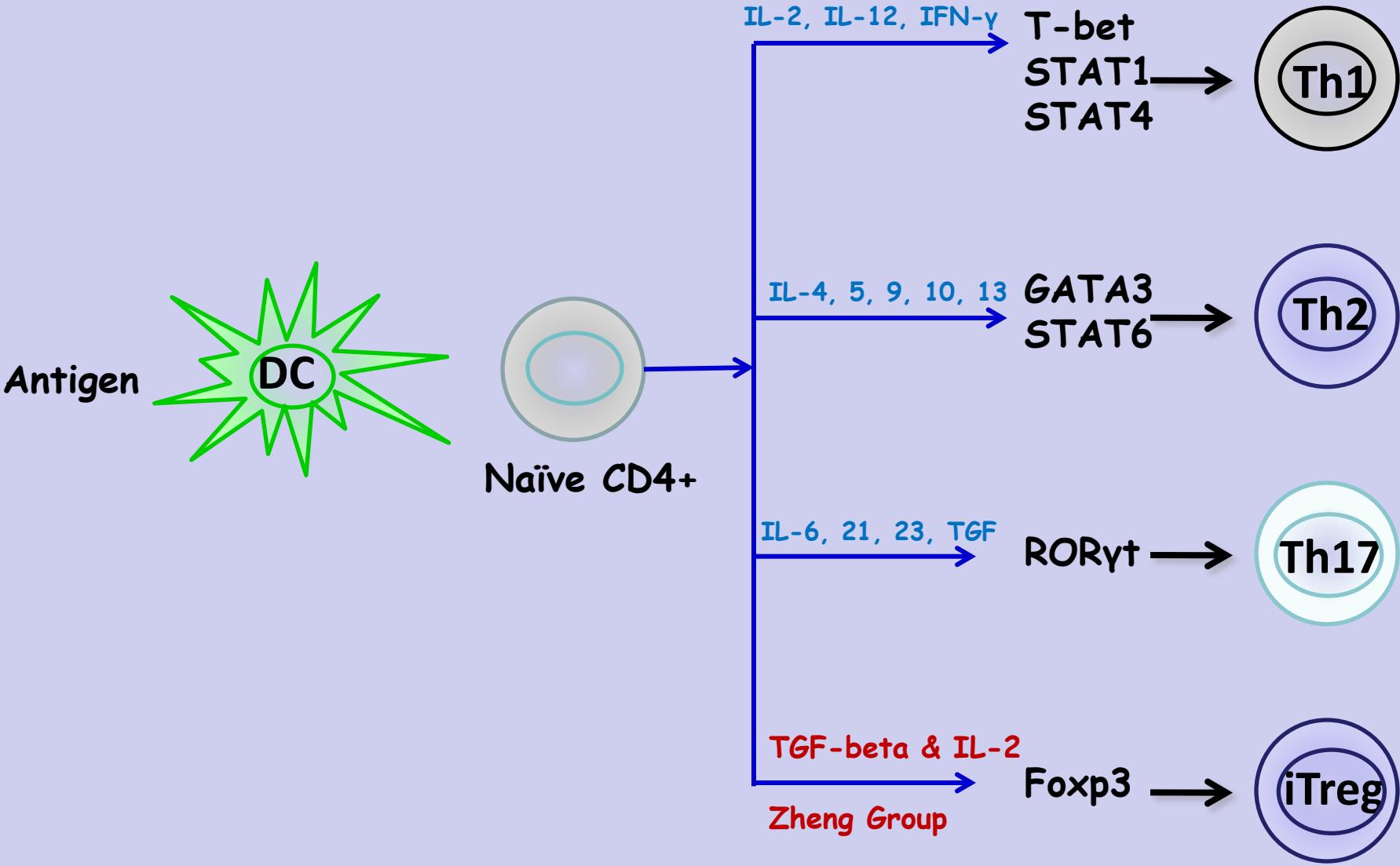
Mucida, Daniel et al., "Reciprocal T(H)17 and regulatory T cell
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Wang, Jun et al., "De novo generation and enhanced suppression of
human CD4+CD25+regulatory T cells by reinoic acid", J. of
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Nolting, Jens, et al., "Retinoic acid can enhance conversion of naive
into regulatory T cells independently of secreted cytokines", The
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Schambach, Felix et al., "Activation of retinoic acid receptor-alpha
favours regulatory T cell induction at the expense of IL-17 secreting
T helper cell differentiation", European Journal of Immunology,
(2007), vol. 37, No. 9, pp. 2396-2399.

Differentiation of T Effector Cells



TGF- β -induced iTregs come from CD4⁺CD25⁻ cells

The Journal of Immunology

2002; 169: 4183-4189

Generation Ex Vivo of TGF- β -Producing Regulatory T Cells from CD4⁺CD25⁻ Precursors¹

Song Guo Zheng, J. Dixon Gray, Kazuo Ohtsuka, Satoshi Yamagiwa, and David A. Horwitz²

Immunity
Article



Positive and Negative Transcriptional Regulation of the *Foxp3* Gene is Mediated by Access and Binding of the Smad3 Protein to Enhancer I

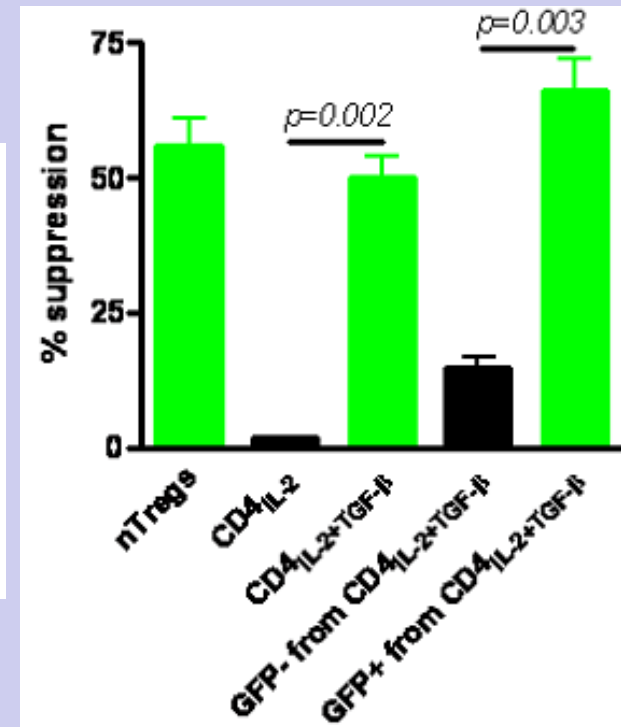
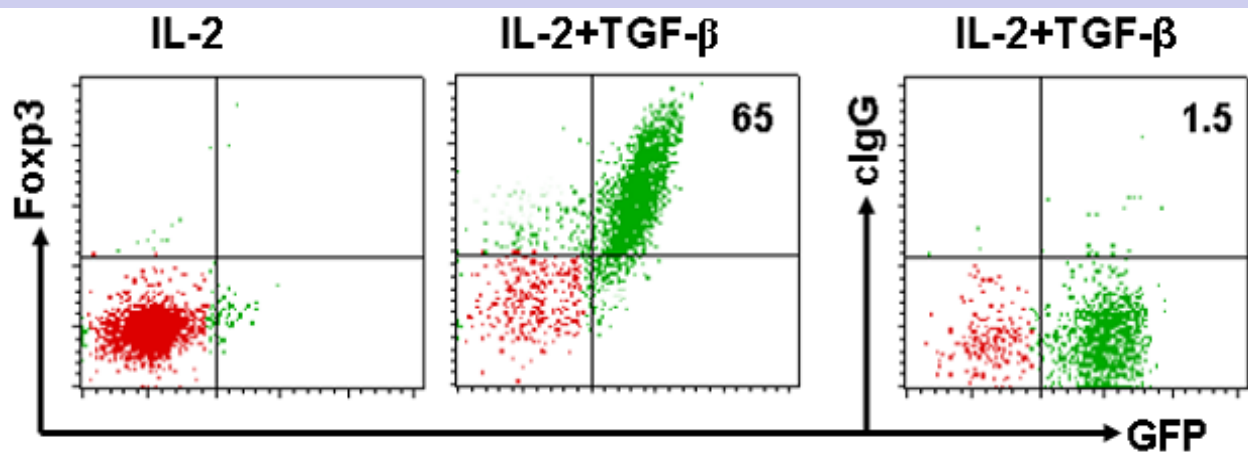
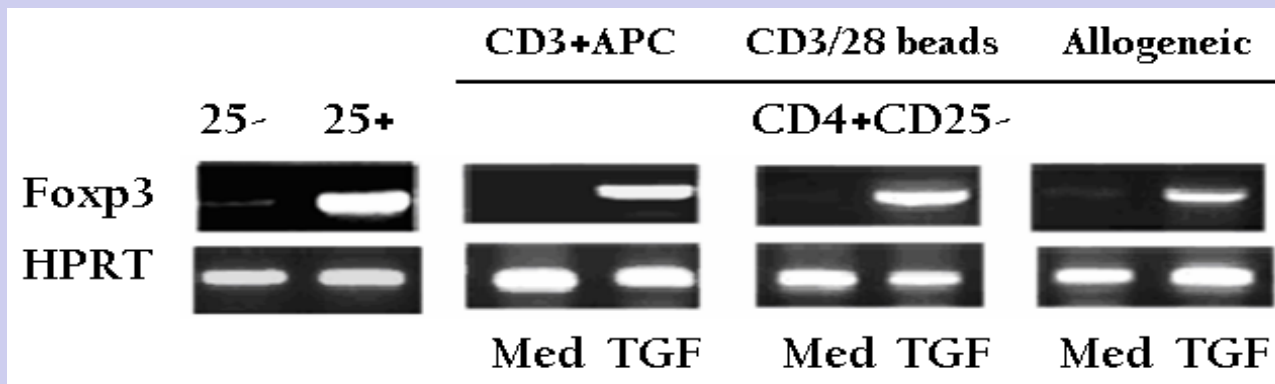
Lili Xu,¹ Atsushi Kitani,¹ Christina Stuelten,² George McGrady,³ Ivan Fuss,¹ and Warren Strober^{1,*}

2010

INTRODUCTION

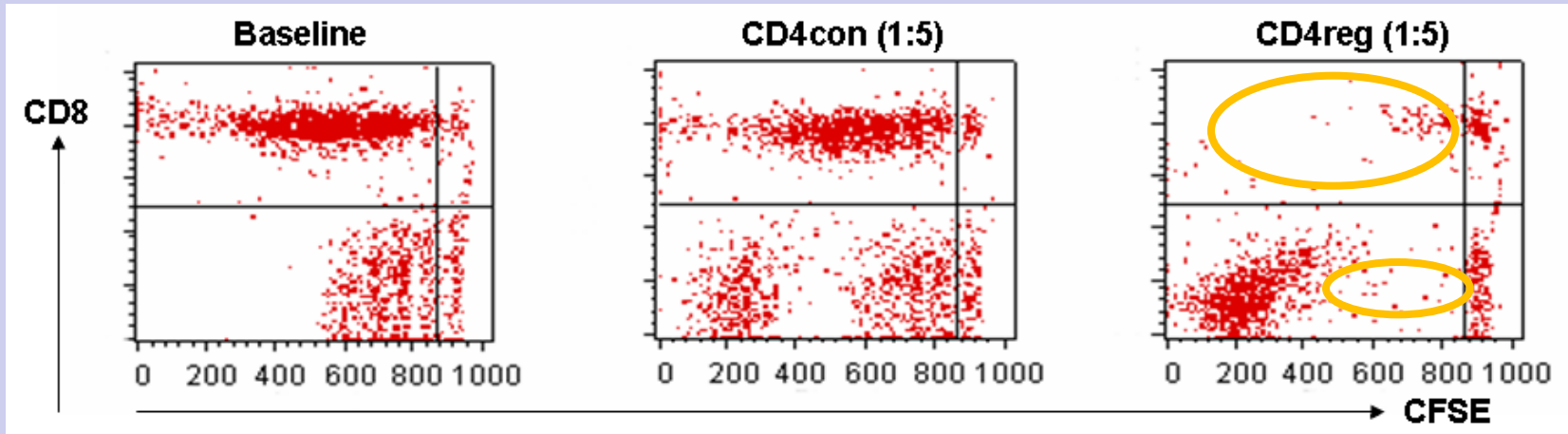
It is now well established that CD4⁺ regulatory T cells (Tregs) can be recruited from unselected CD4⁺ nonregulatory T cells in the peripheral (extra-thymic) lymphoid compartment; the regulatory activity of these cells augment the regulatory activity of thymus-derived Tregs, particularly at inflammatory sites. The major driving force of such recruitment is transforming growth factor- β (TGF- β), which has been shown to induce T cell receptor (TCR)-stimulated CD25⁻ nonregulatory T cells to express the intracellular transcription factor that directs Treg function, Foxp3 (Zheng et al., 2002). Recently, the molecular basis of such induction was clarified, at least in part, by Tone et al., who showed that the *Foxp3* gene was regulated by Smad3 and NFAT transcription factors that bind to sites in an enhancer region (enhancer I) located in an intron between untranslated

Phenotype and Function of iTregs

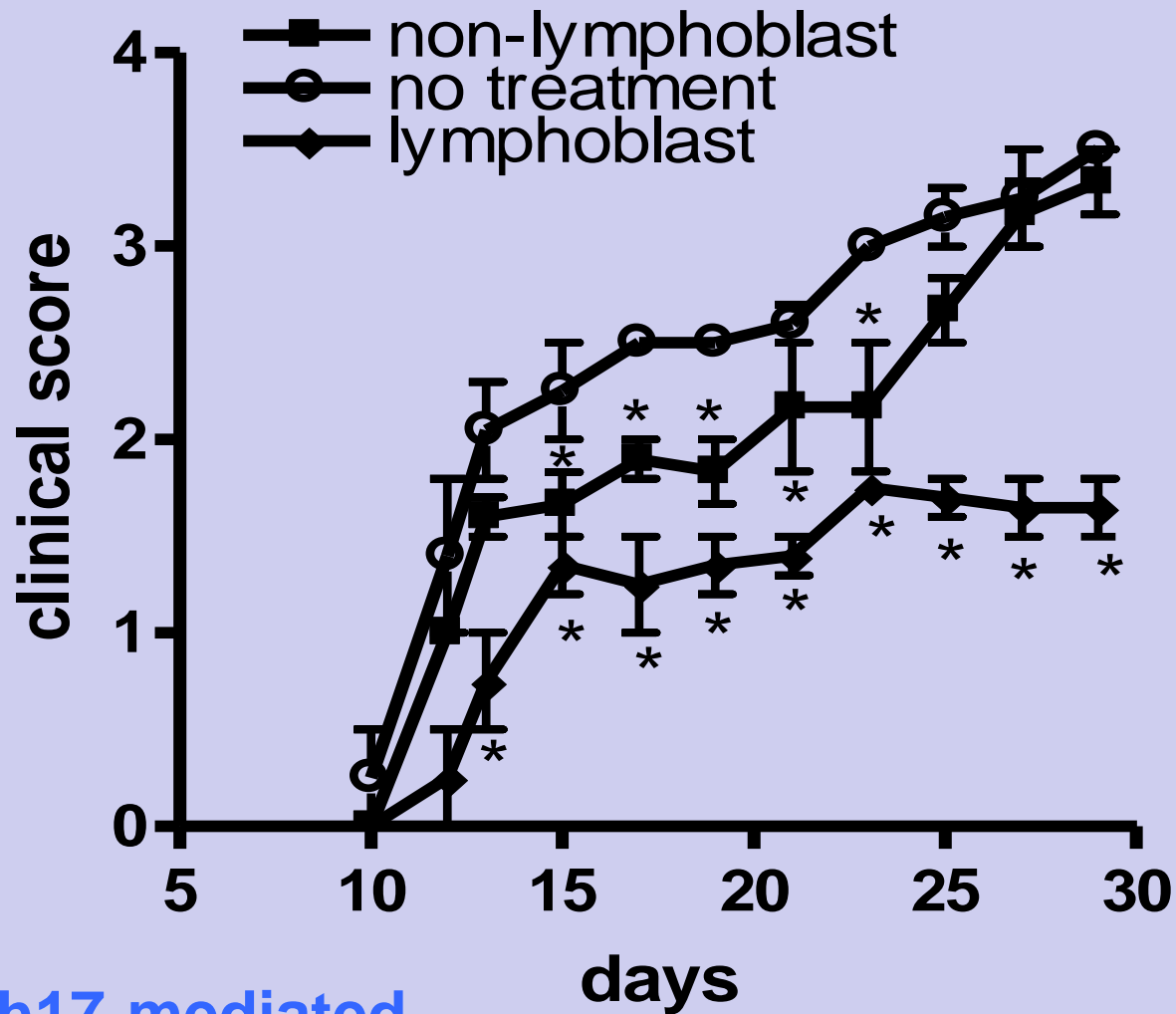


Suppression *in vitro*

iTreg Can Suppress T Cell Proliferation *in vitro*

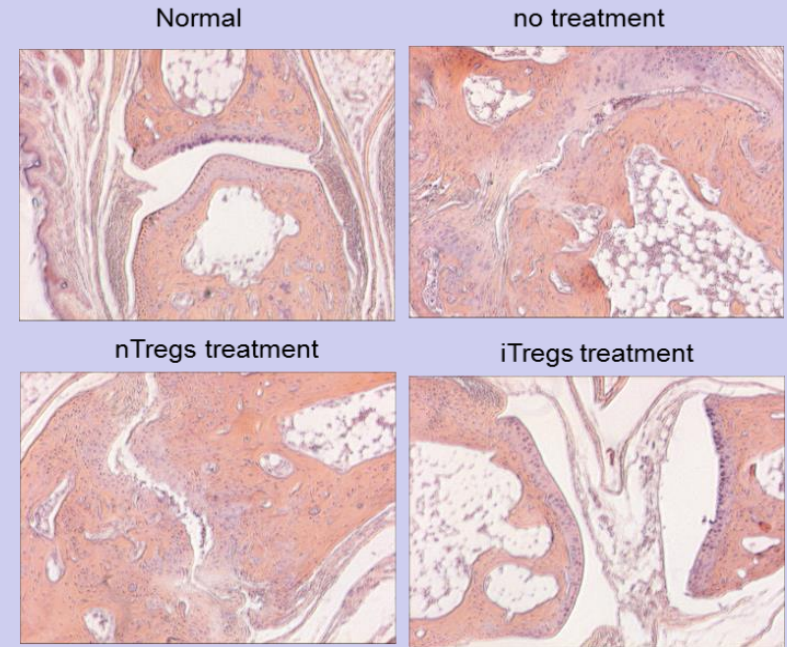
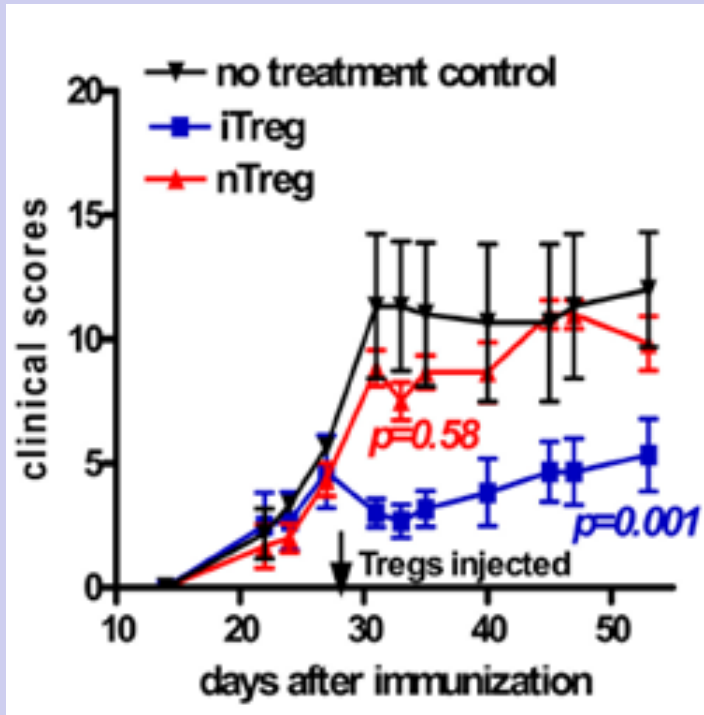


iTregs suppress EAE

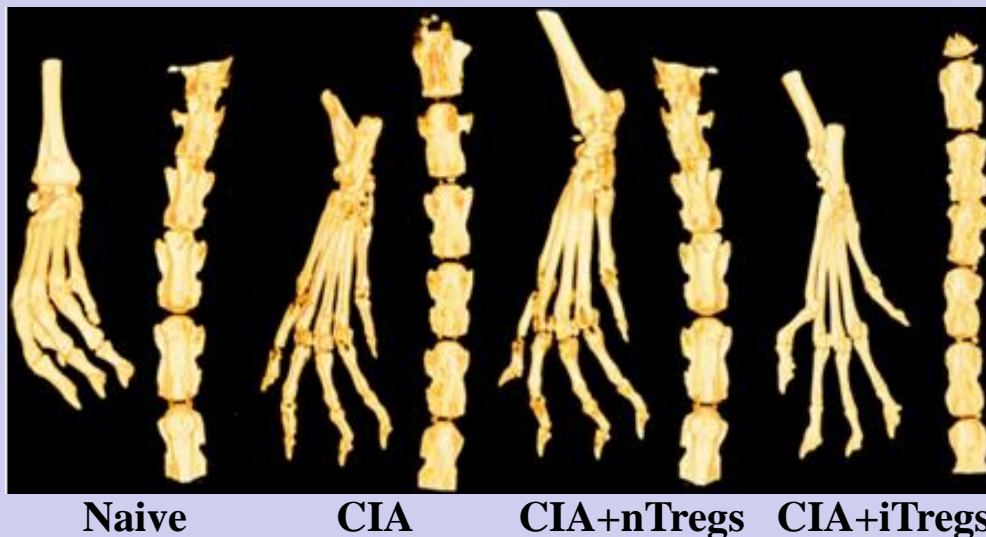


Th1/Th17-mediated

iTregs but not nTreg ameliorate the severity of established CIA



Day 50



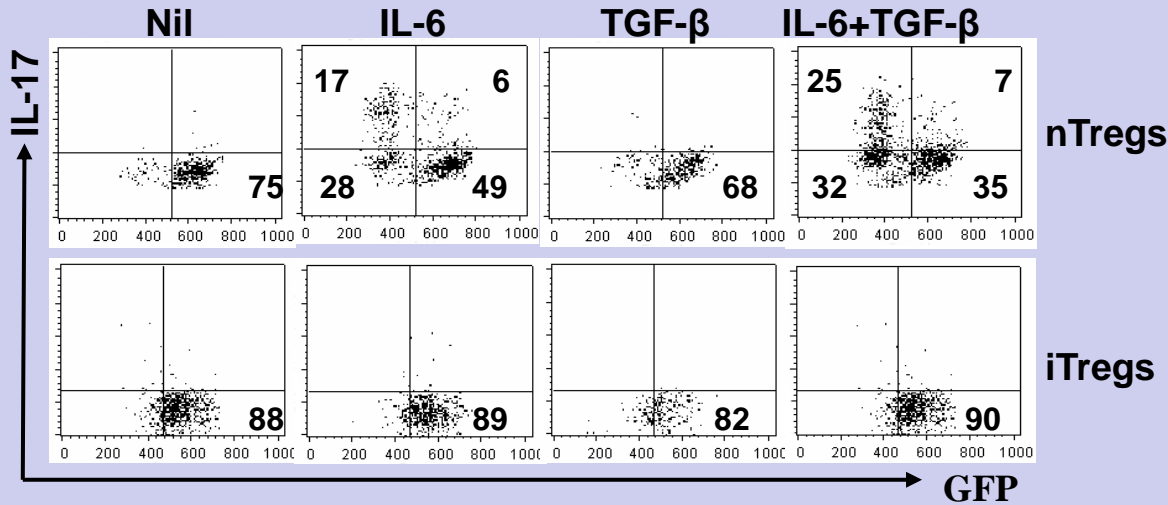
Th17-mediated

Day 56

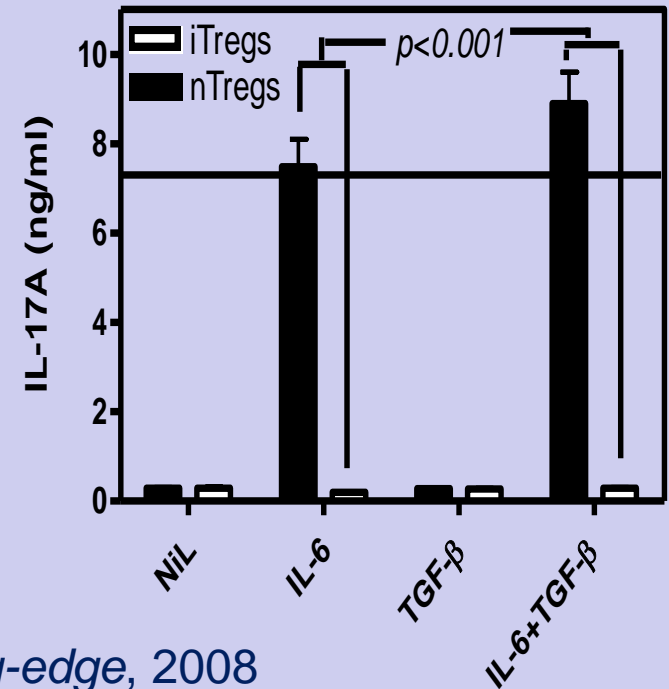
Kong et al. *Ann Rheum Dis*, 2012

Stability and functionality of Treg subsets *in vitro*

Foxp3-GFP Knock-in mice



O'Connor RA, et al and Anderton SM.
J Immunol. 2010; 185:7235-43



Zheng et al. *J Immunol*, cutting-edge, 2008

iTregs and Autoimmune Diseases and Transplantation

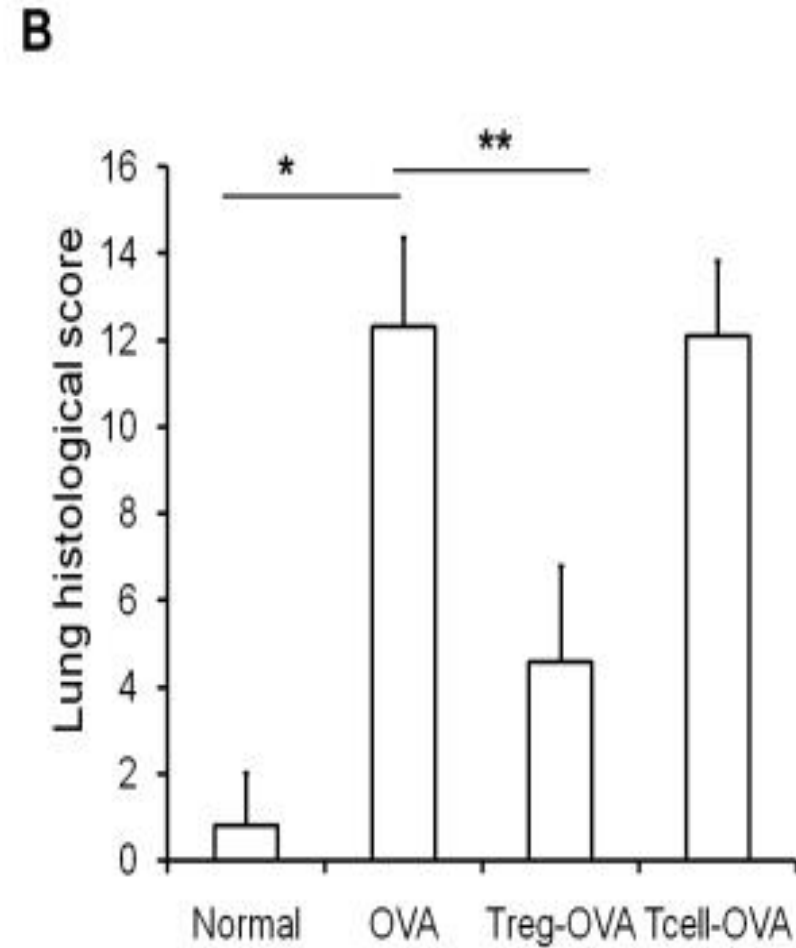
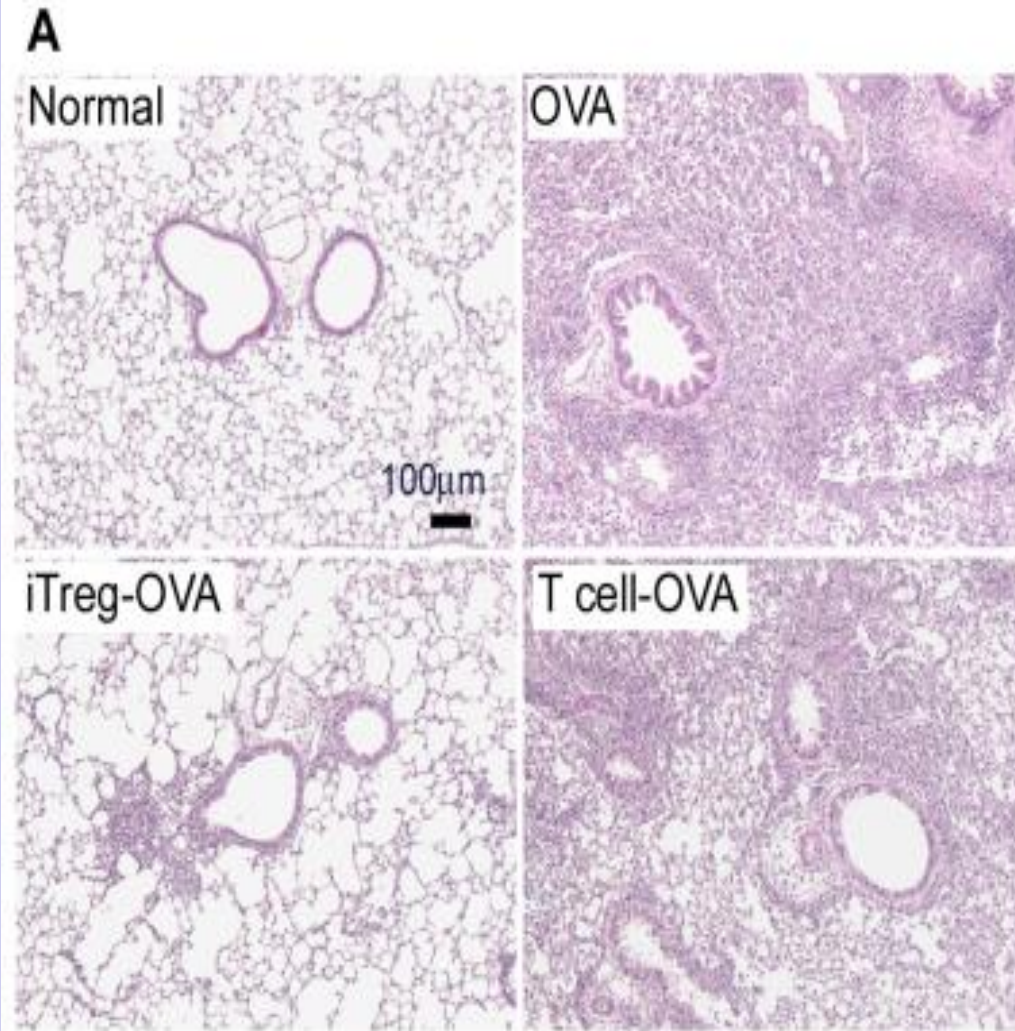
- ❖ Colitis. Fantini MC, et al. *Gut*. 2006; 55:671-80.
- ❖ T1 Diabetes: You S, et al. *PNAS*. 2007;104:6335-40
- ❖ Gastritis: Huter EN, et al. *J Immunol*. 2008;181:8209-13.
- ❖ EAE: Selvaraj RK, Geiger TL. *J Immunol*. 2008;180:2830-8.
- ❖ cGVHD: Su H, et al. *Br J Dermatol*. 2008;158: 1197-209
- ❖ Asthma: Weiss JM, et al. *J Exp Med*. 2012; 209:1723-42
- ❖ Allograft rejection: Zheng et al, *Int Immunol*. 2006; 18:279-89;
Yang H, et al. *Transplantation*. 2012; 93:580-8

Questions?

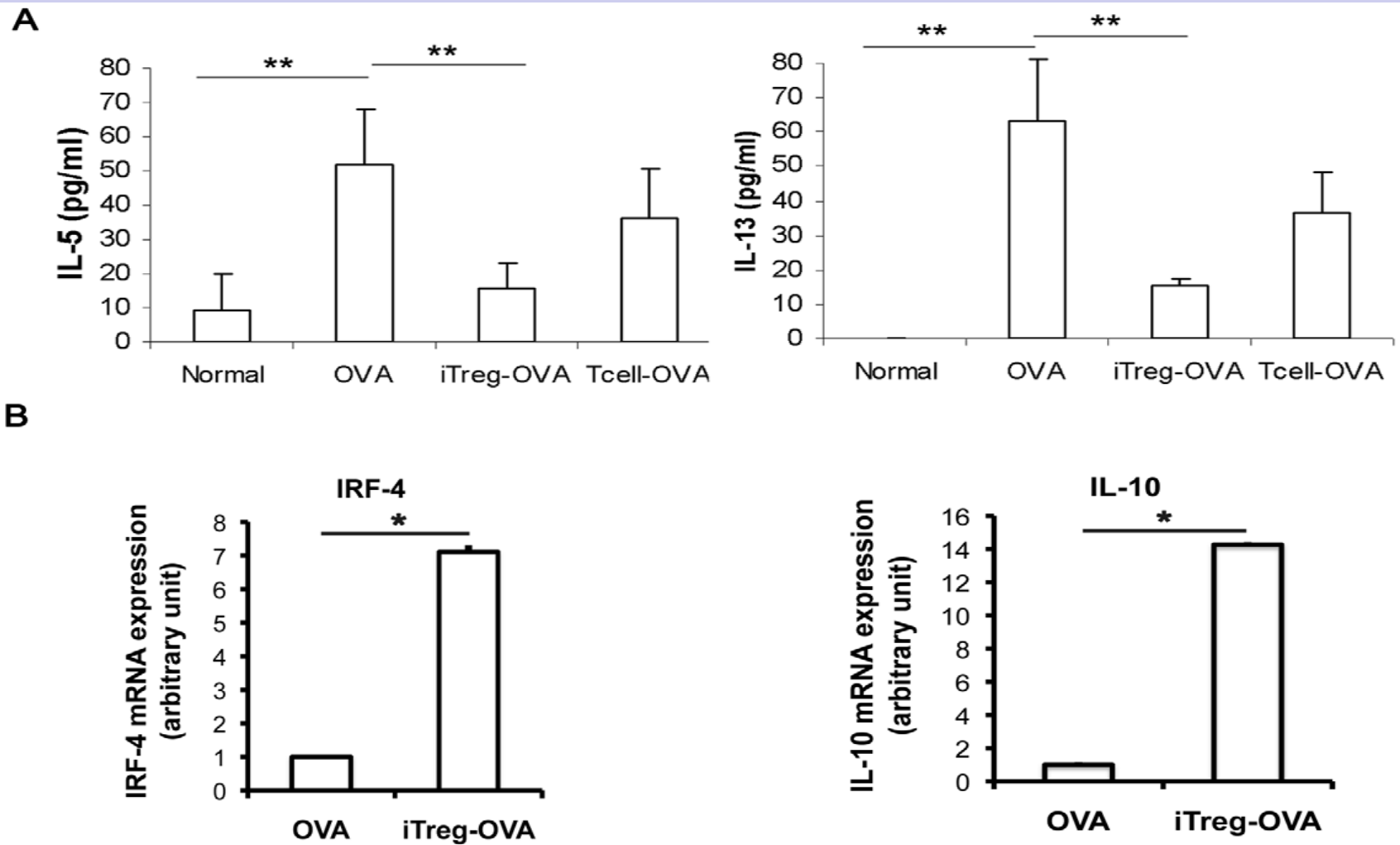
Can iTreg suppress Th2 or B cells?



iTregs suppress asthma

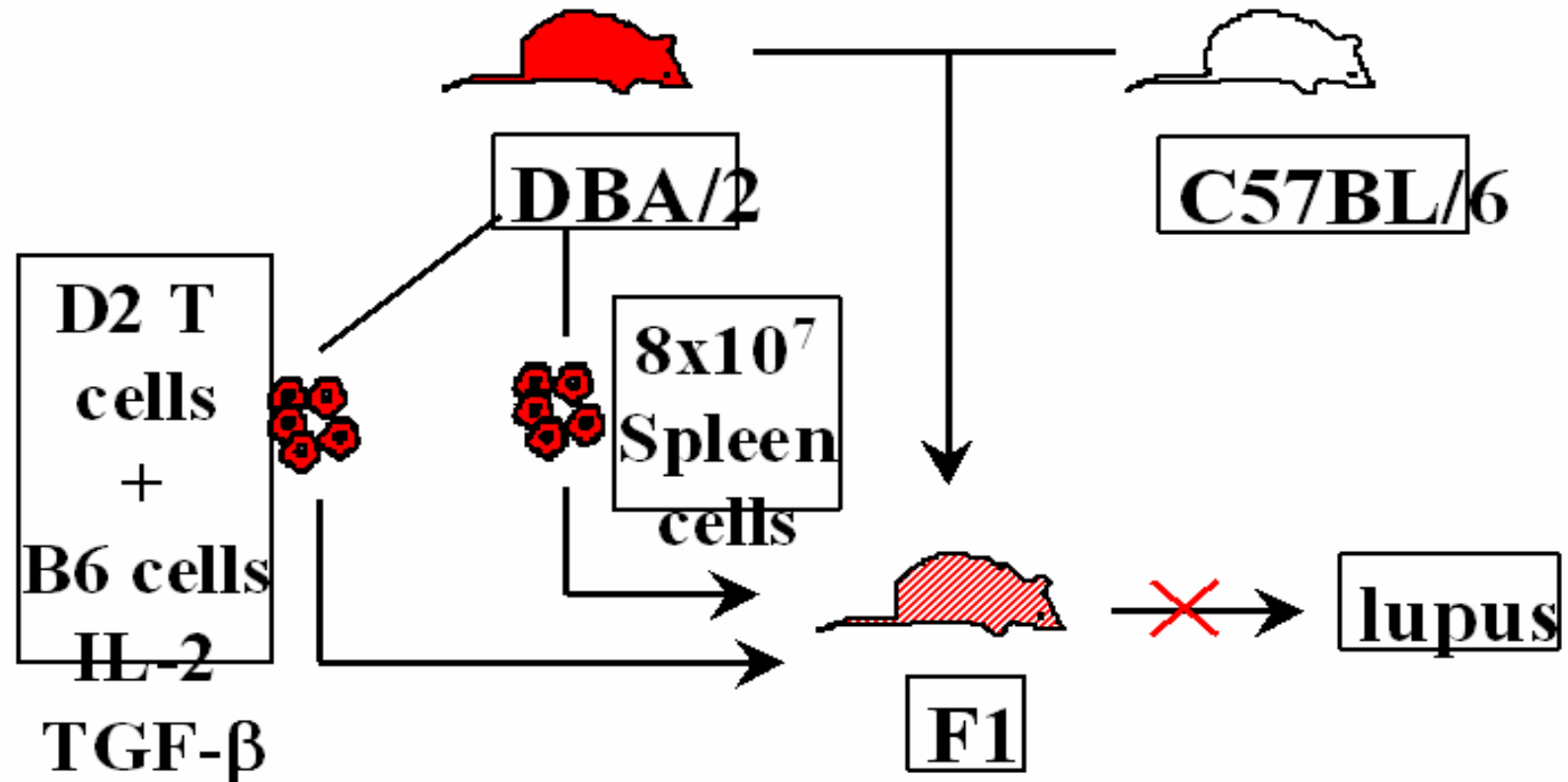


Adoptive Transfer of iTreg Cells Altered Cytokine Production



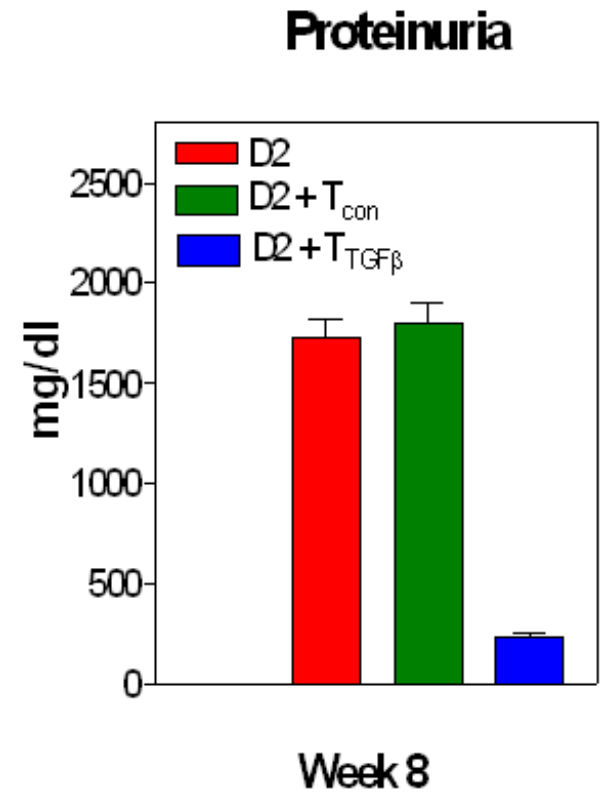
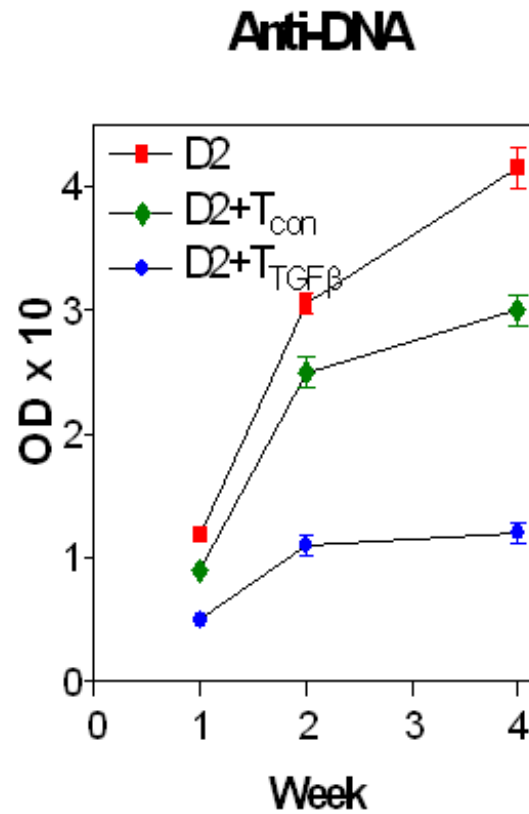
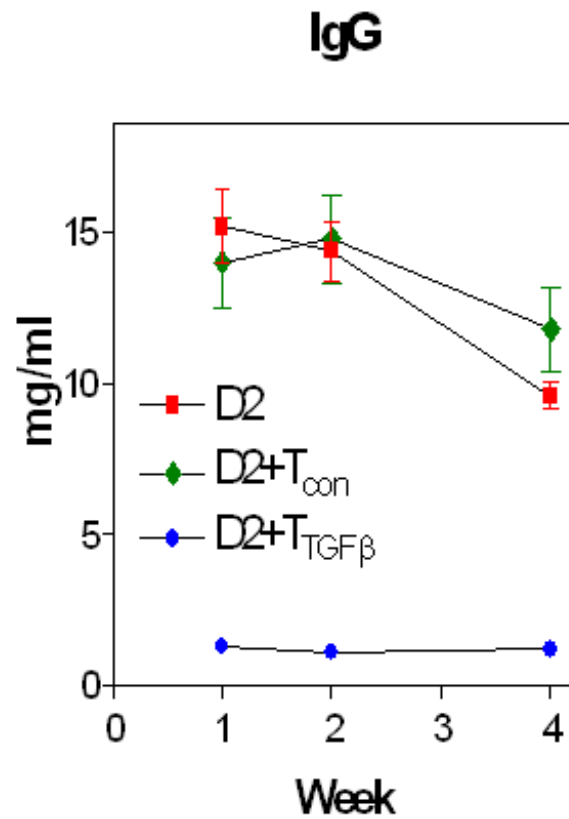
Chronic Graft-versus Host Disease with a typical lupus syndrome

Lupus-like Disease

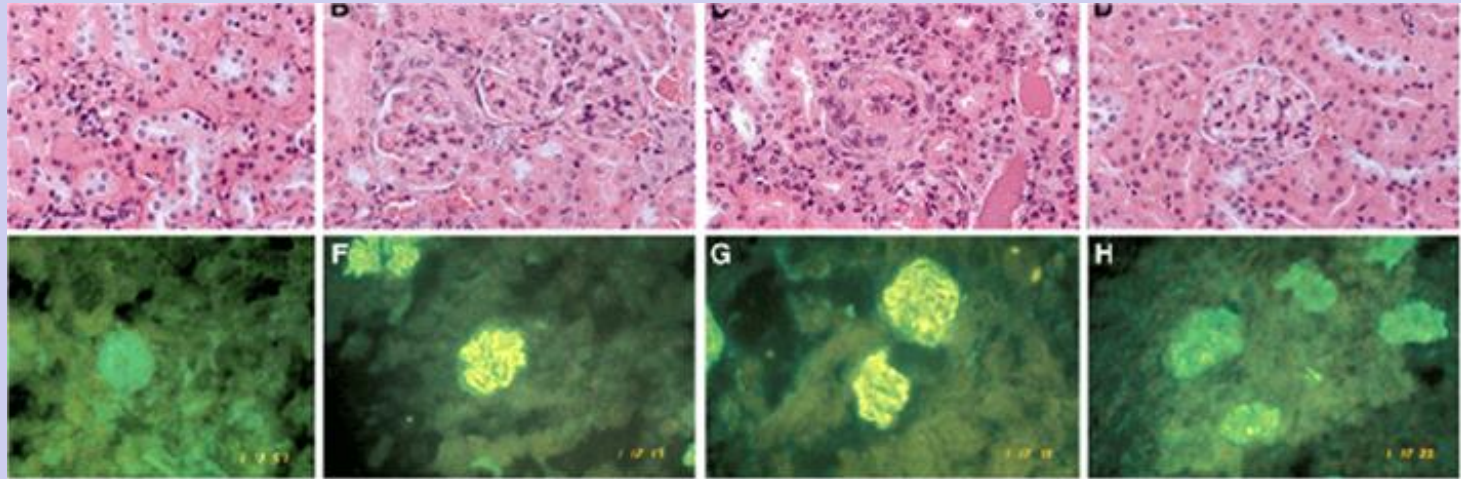


Gleichmann, *J. Exp. Med.* 155:1501; 1982

TGF- β induced iTreg cells suppress autoantibody production



iTreg cells prevent lupus-like nephritis in cGVHD model



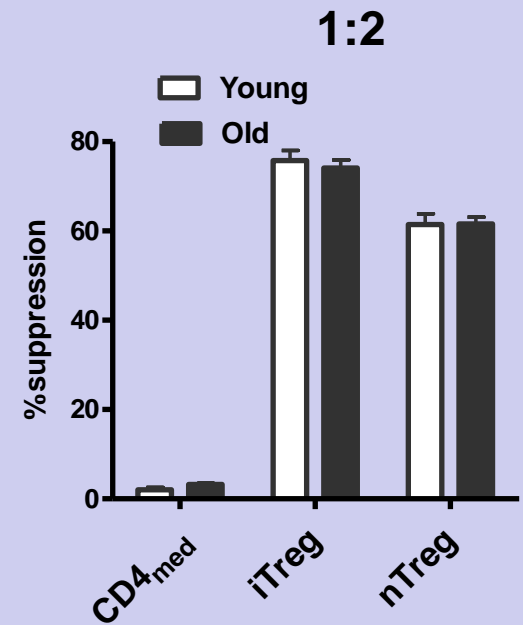
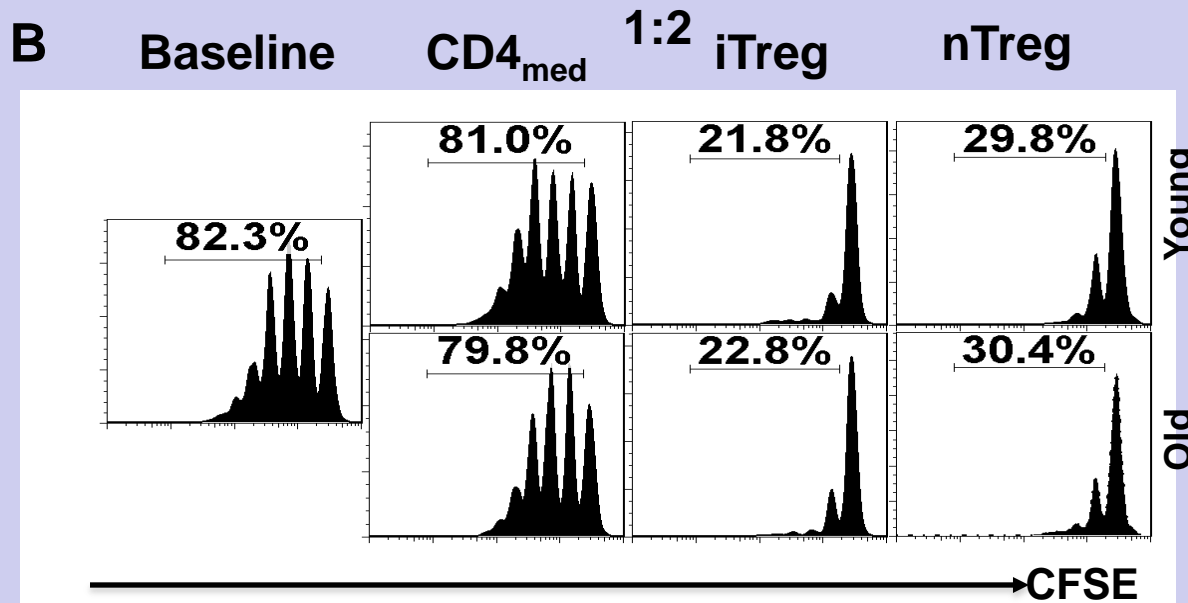
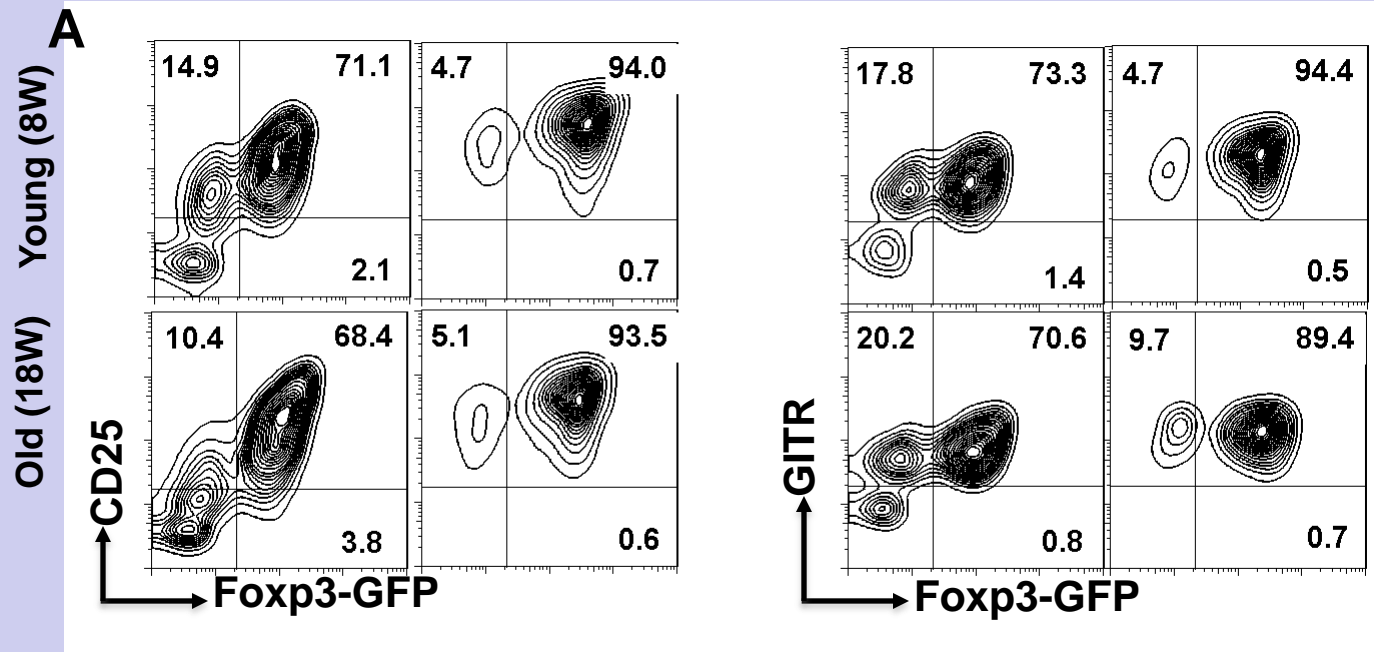
NIL

D2 alone

D2+Tcon

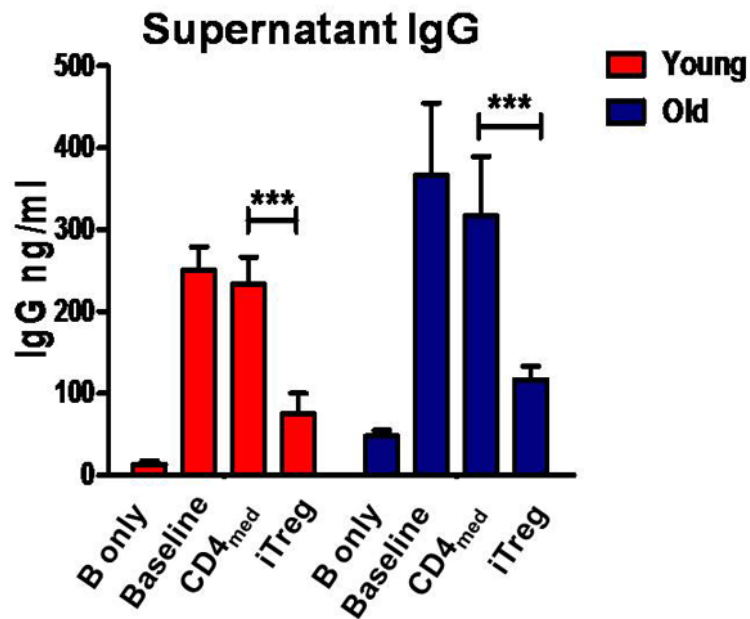
D2+Treg

iTreg can be generated from young or old NZM2328 lupus mice

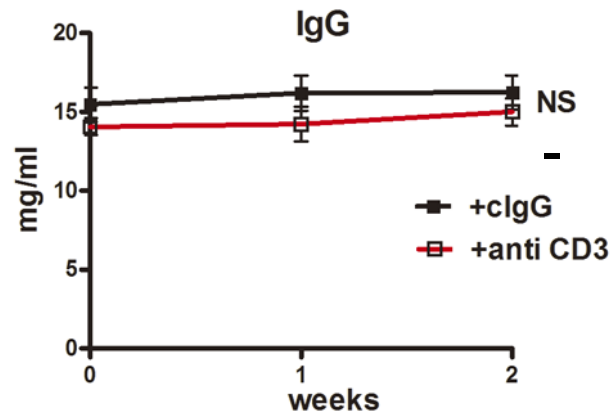


Autologous iTreg can suppress lupus development

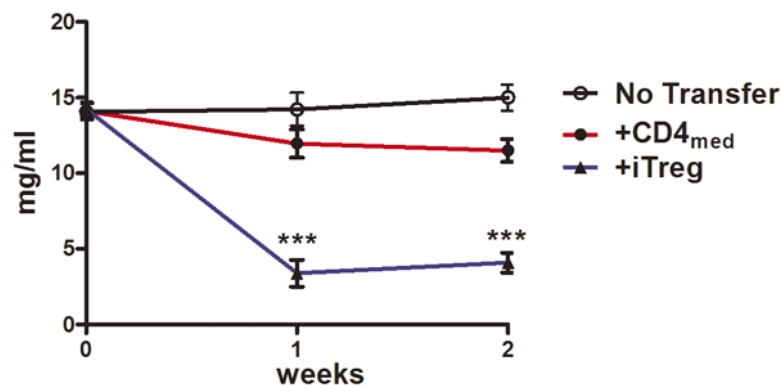
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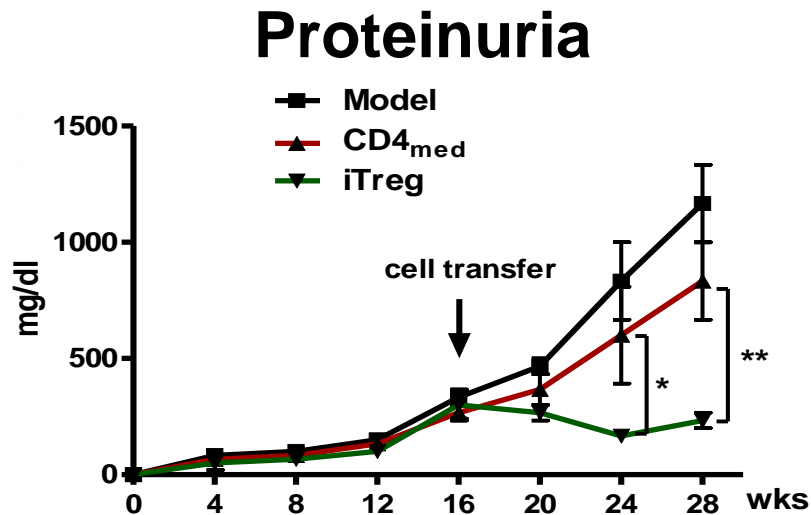
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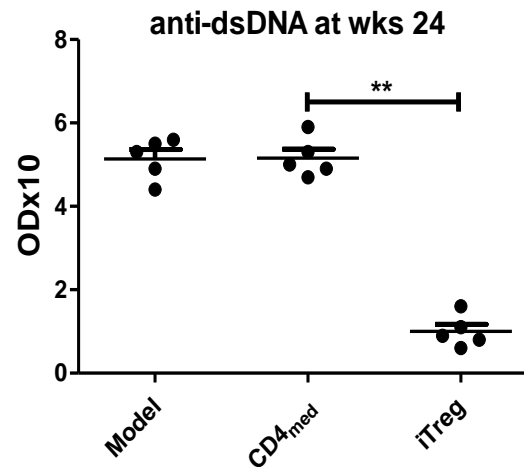
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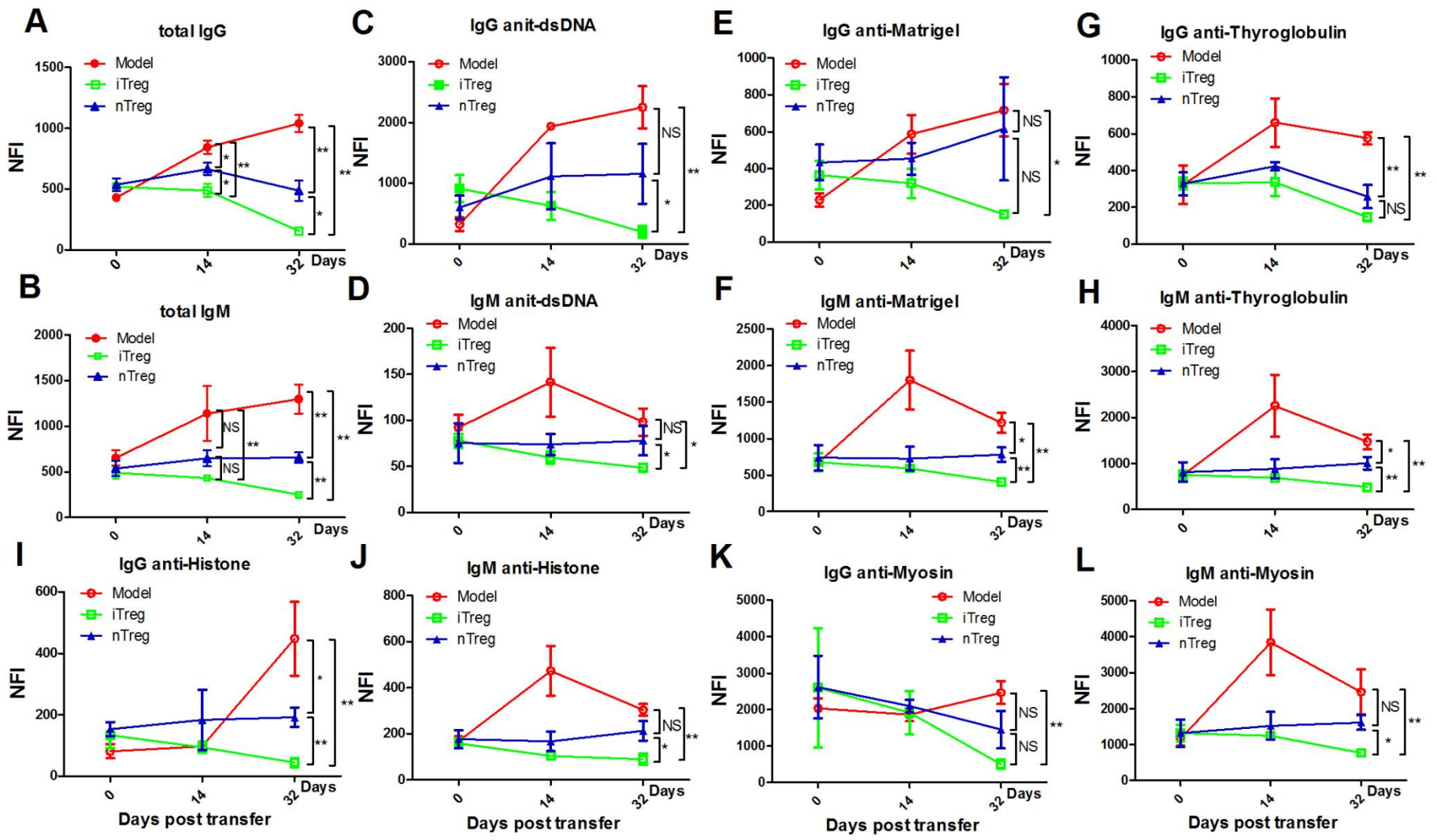
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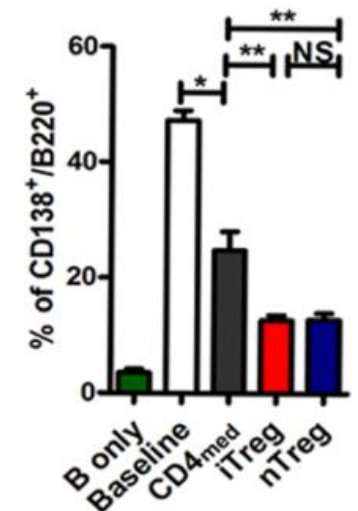
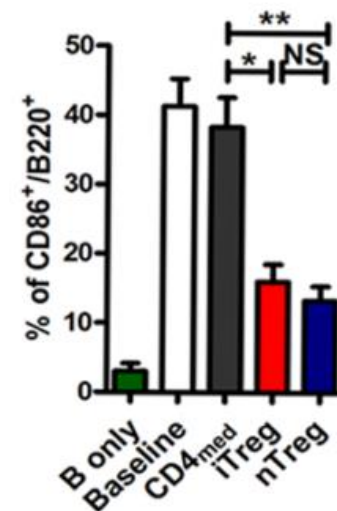
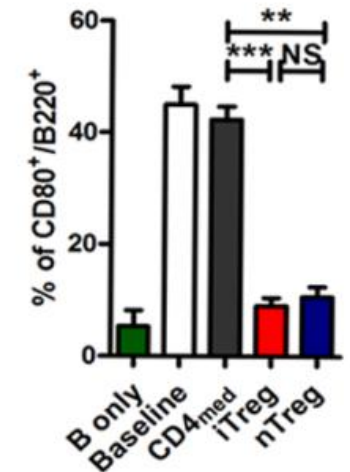
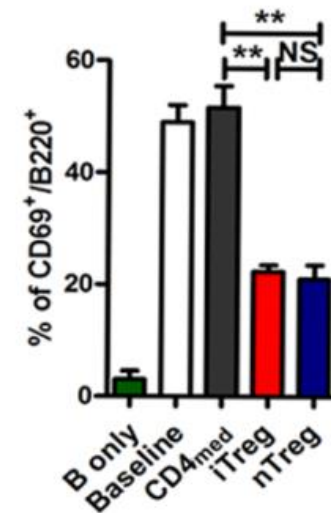
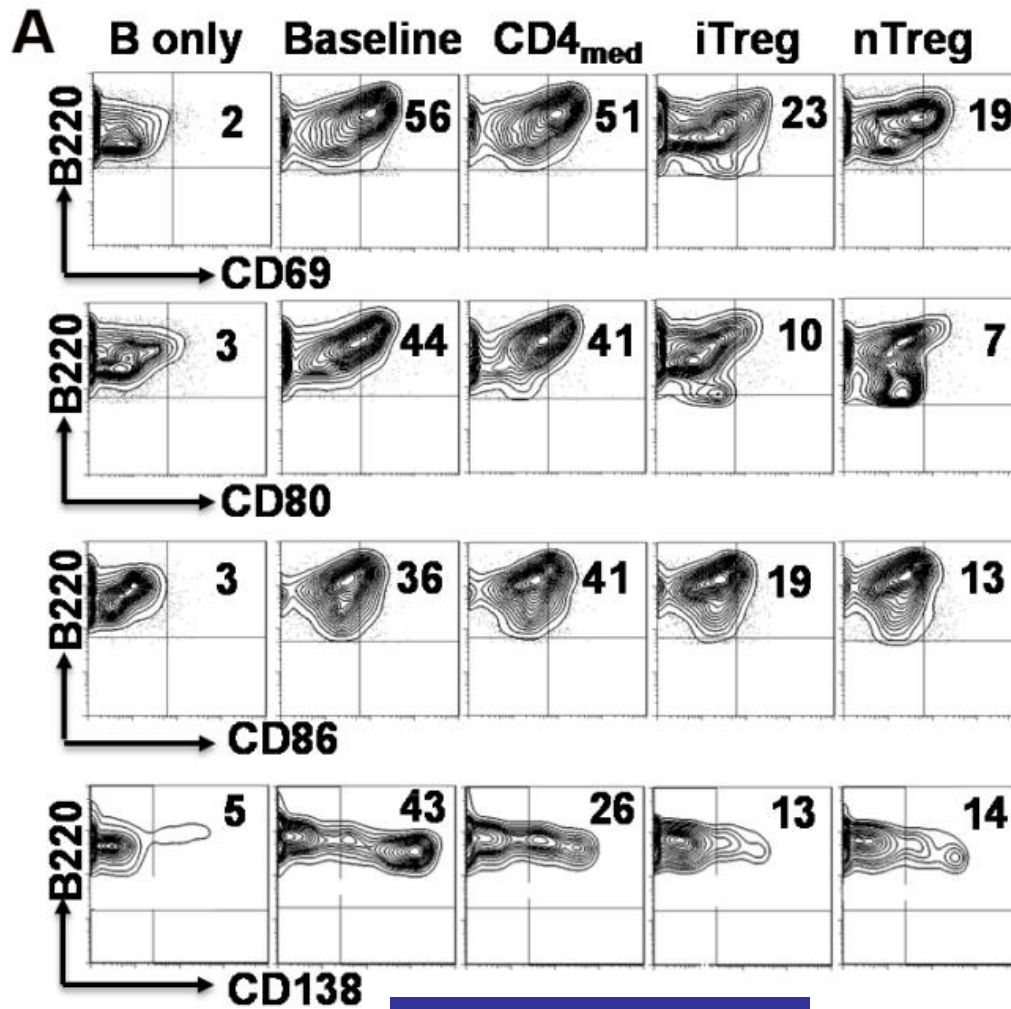
E



iTreg displayed superior effect on the production of antibodies *in vivo*

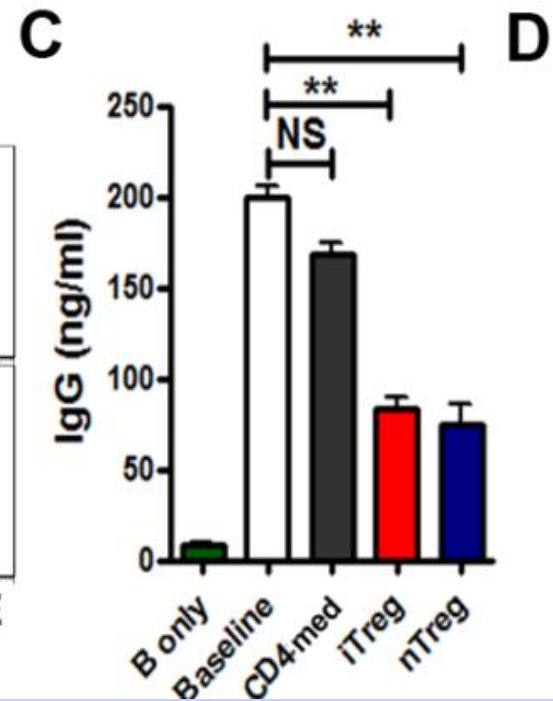
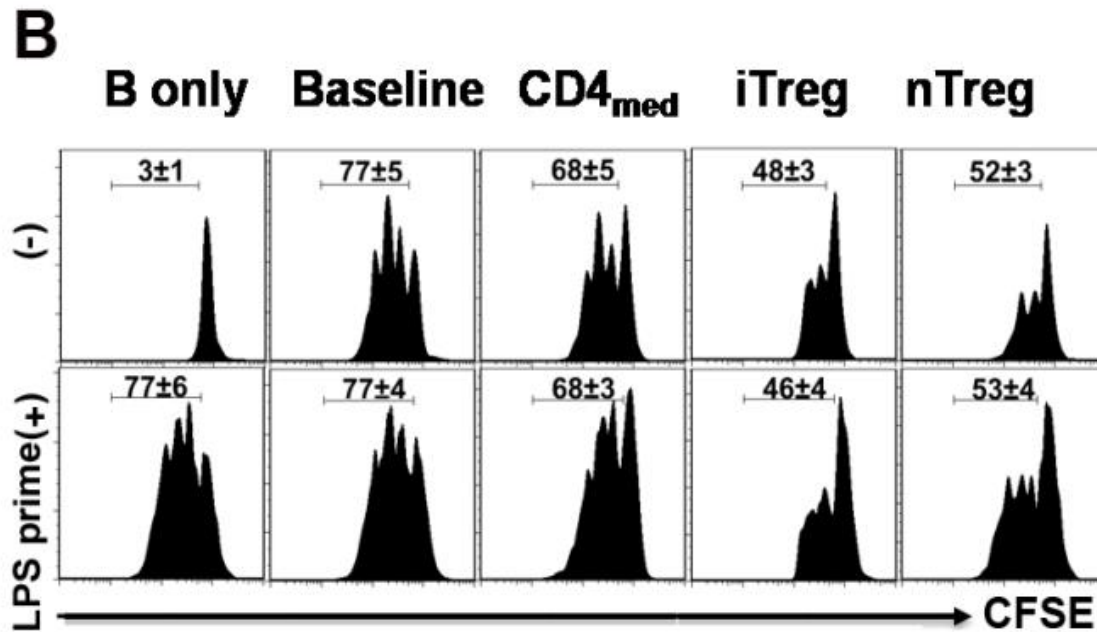


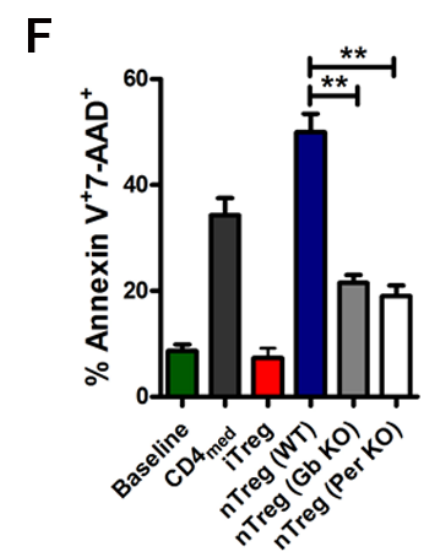
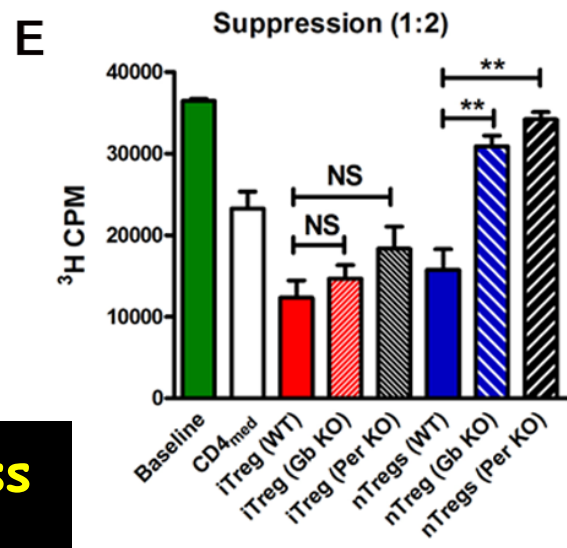
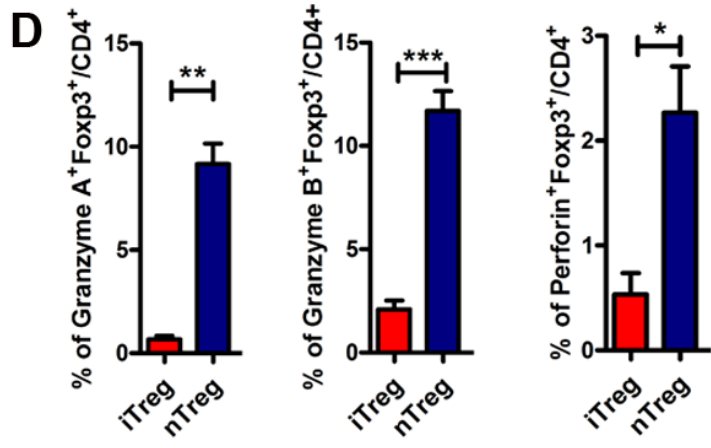
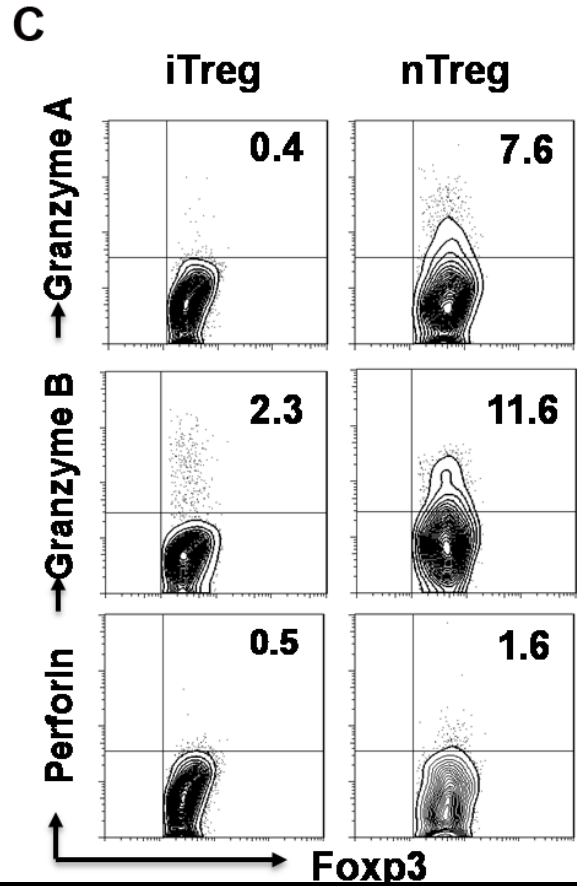
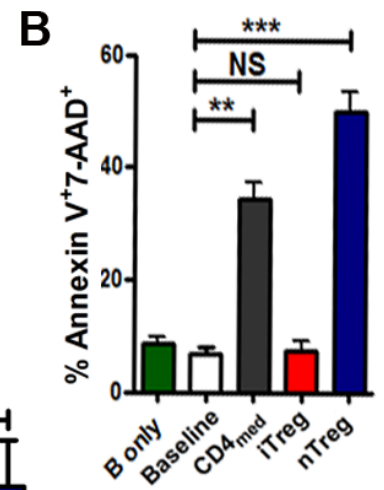
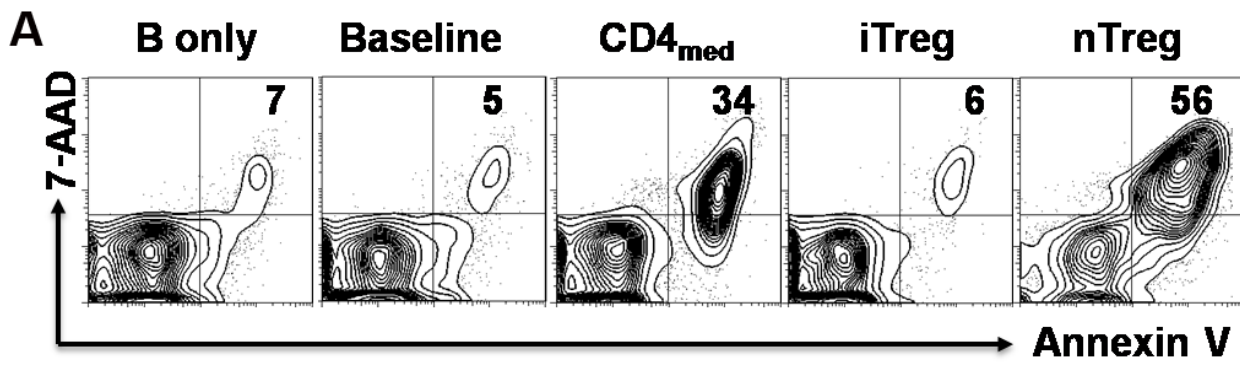
Both nTreg and iTreg suppress B cell activation and differentiation



MS reversion

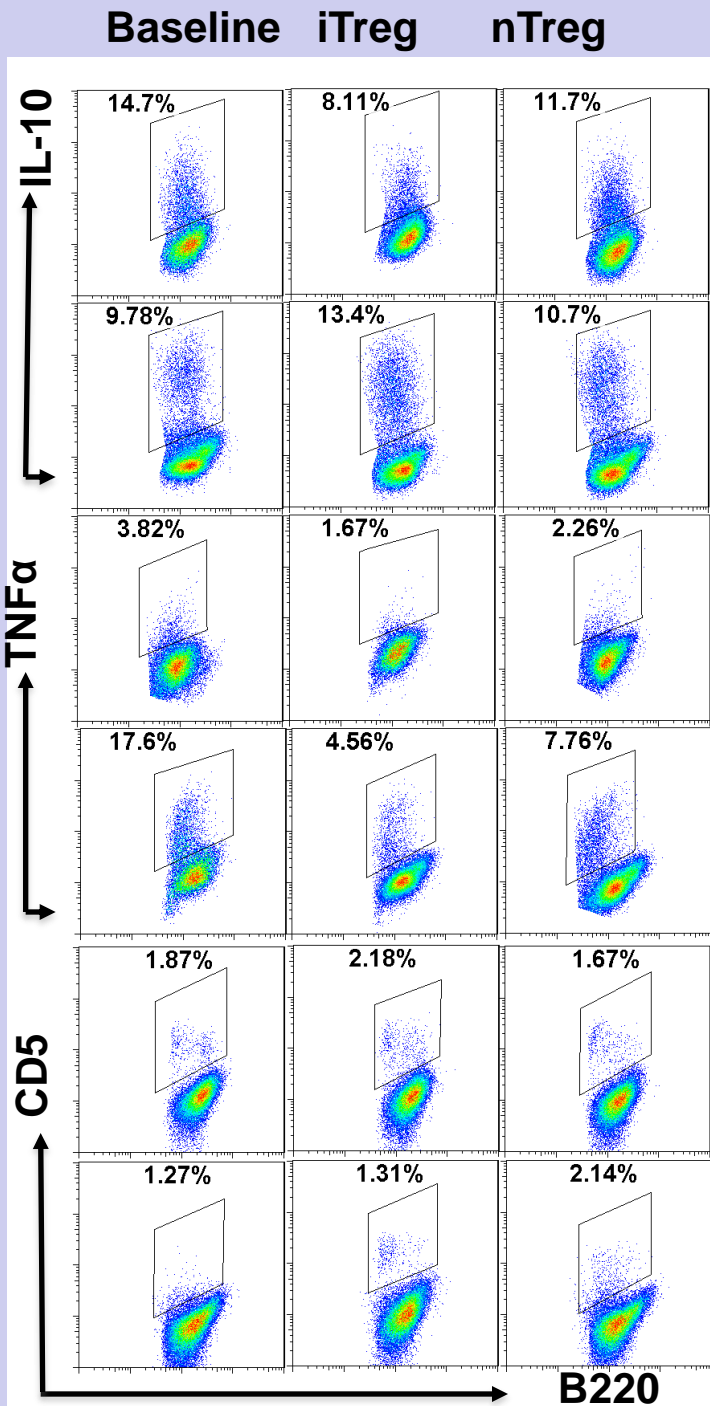
Both nTreg and iTreg suppress B proliferation and IgG production



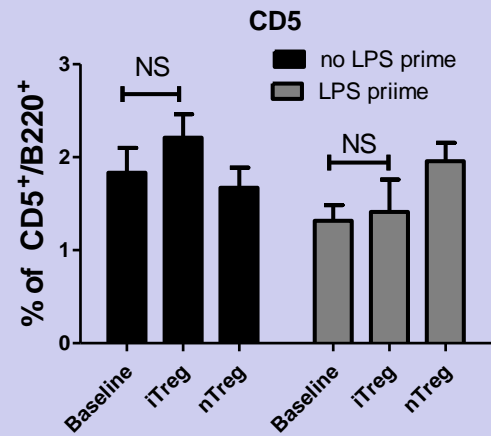
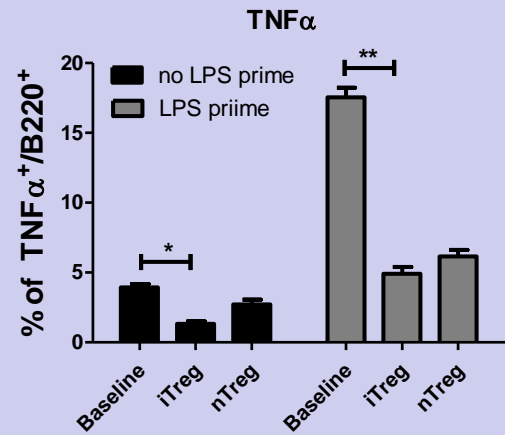
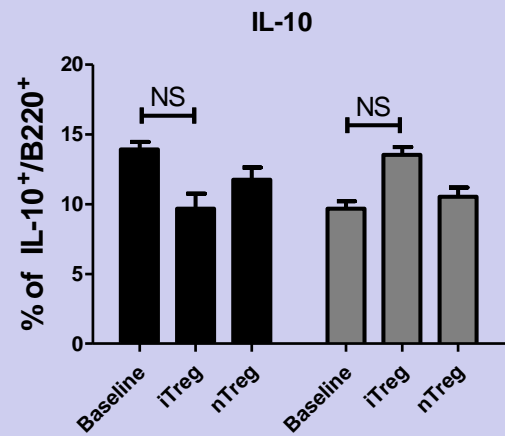


iTreg but not nTreg suppress B cells with cytotoxicity

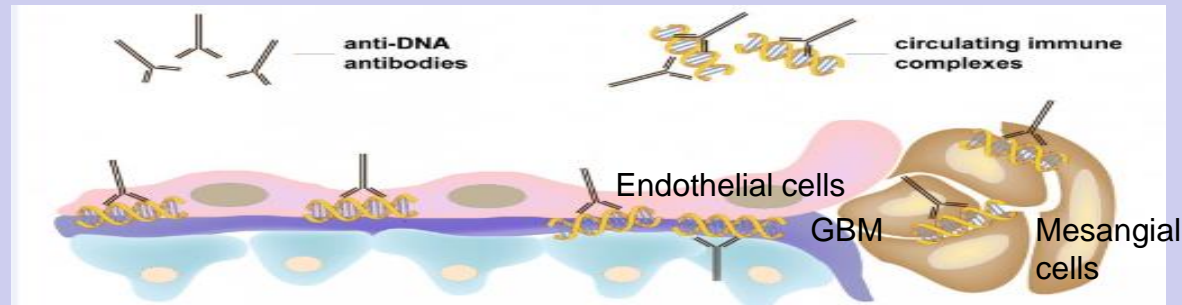
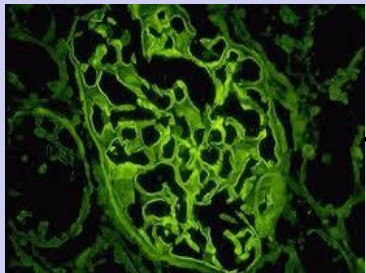
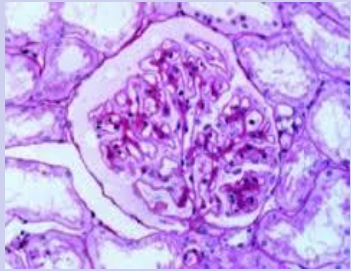
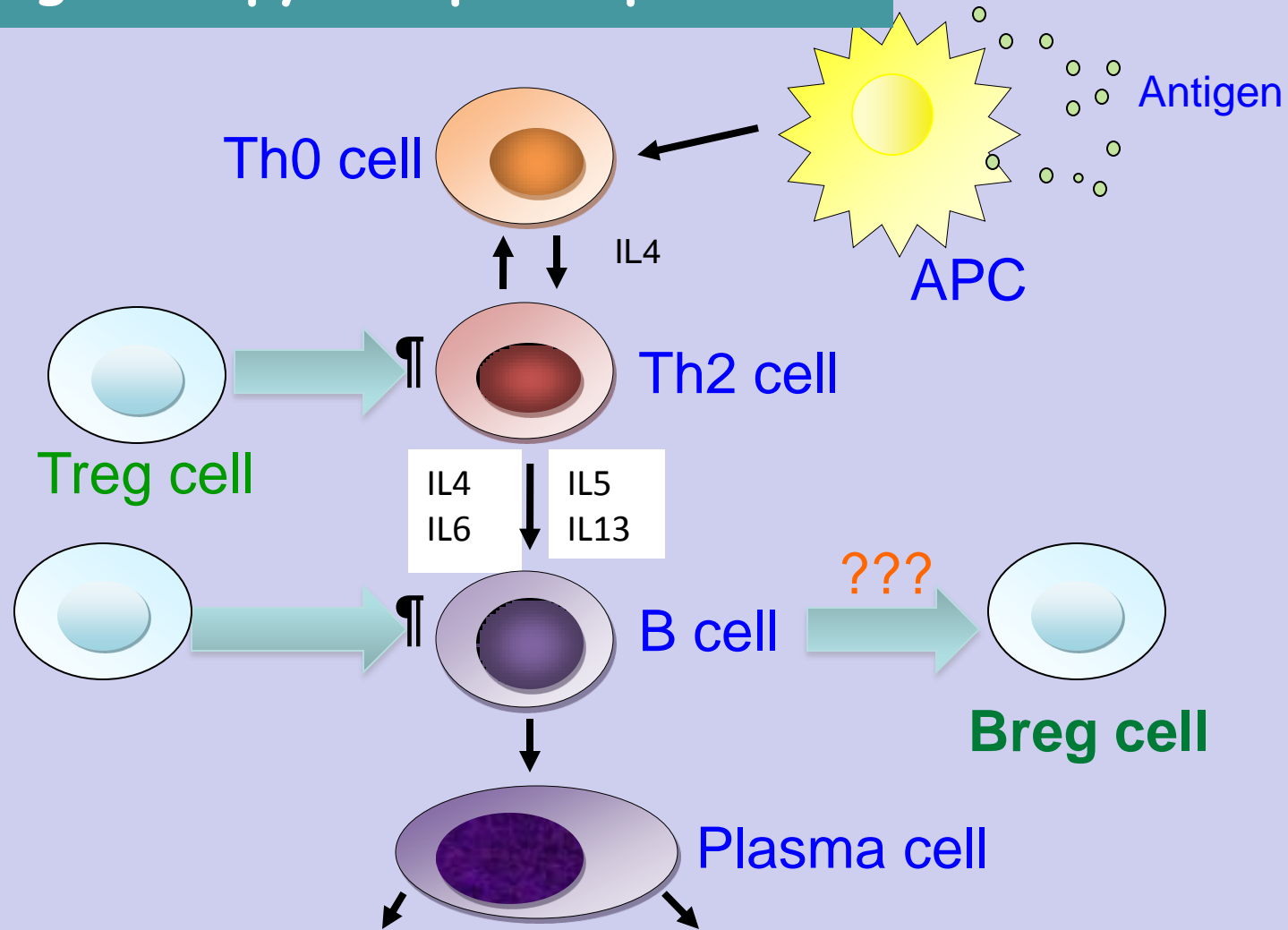
iTreg suppress cytokine production of B cells, Breg?



LPS (+) (-) (+) (-) (+) (-) (+) (-)



Treg therapy in lupus nephritis



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