Th17 and Regulatory T cells in Reproduction

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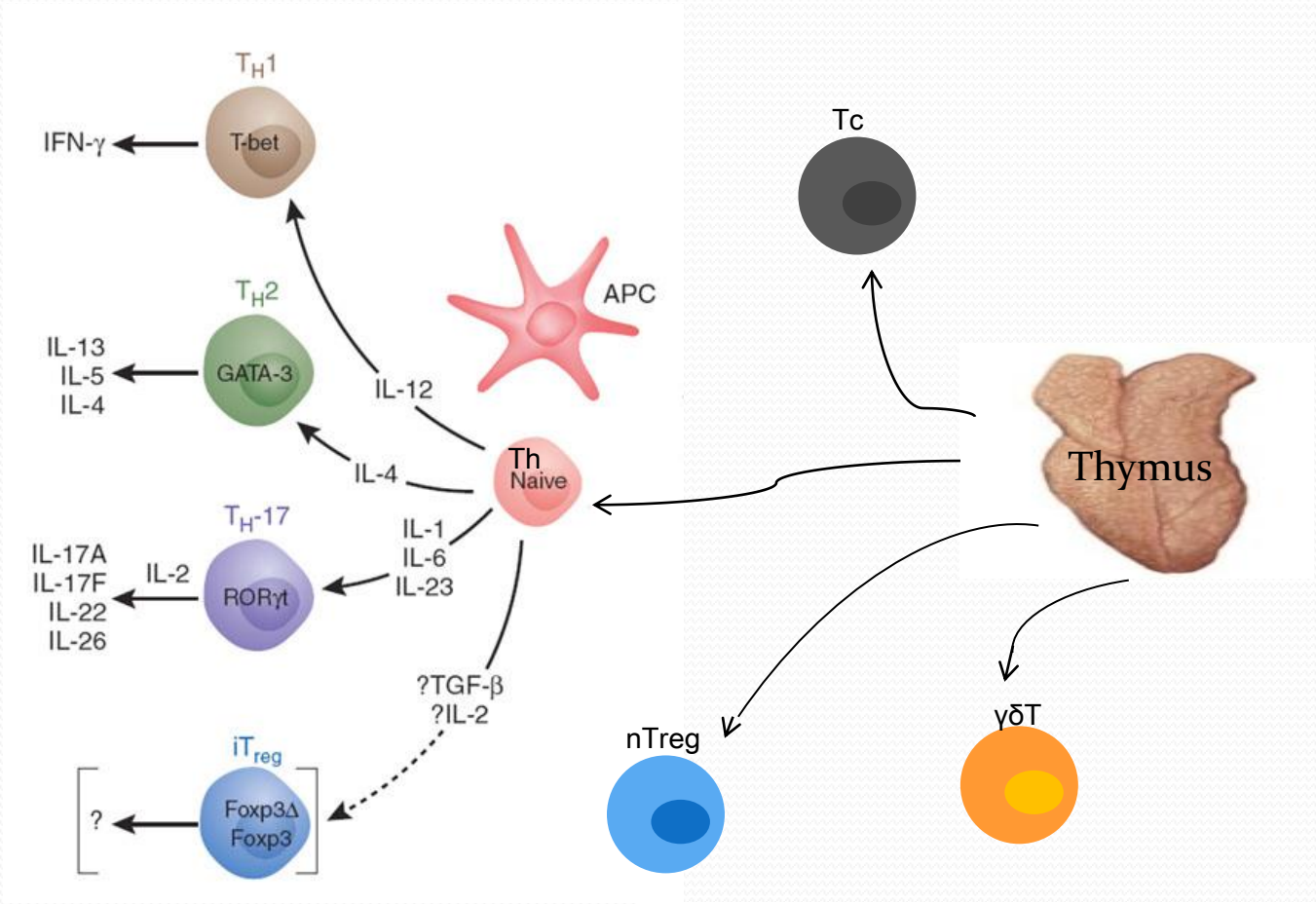
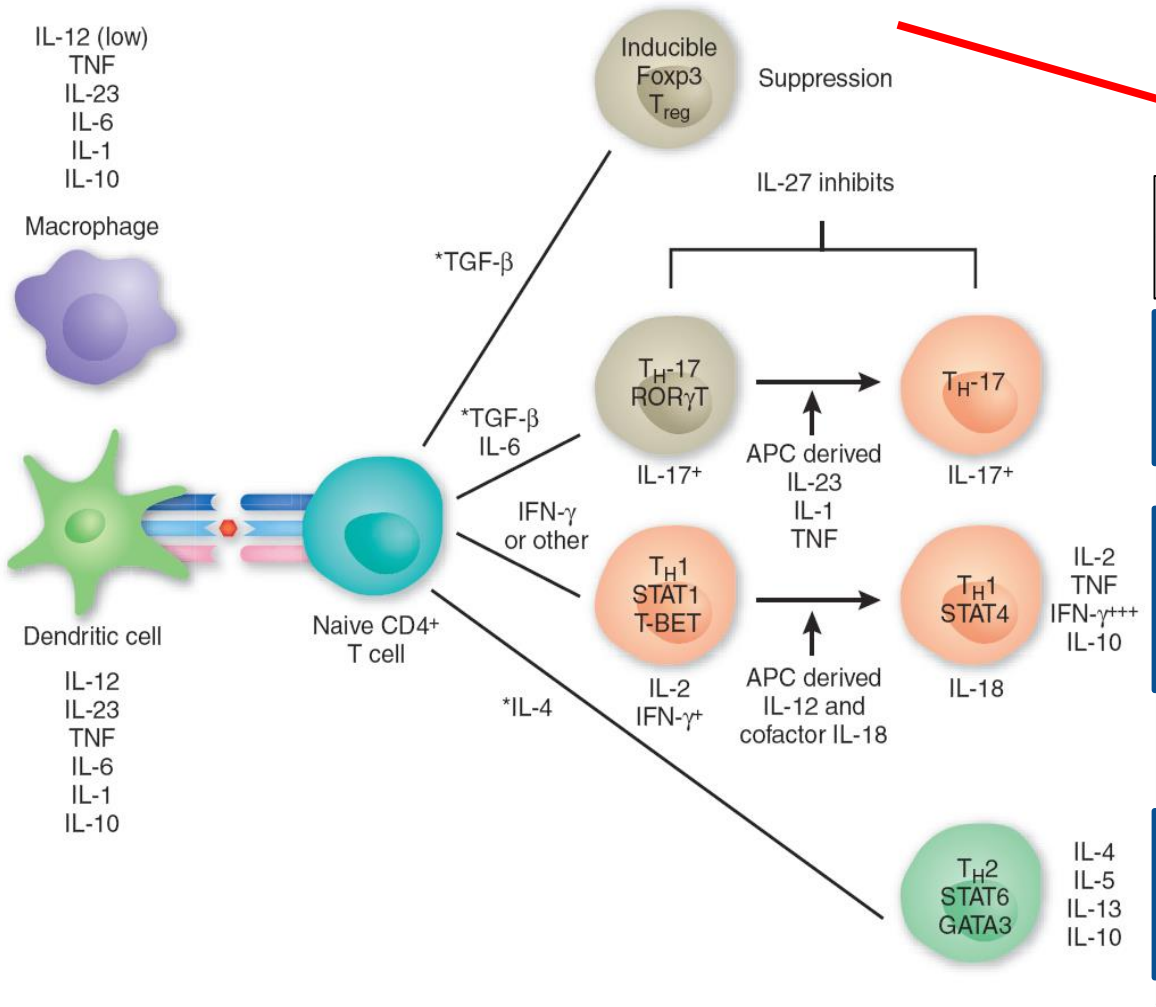
Human T cell subpopulations



NATURE IMMUNOLOGY VOLUME 8 NUMBER 9 SEPTEMBER 2007

Role of T cells



Immune reaction	Target microbes
Acute inflammation: neutrophil infiltration	Acute bacterial and fungal infections
Intracellular pathogens: macrophage activation	Intracellular infection: mycobacteria toxoplasma leishmania listeria
Mucosal barrier: mast cell and eosinophil activation; IgE induction	Helminths

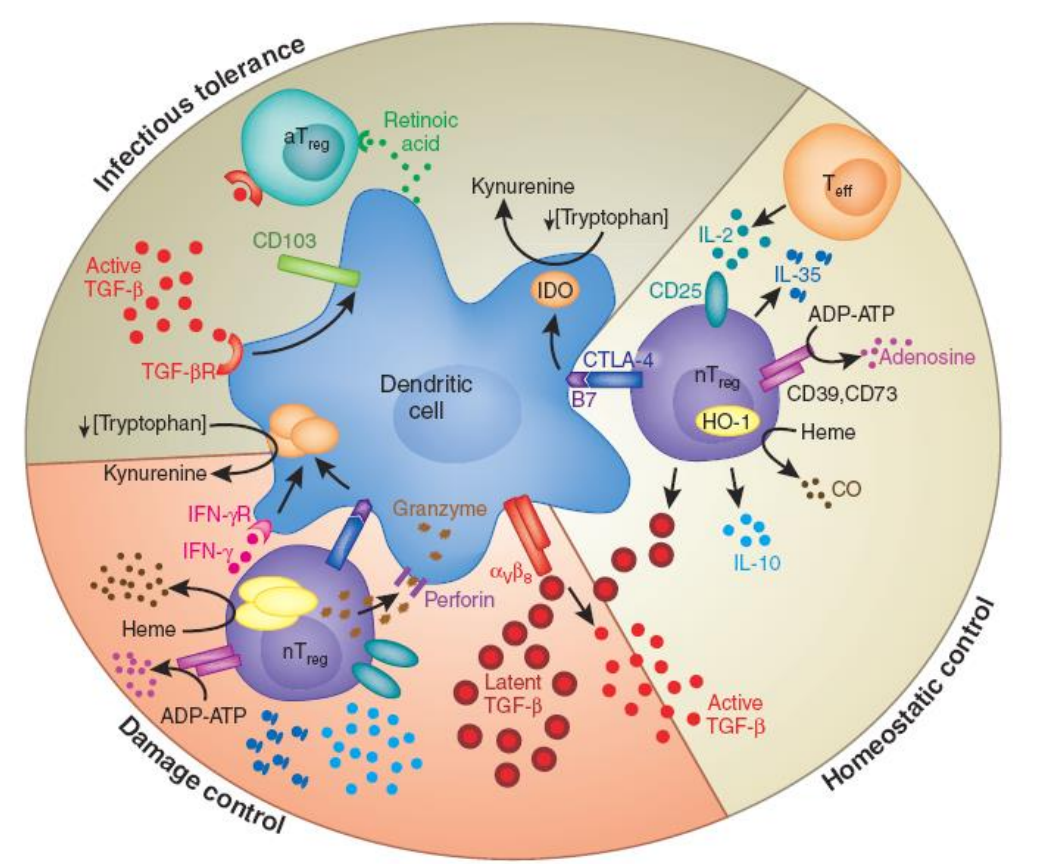
Regulatory cells

- Th3 cells: TGF-β secreting T cells
- Tr1 cells: IL-10 secreting T cells
- $Foxp3^{+}$ Treg cells

$CD4^+CD25^+Foxp3^+$Treg cells

- 5-10% of peripheral $CD4^+$ T cells in human and mice
- Master gene of Treg cells= Foxp3
- Suppress the activation, proliferation and/or effector functions of the T cells, and possibly NK, NKT, B cells, and DCs.

Action mechanism of Treg cells



TGF-β
IL-10
CTLA-4
GranzymeB
Perforin
IDO

IFN-γ
IL-9
HO-1
cAMP
Galectin
CD39-CD73:adenosin
'IL-2 sink'
IL35

Inhibition of

Th1
Th2
Th17
NK
DC
B

Changes in putative pathogenesis of some autoimmune D

- From Th1 theory toTh17 pathogenesis
  - Experimental autoimmune encephalitis
  - Collagen-induced arthritis
  - Some forms of colitis

Th17 cells

- IL-17 secreting T cells
  - First report in 1995
  - Discovery of proinflammatory Th17 cells in 2005
- IL-17
  - 6 forms: A,B,C,D, E, and F
  - IL-17A, commonly referred to as IL-17
- Th17 cells
  - Major transcription factor: retinoic acid receptor-related orphan receptor (ROR)-γt
  - Generation under the influence of IL-6, TGF-β, IL-1
  - Maturation and maintenance by IL-23

IL-17A, IL-17F and IL-22 from Th17 cells

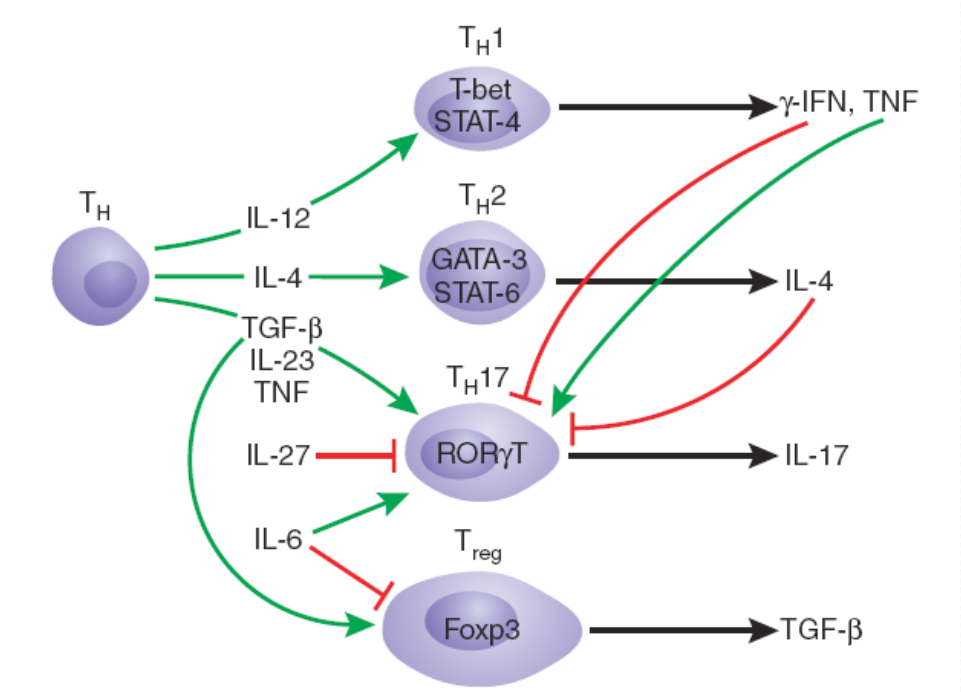
- Neutrophilia
- Tissue remodeling and repair: MMP3, MMP13
- Production of antimicrobial proteins
  - β-defensin 2, β-defensin 3, psoriasin

T cells response following infection

Microbes	Cytokine from innate immune cells	T cells response
Intracellular bacteria & viruses	IL-12	Th1 cells (IFN-γ), $CD8^+$Tc cells
Parasites	IL-4	Th2 cells (IL-4, IL-5, IL-13)
Bacteria and fungi	IL6, IL-21, IL-23, TGF-β, IL-1β	Th17 cells (IL-17A, IL-17F, IL-22)

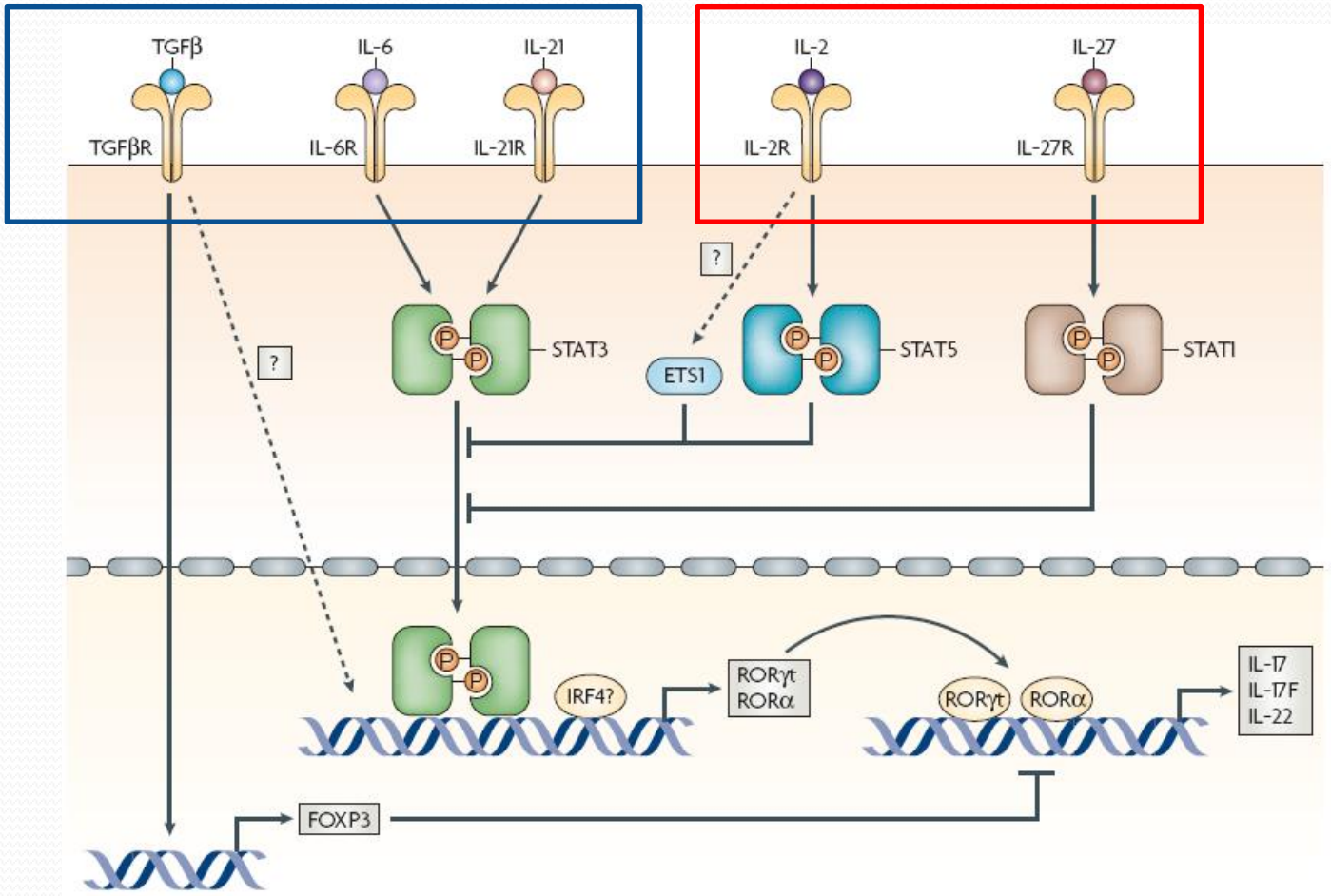
Relationship between Th17 and Th1-Th2 cells

- Crossregulation
  - Th1 Vs. Th2
  - Th1 Vs. Th17
  - Th2 Vs. Th17
  - iTreg Vs. Th17



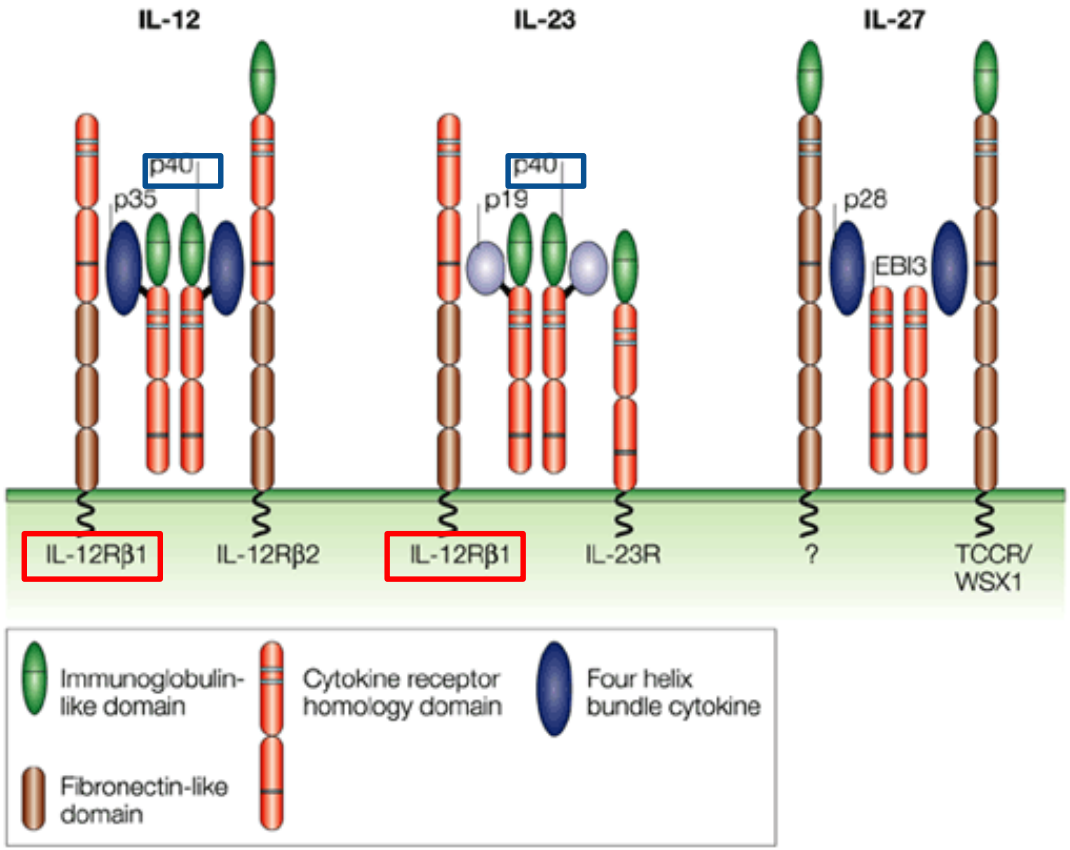
Nat Med 2007;13:139

Transcriptional regulation of Th17 cell differentiation



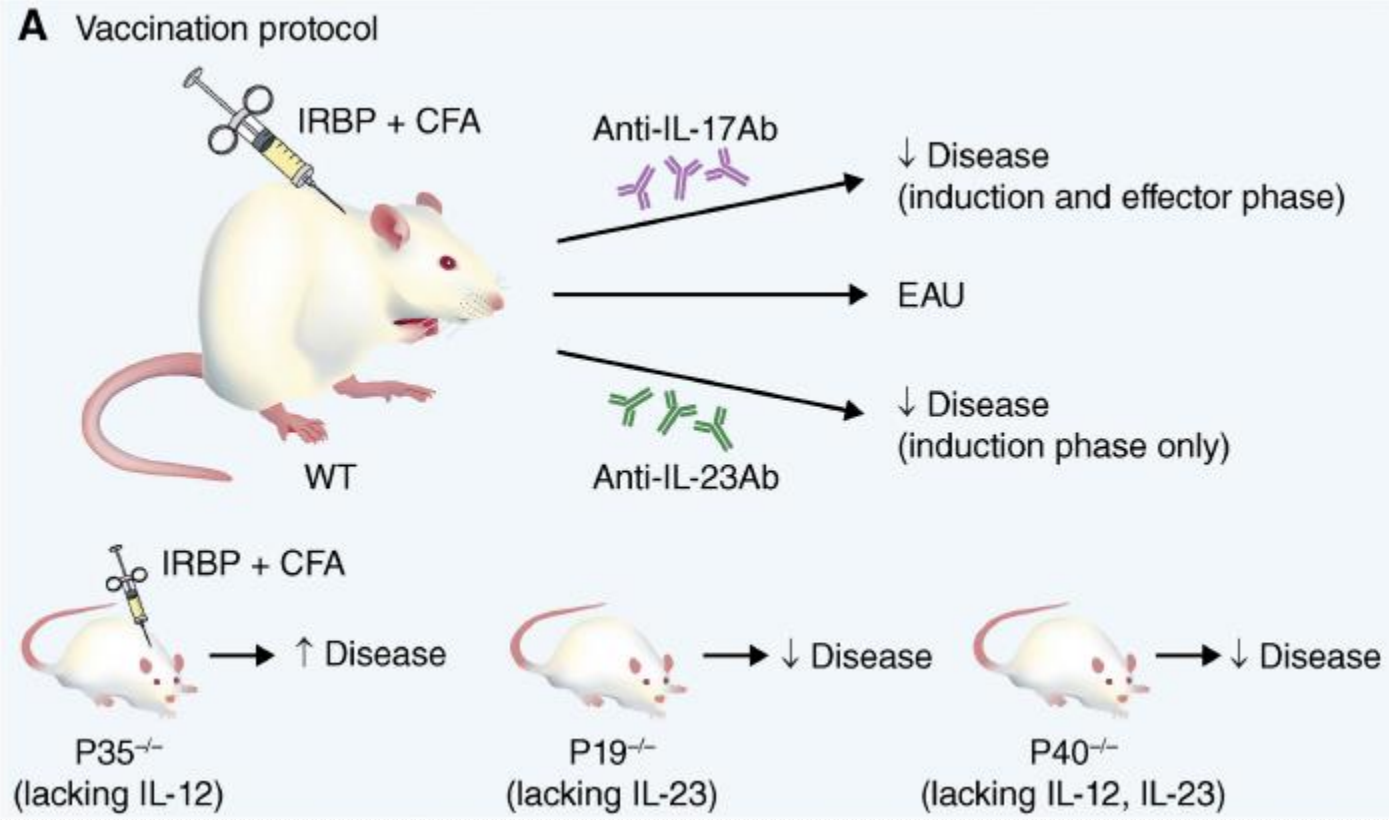
Nat Rev Immunol 2008;8:337

IL-12 is the prototype of a family of heterodimeric cytokines



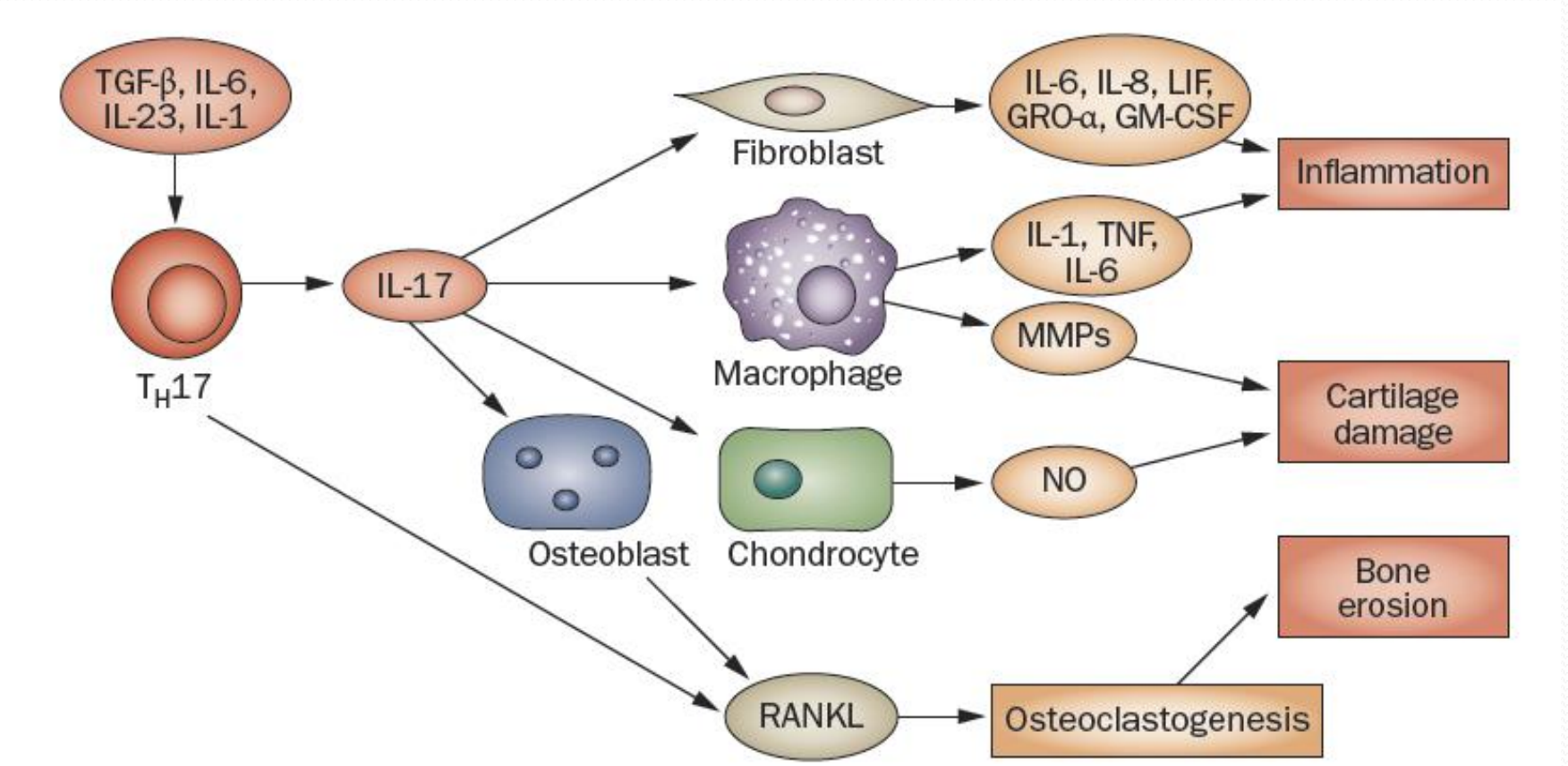
Nature Reviews Immunology ***3****, 133-146 (February 2003)*

Th17 or Th1 cells can induce EAU depending on the method of disease induction



JEM 2008;205:1517

Action of Th17 cells in Rheumatoid Arthritis



Cytokines produced by Th17 cells

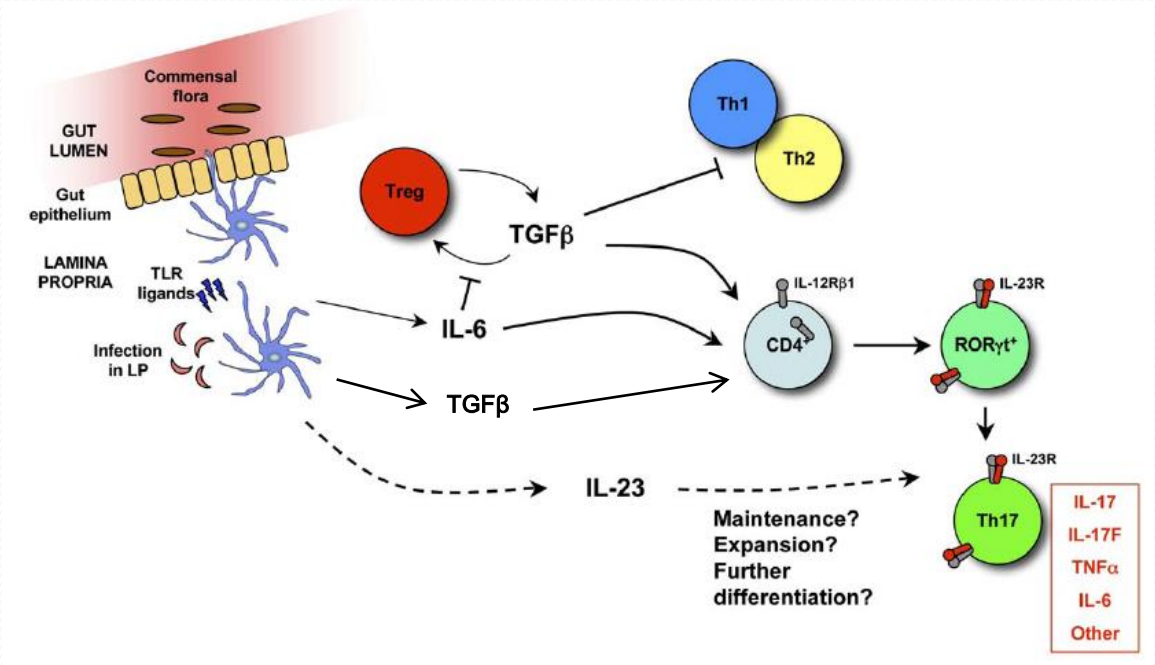
	Function
IL-17A	Fibroblast: chemokine , MMP production
IL-17F	Unknown, weak activity
IL-22	Antimicrbial peptides: β-defensin-2, β-defensin-3
IL-21	Potent regulator of $CD8^+$ Tc cells, regulation of Th17 differentiation
CCL20 (ligand for CCR6)	Produced by Th17 cells and intestinal tissue. Recruitment of Th17 cells to inflamed tissues
IL-23	Expressed by activated DCs and macrophages Important to expand Th17 cells

Clinical significance of Th17 cells

- Infectious Ds: several bacterial, viral, and fungal inf.
- Several autoimmune Ds
  - IBD, MS, systemic scleroderma, asthma, RA, uveitis, SLE, Sjögren's syndrome, allergic contact dermatitis, etc.
- Th17-associated cytokines
  - Contribution to Metabolic D via IL-17
    - Atherosclerosis
    - Diet-induced obesity in mice
  - Promotion of tumors
    - Chemically induced papillary skin tumors in mice (IL-23)
    - B16 melanoma and MB49 bladder ca (IL-17)

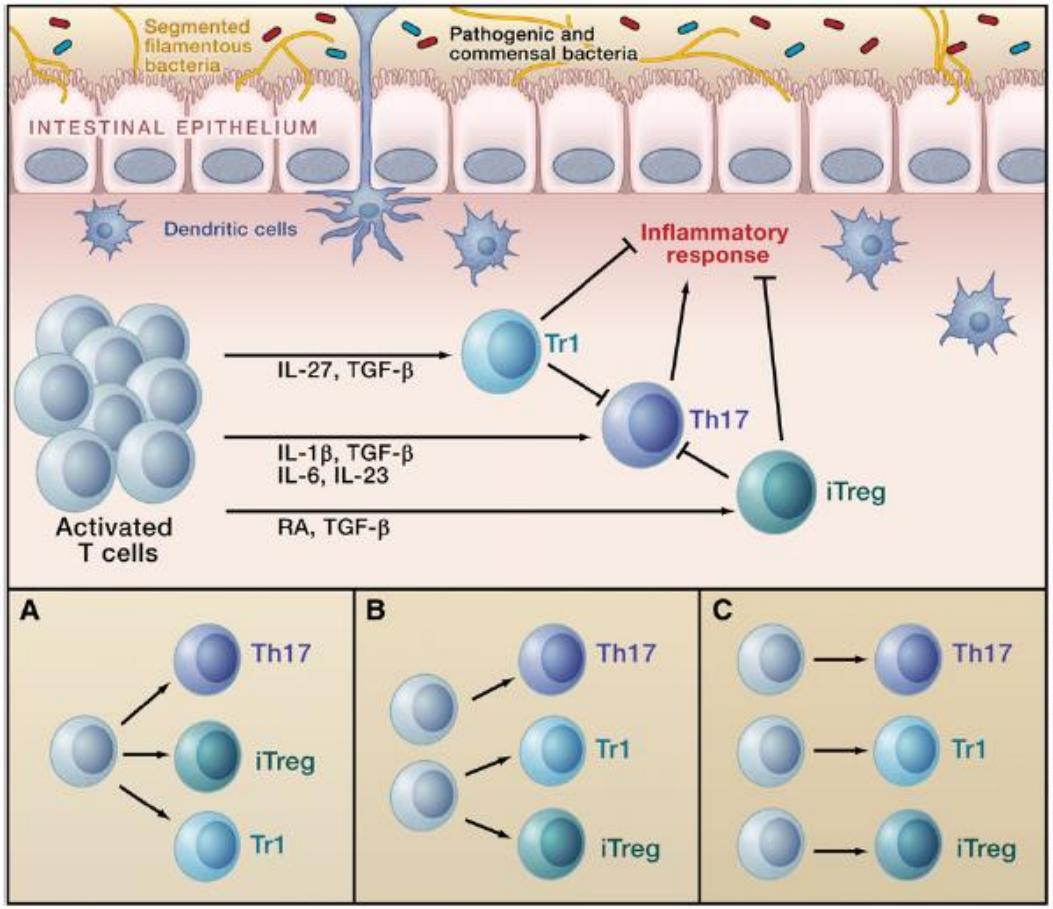
Th17 and Treg cells are most abundant in intestinal LP

- Accumulation of T cells in LP caused by
  - Self Ag, food Ag, microbial Ag
  - Commensal-derived signal



Cell, 2006;126:1121

Possible Relationship between Precursors Differentiating into Proinflammatory and Protective T Cells in the Gut: 3 possible ways



* Gut DCs produce RA.

Cell, 2010;140:845

IL-17 producing cells

- $CD4^+$ and $CD8^+$ cells
- γδ T cells
- LTi (lymphoid tissue inducer) cells: $lineage^-$ (Lin^-) $CD127^+RORC^+$ LTi-like cells
- NK-like cells: $ROR\gamma t^+$ $NKp46^+$ NK-LTi cells

Balance of Effector & Treg cells

- Can be achieved by
  - Influx of predifferentiated T cells
  - Local induction of distinct effector and/or regulatory T cell profiles
  - Redirection of the differentiation program of T cells

Proinflammatory cytokines upregulate adhesion molecules

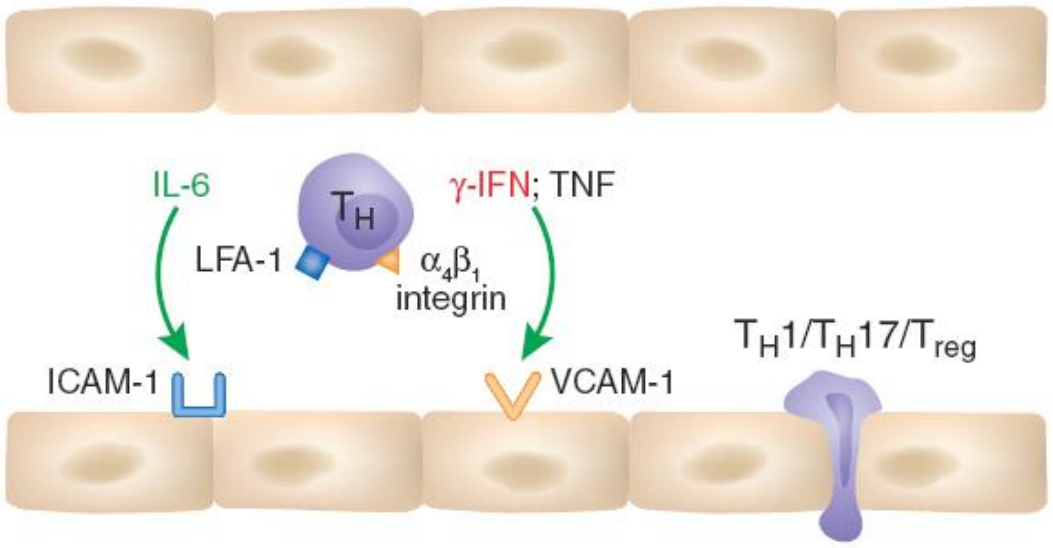
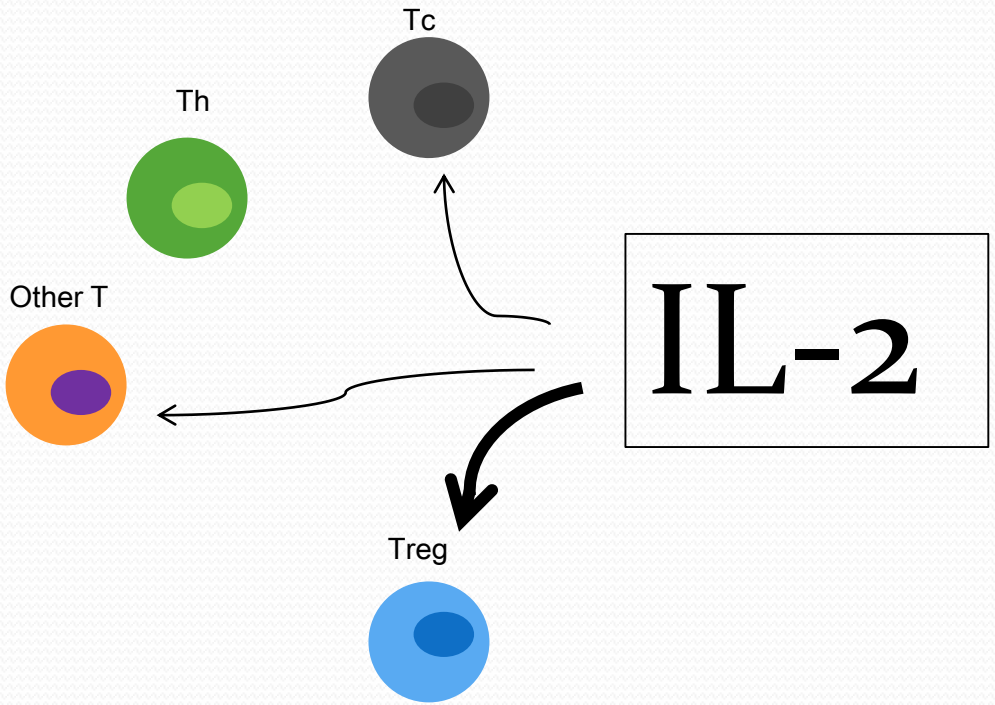


Figure 2 IL-6, TNF and γ-interferon upregulate adhesion molecules, allowing T-helper cells to gain access to critical organs in autoimmune disease.

Nat Med 2007;13:139

Immune equilibrium by a negative regulatory loop



Th17 and Treg cells in idiopathic RPL women

- Hypothesis: ↑inflammatory response and ↓immune regulation in women with idiopathic RPL

- We investigated
  - Levels of Type 1 T, Type 2 T, Th17, and Treg cells
  - Ratios of Pro-inflammatory cells to Anti-inflammatory cells
  - Correlations of Th17 cell level with other T cell subset levels

Materials and Methods (1)

- Idiopathic RPL women (n=42)
  - 2 or more SABs
  - No abnormality in genetic, anatomic, endocrine and infectious factors
- Healthy women in reproductive age (n=34)
  - Fertile or Unmarried women
  - No Hx of SAB

Materials and Methods (2)

- PBMC in the early or mid-follicular phase cycle
- Flow cytometric study
  - Type 1 and type 2 intracellular cytokine study:
    - anti-CD3, anti-CD8, anti-TNF-α, anti-IFN-γ, anti-IL-10
  - Th17 cell study: anti-CD3, anti-IL-17A mAbs
  - Treg cell study: anti-CD4, anti-CD25, anti-Foxp3
- Statistics
  - Student t-test
  - Partial correlation adjusted by age

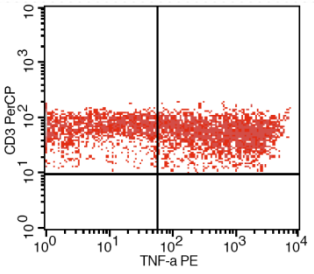
Age and Obstetric histories of subjects

	RPL (n=42)	Controls (n=34)	P-value
Age, years	32.5 ± 4.0	31.1 ± 3.7	NS
Parity (range)	0.1 ± 0.3 (0-1)	1.2 ± 1.0 (0-3)	<0.001
No. SAB (range)	3.3 ± 1.5 (2-10)	0.0 ± 0.0 (0)	<0.001

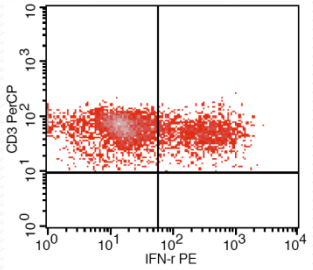
Values are mean ± SD

FACS analysis of T cell subsets

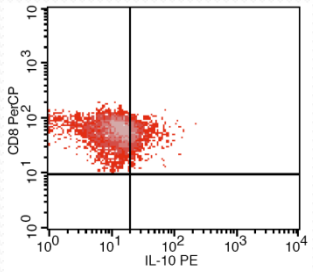
- Th1 and Th2 cytokine producing T cells



TNF-α⁺ cells

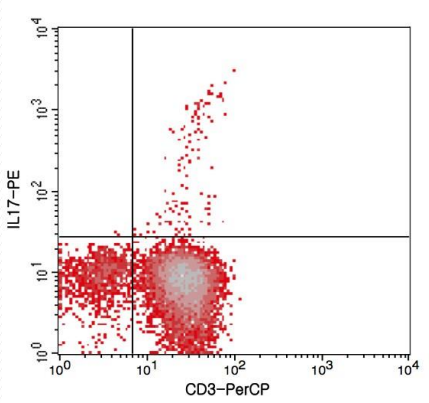


IFN-γ⁺ cells

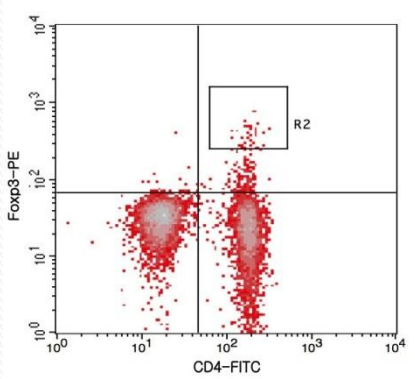


IL-10⁺ cells

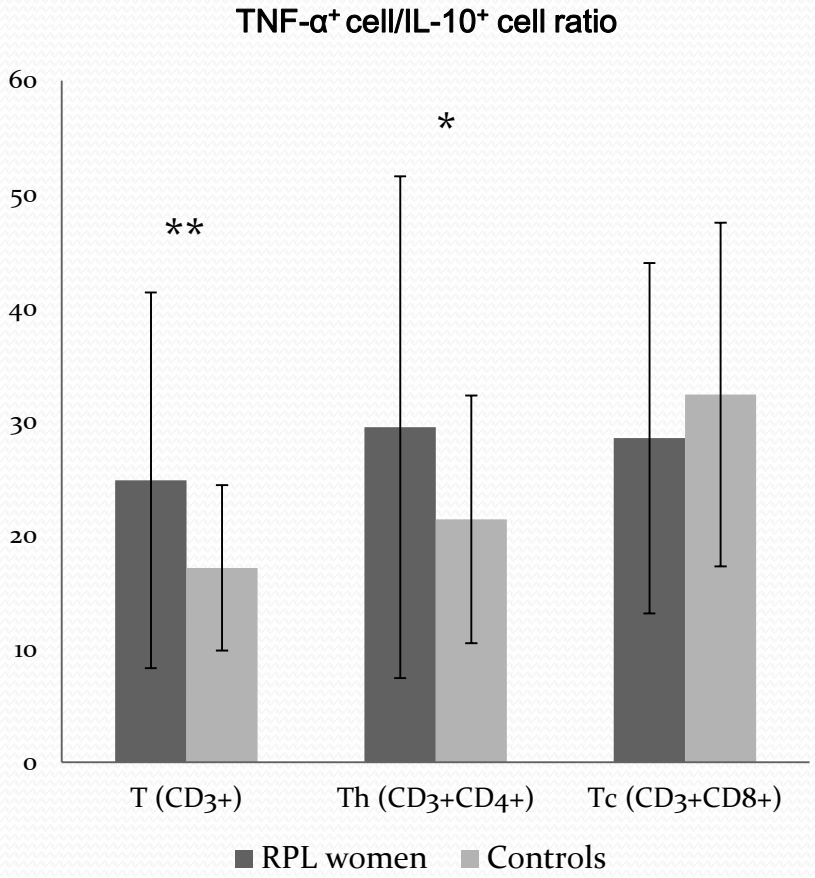
- Th17 cells

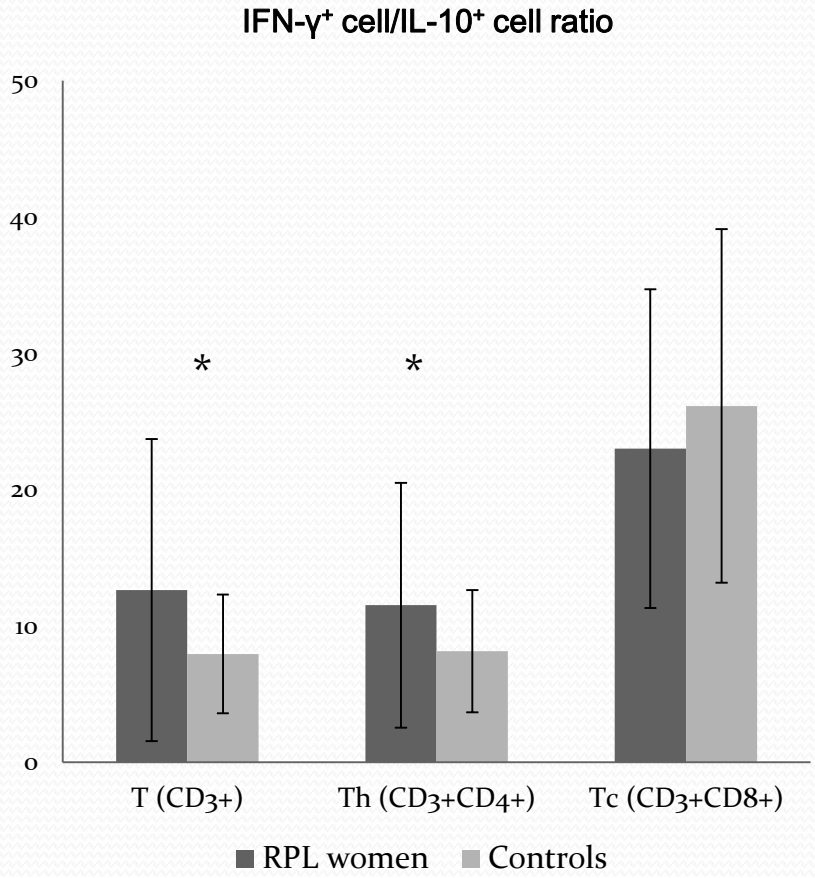


- Treg cells



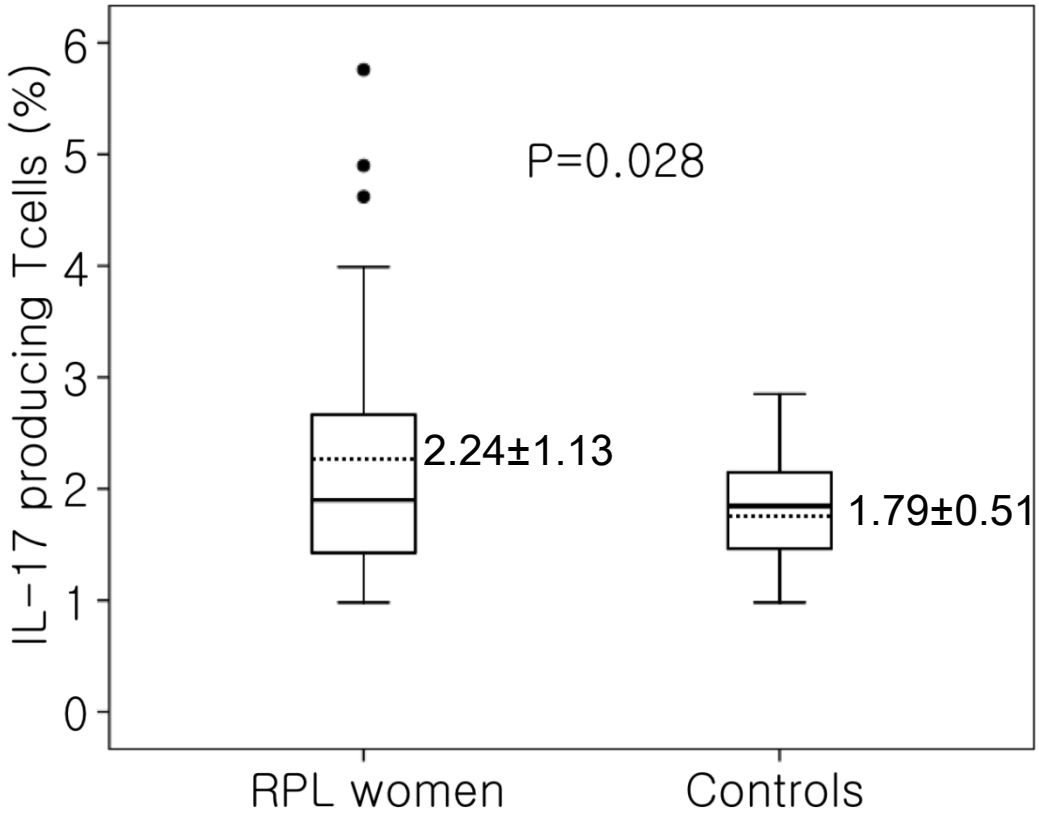
Comparison of Type 1/Type 2 cytokine-producing T cell ratios between RPL women and Controls



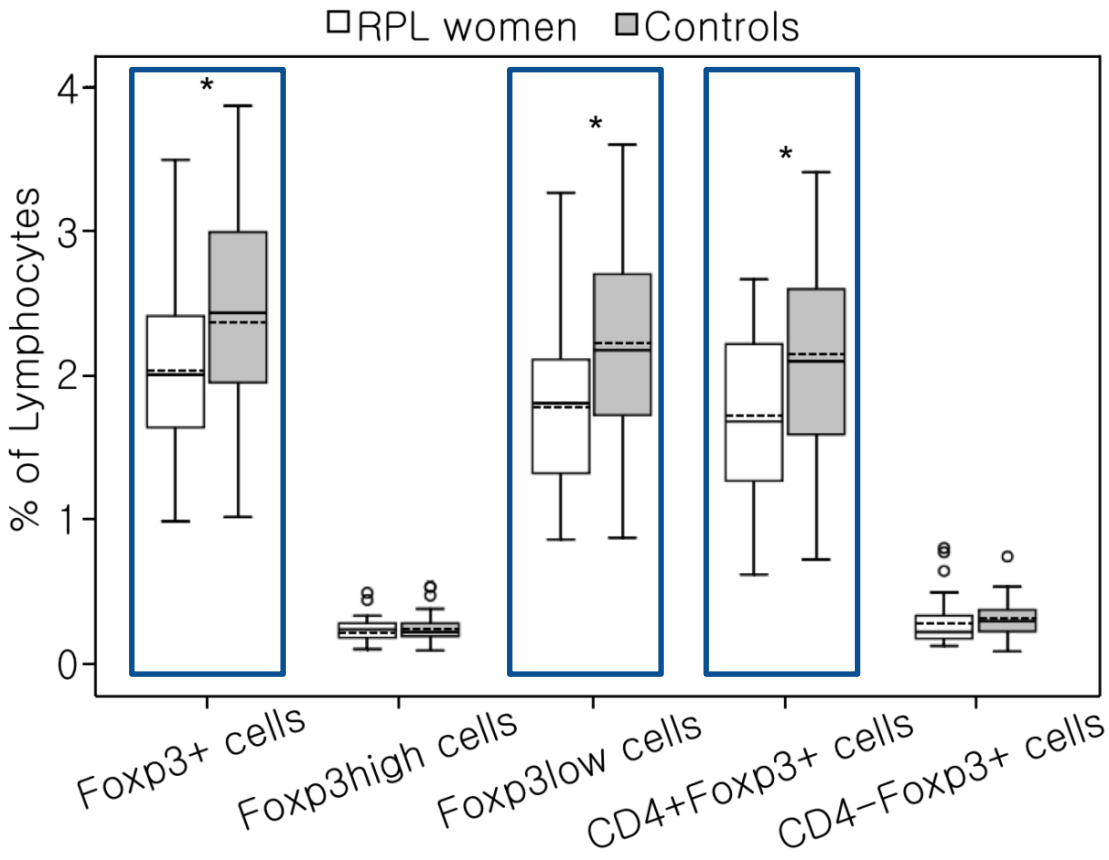


* $p<0.05$, ** $p<0.01$

Comparison of Th17 cell levels between RPL women and Controls

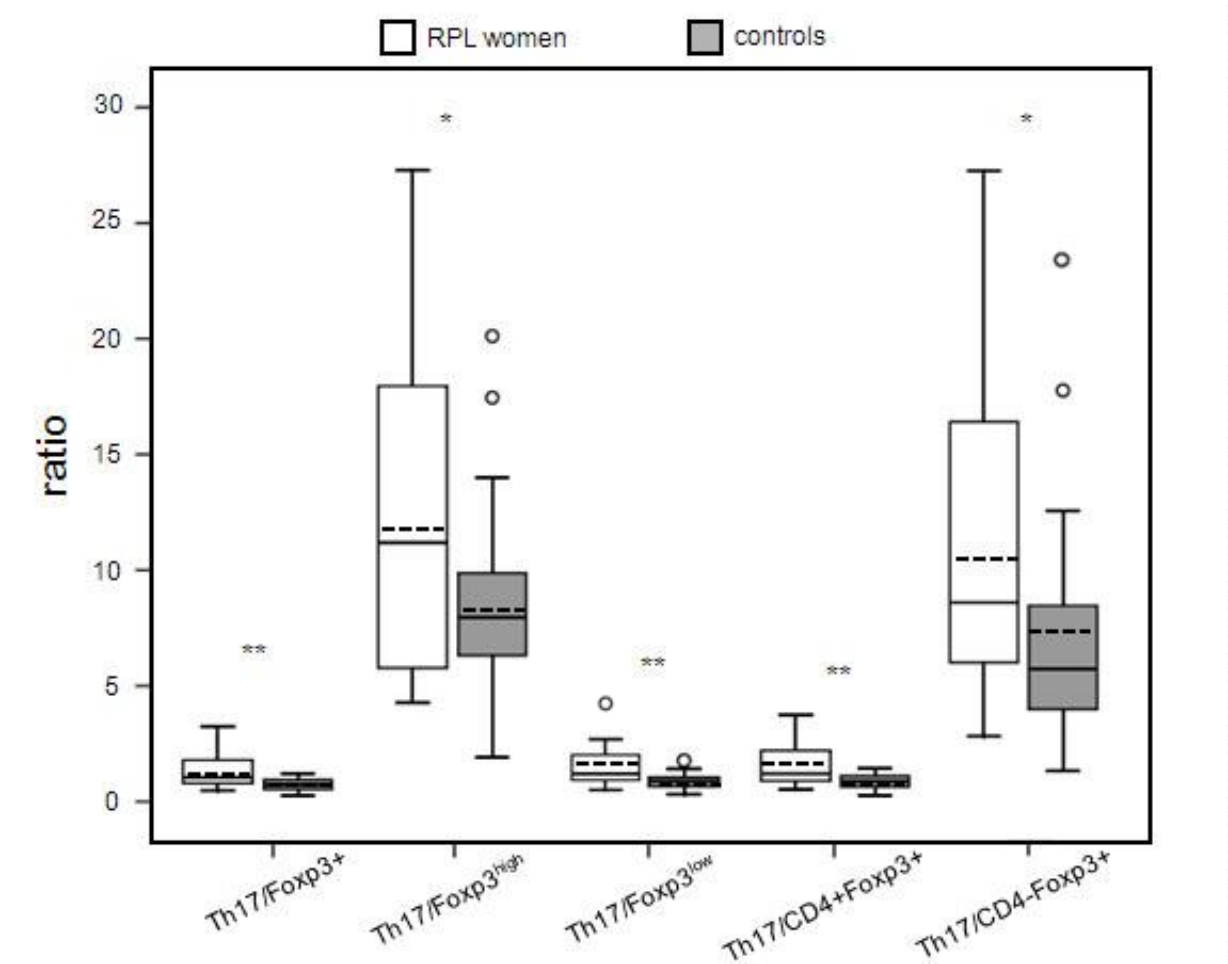


Comparison of Treg cell subsets between RPL women and Controls



* p<0.05

Th17/Treg cell ratios in RPL



* P<0.05, ** p<0.01

Correlation of Th17 cells with Treg cell subsets

		Th17 cells	
		R	P-value
Regulatory T cell subsets			
	$Foxp3^{+}$ cells	-0.029	NS
	$Foxp3^{high}$ cells	-0.001	NS
	$Foxp3^{low}$ cells	-0.030	NS
	$CD4^{+}Foxp3^{+}$ cells	-0.051	NS
	$CD8^{+}Foxp3^{+}$ cells	0.085	NS

Correlation of Th17 cells with Cytokine-producing T cells

		Th17 cells	
		R	P-value
Cytokine-producing T cells			
T cells	$CD3^+TNF\text{-}\alpha^+$ cells	0.113	NS
	$CD3^+IFN\text{-}\gamma^+$ cells	-0.014	NS
	$CD3^+IL10^+$ cells	-0.120	NS
Th cells	**$CD3^+CD4^+TNF\text{-}\alpha^+$ cells**	**0.278**	**0.017**
	$CD3^+CD4^+IFN\text{-}\gamma^+$ cells	0.093	NS
	$CD3^+CD4^+IL\text{-}10^+$ cells	-0.126	NS
Tc cells	$CD3^+CD8^+TNF\text{-}\alpha^+$ cells	0.020	NS
	$CD3^+CD8^+IFN\text{-}\gamma^+$ cells	-0.040	NS
	$CD3^+CD8^+IL\text{-}10^+$ cells	0.193	NS

Correlation of Th17 cells with Ratios of Type 1 and Type 2 cytokine-producing T cells

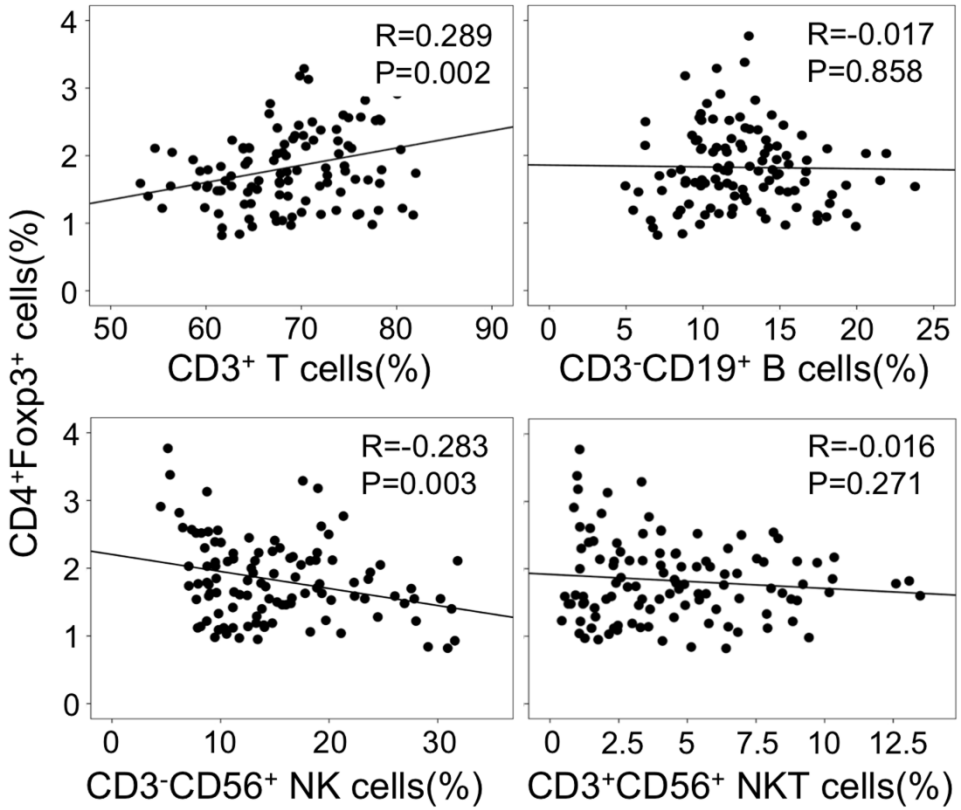
		Th17 cells	
		R	P-value
Ratios of type 1 to type 2 cytokine-producing T cells			
T cells	$CD3^+TNF\text{-}\alpha^+/CD3^+IL\text{-}10^+$ cell ratio	0.153	NS
	$CD3^+IFN\text{-}\gamma^+/CD3^+IL\text{-}10^+$ cell ratio	0.070	NS
Th cells	**$CD3^+CD4^+TNF\text{-}\alpha^+/CD3^+CD4^+IL\text{-}10^+$ cell ratio**	**0.275**	**0.018**
	$CD3^+CD4^+IFN\text{-}\gamma^+/CD3^+CD4^+IL\text{-}10^+$ cell ratio	**0.255**	**0.029**
Tc cells	$CD3^+CD8^+TNF\text{-}\alpha^+/CD3^+CD8^+IL\text{-}10^+$ cell ratio	-0.122	NS
	$CD3^+CD8^+IFN\text{-}\gamma^+/CD3^+CD8^+IL\text{-}10^+$ cell ratio	-0.123	NS

Conclusion

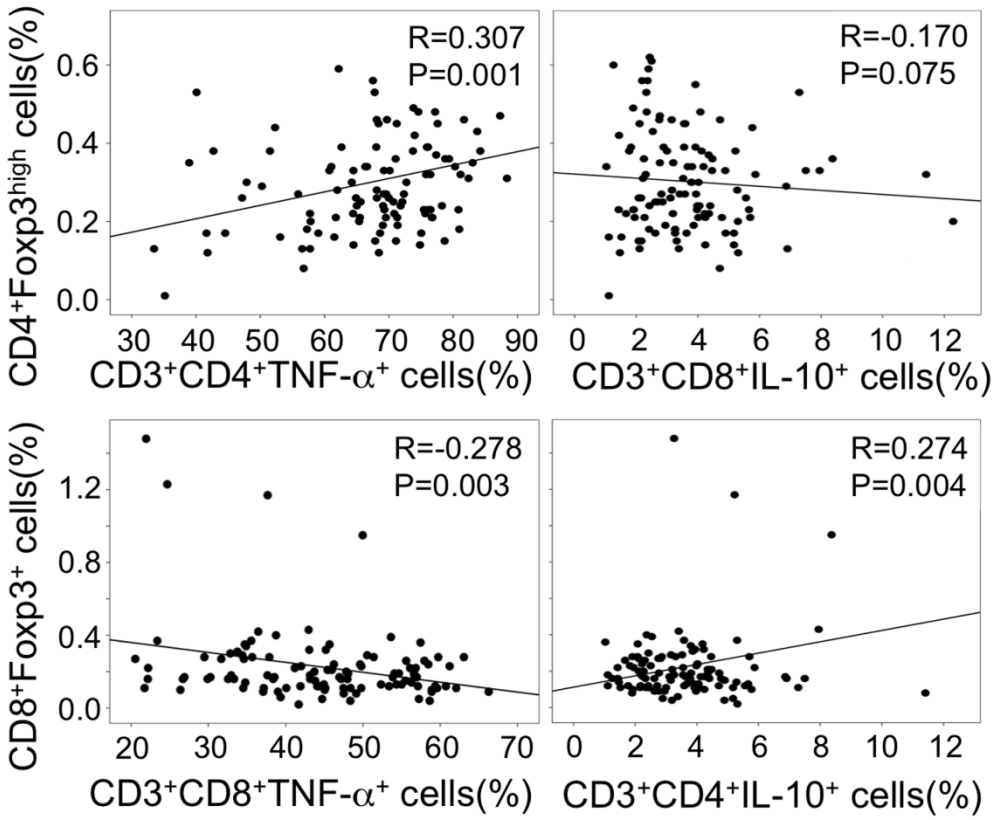
- Enhanced pro-inflammatory immune responses and diminished immune regulation in idiopathic RPL women even before conception.
  - Higher Th1/Th2 cytokine producing cell ratios
  - Higher Th17 cell proportion
  - Decreased $Foxp3^{+}$ Treg cell subsets
  - Higher Th17/Treg cell ratios
- This may be the first report of Th17 cell study obtained from PB of RPL women

Correlation of Treg cells with other immune cells

Correlation of Treg cells with Lymphocytes



$CD4^+Foxp3^{high}$ and $CD8^+Foxp3^+$ cells with Cytokine-producing cells



The relationship Foxp3$^+$ cell subsets and T, B, and NK cell subsets and NK cytotoxicity. The beta regression coefficients (B) are calculated under the control of age and menstrual phases by multiple linear regressions.

Lymphocyte subsets	Foxp3$^+$ cells	Foxp3^{low} cells	CD4$^+$Foxp3$^+$ cells	CD4$^+$Foxp3^{high} cells	CD8$^+$Foxp3$^+$ cells	CD4$^+$ Foxp3$^+$ cell /CD4$^+$ cell ratio	CD8$^+$ Foxp3$^+$ cell /CD8$^+$ cell ratio
CD3$^+$ T cells	0.271**	0.276**	0.291**	0.013	-0.001	0.008	0.004
CD3$^-$CD19$^+$ B cells	-0.028	-0.010	0.002	-0.086	-0.087	0.146	-0.019
CD3$^+$CD4$^+$ T cells	0.260**	0.225*	0.332***	0.203*	-0.146	-0.178	-0.047
CD3$^+$CD8$^+$ T cells	-0.013	0.036	-0.066	-0.237*	0.144	0.153	0.048
CD3$^+$CD4$^+$ T cell/CD3$^+$CD8$^+$ T cell ratio	0.150	0.090	0.205*	0.310***	-0.122	-0.221*	-0.022
CD3$^-$CD56$^+$ NK cells	-0.258**	-0.253**	-0.290**	-0.059	0.037	-0.108	-0.015
CD3$^-$CD56^{dim} NK cells	-0.257**	-0.252**	-0.286**	-0.061	0.027	-0.108	-0.021
CD3$^-$CD56^{bright} NK cells	-0.084	-0.097	-0.170	0.046	0.221*	-0.053	0.143
CD3$^+$CD56$^+$ NKT cells	-0.176	-0.181	-0.167	-0.003	-0.063	-0.037	-0.048
NK cell cytotoxicity							
NK cell activity at E:T=50:1	-0.117	-0.107	-0.040	-0.063	-0.071	-0.050	-0.118
NK cell activity at E:T=25:1	-0.118	-0.111	-0.097	-0.050	-0.084	-0.105	-0.116
NK cell activity at E:T=12.5:1	-0.084	-0.078	-0.072	-0.040	-0.050	-0.079	-0.086

E:T, effector to target ratio

* $p<0.05$, ** $p<0.01$, *** $p\leq0.001$

The relationship Foxp3$^+$ cell subsets and cytokine-producing T cells. The beta regression coefficients (B) are calculated under the control of age and menstrual phases by multiple linear regressions.

Cytokine-producing cells	Foxp3$^+$ cells	Foxp3^{low} cells	CD4$^+$Foxp3$^+$ cells	CD4$^+$Foxp3^{high} cells	CD8$^+$Foxp3$^+$ cells	CD4$^+$Foxp3$^+$ cell/CD4$^+$ cell ratio	CD8$^+$Foxp3$^+$ cell/CD8$^+$ cell ratio
CD3$^+$TNF-α$^+$ cells	0.135	0.102	0.116	0.181	0.080	0.139	0.059
CD3$^+$IFN-γ$^+$ cells	-0.022	-0.037	-0.025	0.069	0.003	0.088	0.002
CD3$^+$IL-10$^+$ cells	-0.058	-0.064	-0.054	0.017	-0.024	0.007	-0.055
CD3$^+$CD4$^+$TNF-α+ cells	0.047	-0.009	0.033	0.273*	-0.027	0.196	0.078
CD3$^+$ CD4$^+$IFN-γ$^+$ cells	0.116	0.084	0.071	0.169	0.150	0.201	0.187
CD3$^+$ CD4$^+$IL-10$^+$ cells	0.141	0.159	0.056	-0.068	0.266**	0.120	0.246*
CD3$^+$CD8$^+$TNF-α$^+$ cells	0.009	-0.006	0.108	0.071	-0.275**	0.163	-0.199
CD3$^+$CD8$^+$IFN-γ$^+$ cells	0.028	0.025	0.084	0.017	-0.149	0.072	-0.074
CD3$^+$CD8$^+$IL-10$^+$ cells	-0.038	0.033	-0.082	-0.346***	0.114	0.054	0.059

* $p<0.05$, ** $p<0.01$, *** $p\leq0.001$

The relationship of Foxp3+ cell subsets and ratios of type 1 to type 2 cytokine-producing T cells. The beta regression coefficients (B) are calculated under the control of age and menstrual phases by multiple linear regressions.

Ratios of type 1 to type 2 cytokine-producing T cells	$Foxp3^+$ cells	$Foxp3^{low}$ cells	$CD4^+Foxp3^+$ cells	$CD4^+Foxp3^{high}$ cells	$CD8^+Foxp3^+$ cells	$CD4^+Foxp3^+$ cell /$CD4^+$ cell ratio	$CD8^+Foxp3^+$ cell/$CD8^+$ cell ratio
$CD3^+TNF\text{-}\alpha^+$ cell /$CD3^+IL\text{-}10^+$ cell ratio	0.094	0.091	0.105	0.026	-0.012	0.049	0.004
$CD3^+IFN\text{-}\gamma^+$ cell /$CD3^+IL\text{-}10^+$ cell ratio	0.027	0.021	0.034	0.031	-0.016	0.056	-0.002
$CD3^+CD4^+TNF\text{-}\alpha^+$ cell /$CD3^+CD4^+IL\text{-}10^+$ cell ratio	-0.080	-0.107	-0.052	0.121	-0.093	-0.034	-0.103
$CD3^+CD4^+IFN\text{-}\gamma^+$ cell /$CD3^+CD4^+IL\text{-}10^+$ cell ratio	0.000	-0.026	0.008	0.124	-0.022	0.065	-0.025
$CD3^+CD8^+TNF\text{-}\alpha^+$ cell /$CD3^+CD8^+IL\text{-}10^+$ cell ratio	0.019	-0.045	0.077	0.313***	-0.156	0.104	-0.090
$CD3^+CD8^+IFN\text{-}\gamma^+$ cell /$CD3^+CD8^+IL\text{-}10^+$ cell ratio	0.005	-0.047	0.046	0.250**	-0.113	0.066	-0.043

* $p<0.05$, ** $p<0.01$, *** $p\leq0.001$

New concept in T cells

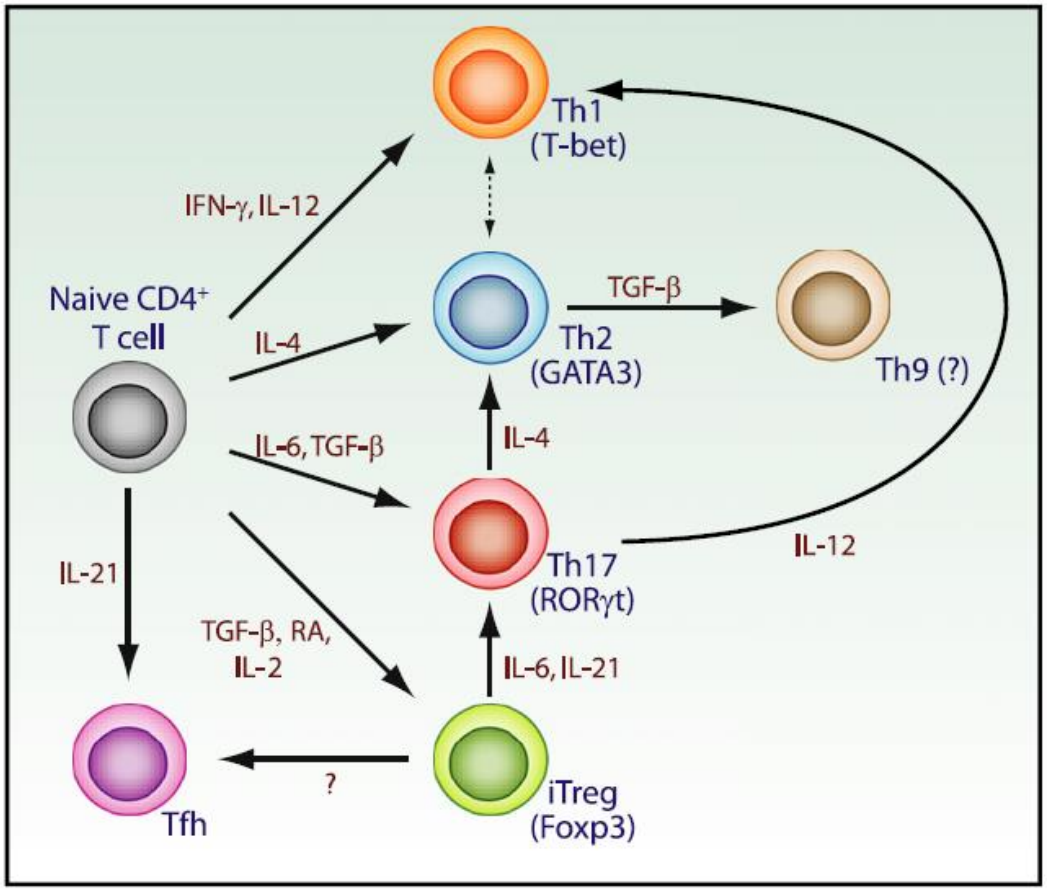
Plasticity of $CD4^+$ T cells

- T cell differentiation: Reversible or Irreversible ?
  - $CD4^+$ T cells, particularly iTreg and Th17 cells, are more plastic than previously appreciated.
  - Expression of Foxp3 by iTreg cells or IL-17 by Th17 cells may not be stable.
    - Conversion of Treg cells to Th1, Tfh or Th17 cells by inflammatory cytokines
    - Conversion of Th17 cells to Th1 or Th2 cells by IL-12 and IL-4, respectively

Immunity, 2009;30:646

The Cytokine Milieu Determines CD4$^+$ T Cell Differentiation and Conversion

Dendritic cell

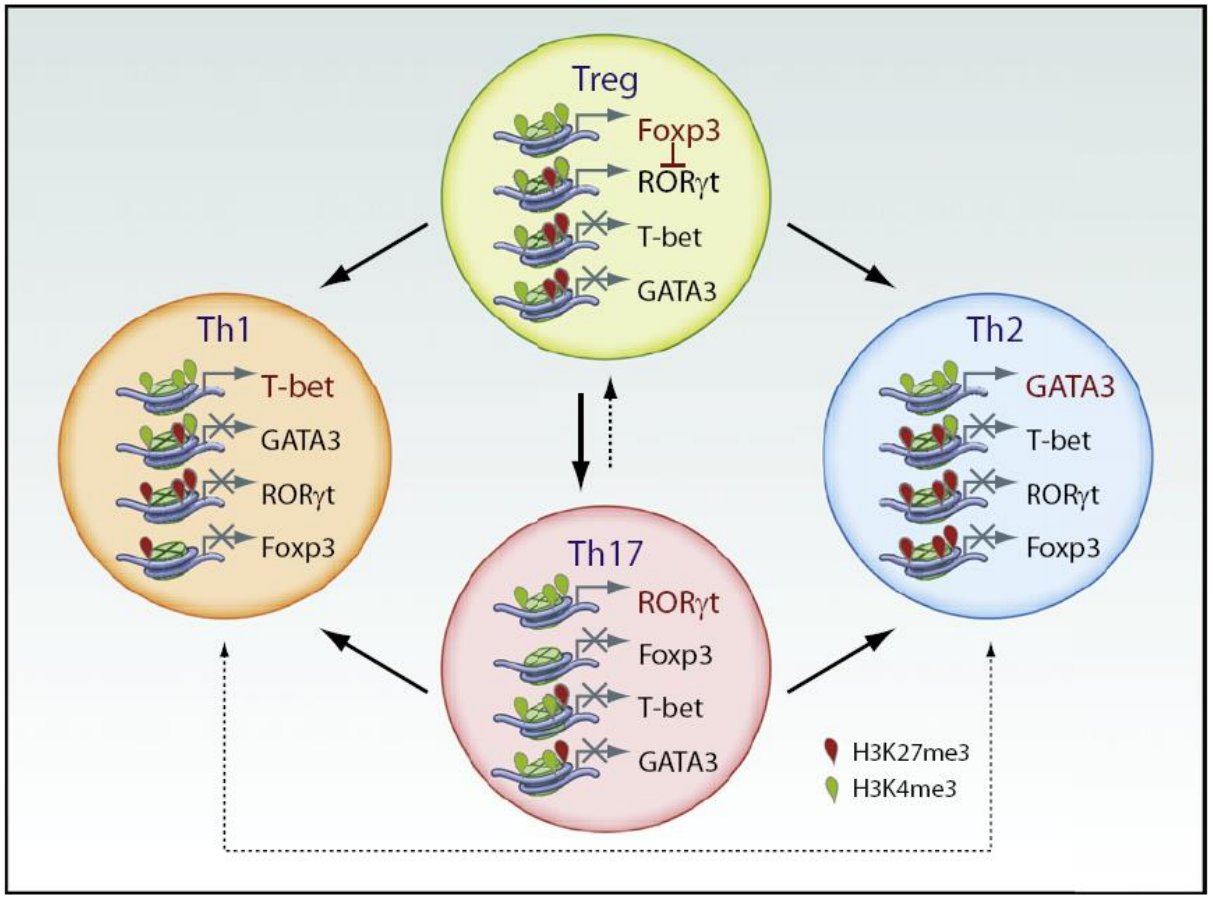


T cell differentiation decision by **cytokine milieu** and strength of **Ag-TCR interaction**

Tfh, follicular helper T cells
Th9, IL-9 producing helper T cells

Immunity, 2009;30:646

Epigenetic Status of Lineage-Specific Transcriptional Regulator Gene Loci in CD4$^+$ T Cells



High plasticity

Low plasticity

H3K27me3: repressive mark
H3K4me3: permissive mark

Immunity, 2009;30:646

Influence of proinflammatory conditions on Treg cells

- nTreg cells → $Foxp3^{+}IL\text{-}17^{+}$ or $Foxp3^{+}IFN\text{-}\gamma^{+}$ cells

Th17 or Th1 polarizing condition

- iTreg cells → $Foxp3^{-}IFN\text{-}\gamma^{+}$ cells

Difference in stability of Foxp3 expression between nTreg and iTreg cells
-nTreg cells: Treg cell-specific demethylated region, fully demethylated
-iTreg cells: TSDR, partially demethylated

Immunity, 2009;30:646

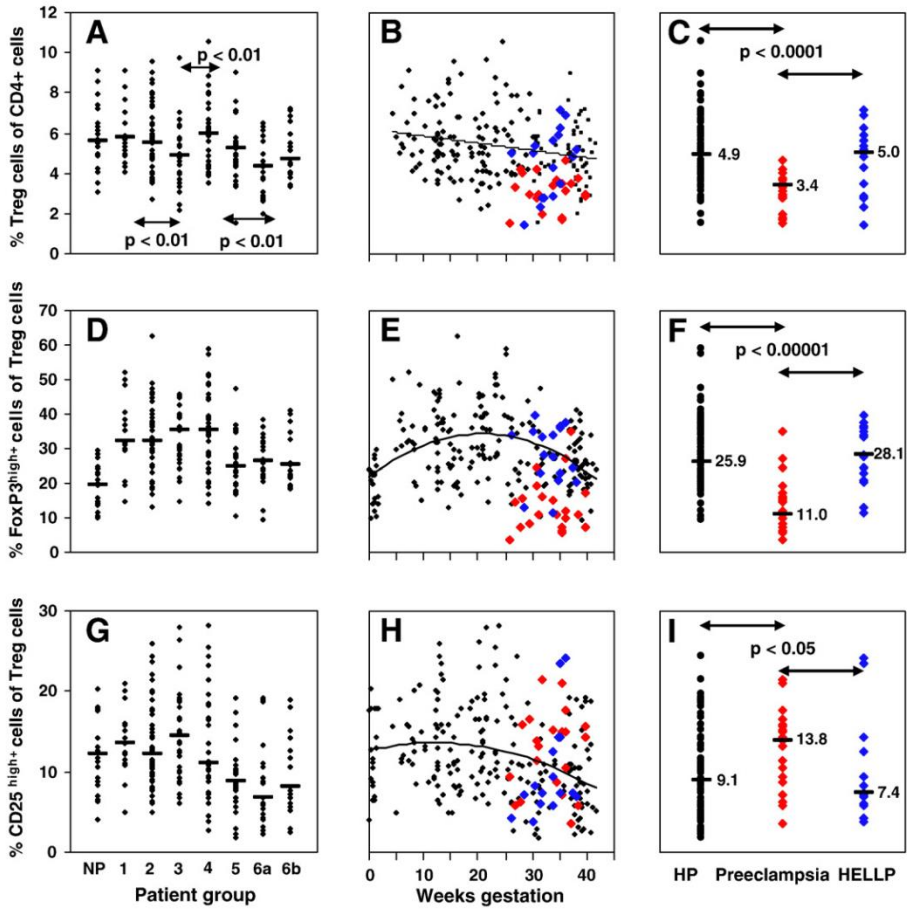
Redifferentiation of Treg cells

- Treg → other effector T cells ?
  - Conversion of nTreg to Th1 cells (Wei et al., 2009)
  - From Foxp3$^+$ cells to Th1 or Th2 phenotypes (Zhou et al., 2008)
  - Foxp3$^+$ cells to Tfh cells (Tsuji et al., 2009)
- Existence of Foxp3$^+$ROTγt$^+$ cells
  - Reduced amount of IL-17
  - Suppressive activity of human Foxp3$^+$ROTγt$^+$ cells
  - A transient population for either iTreg or Th17 cells?

Immunity, 2009;30:646

Supplement

Proportion of Treg cells in PIH



Preeclampsia (•)

HELLP synd (•)

Clin Immunol, 2008;129:401

Journal of Reproductive Immunology 84 (2010) 164–170

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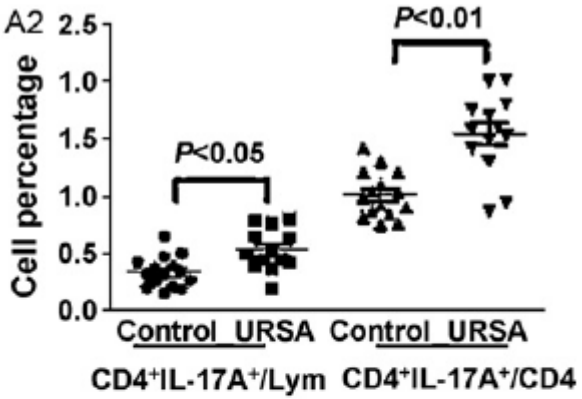
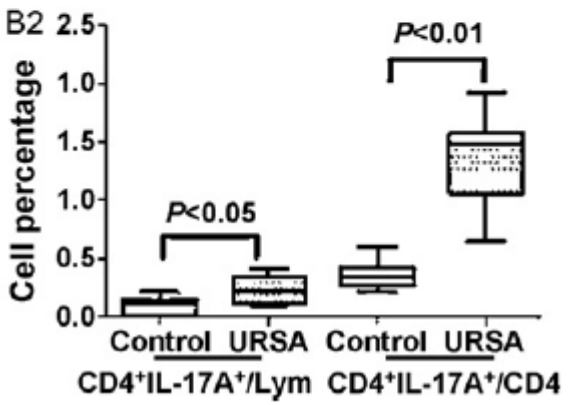
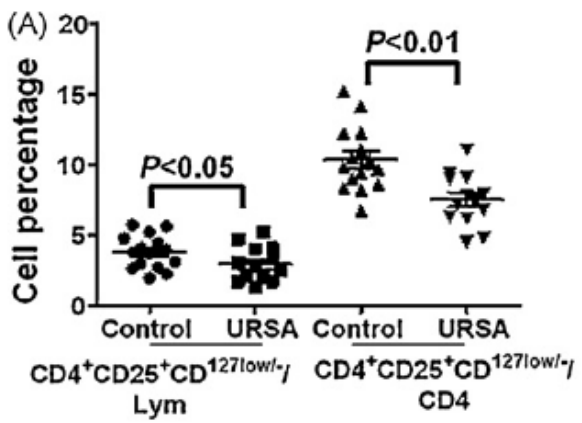
PB

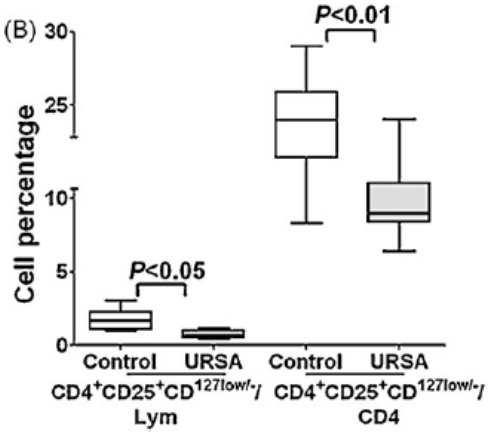
Decidua





TH17 cell study





Treg cell study

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