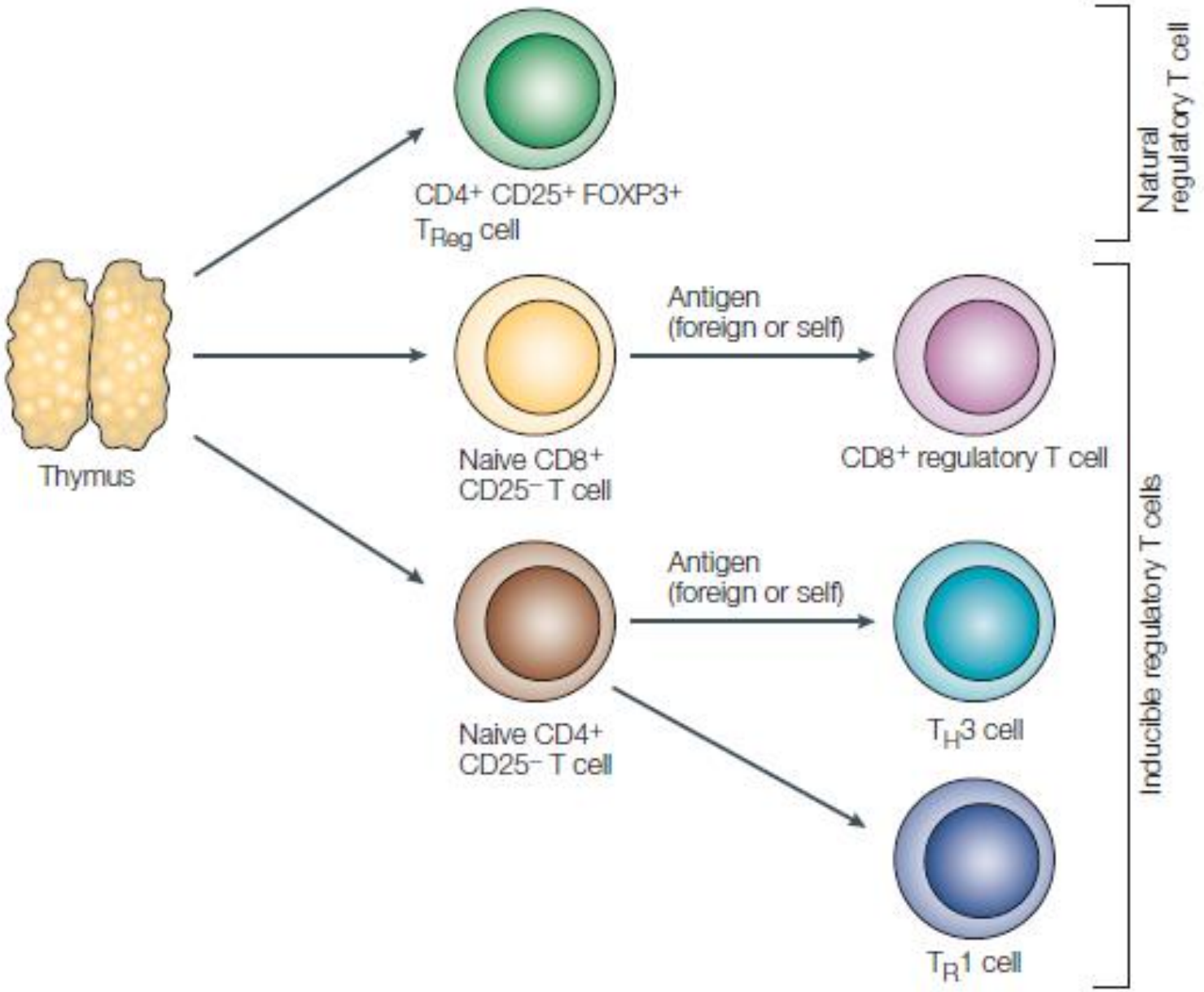
New protocol for the generation of insulin-specific T regulatory cells

Ivana Stojanović, PhD

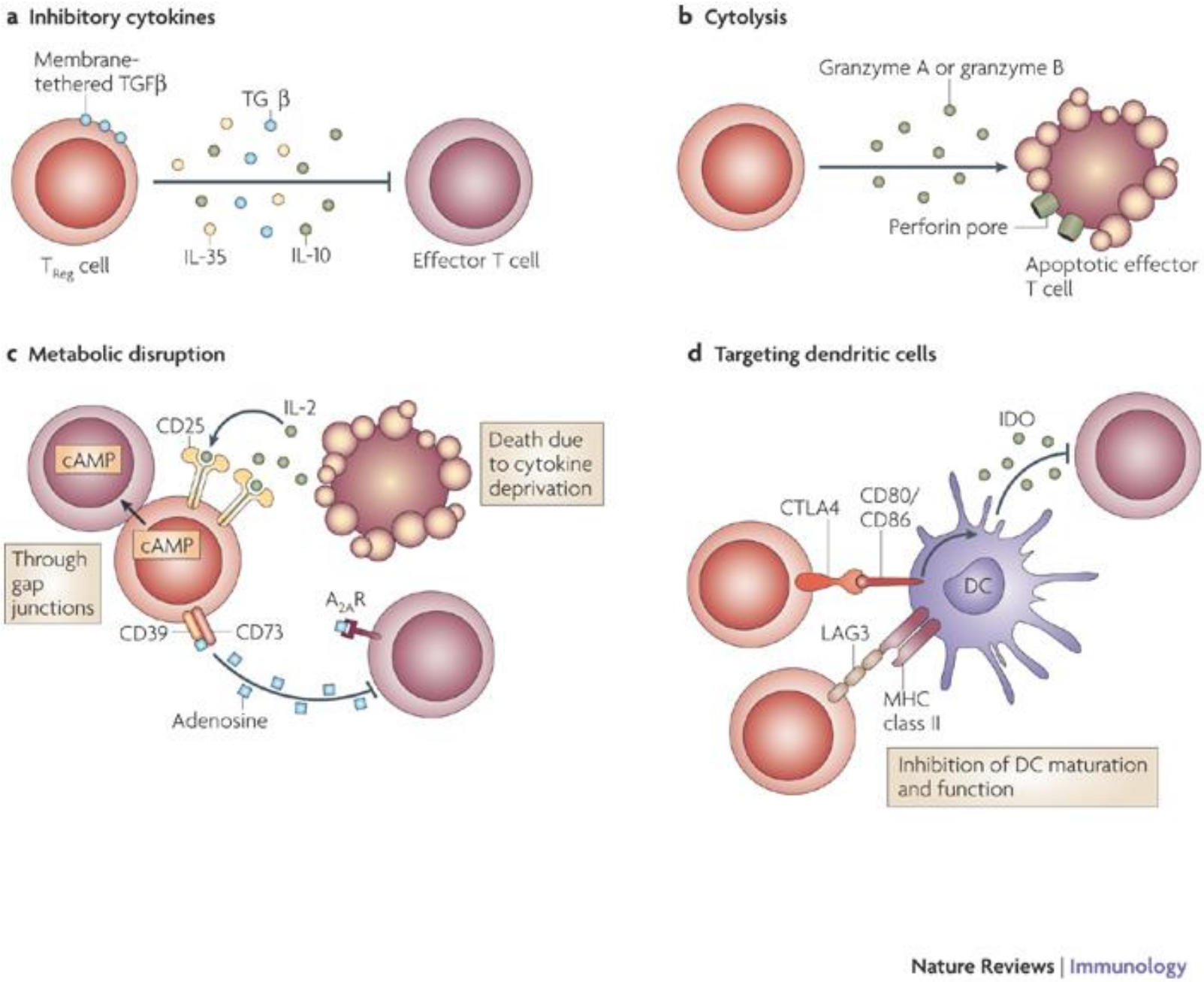
Diabetology group leader

Institute for Biological Research “Sinisa Stankovic”, University of Belgrade

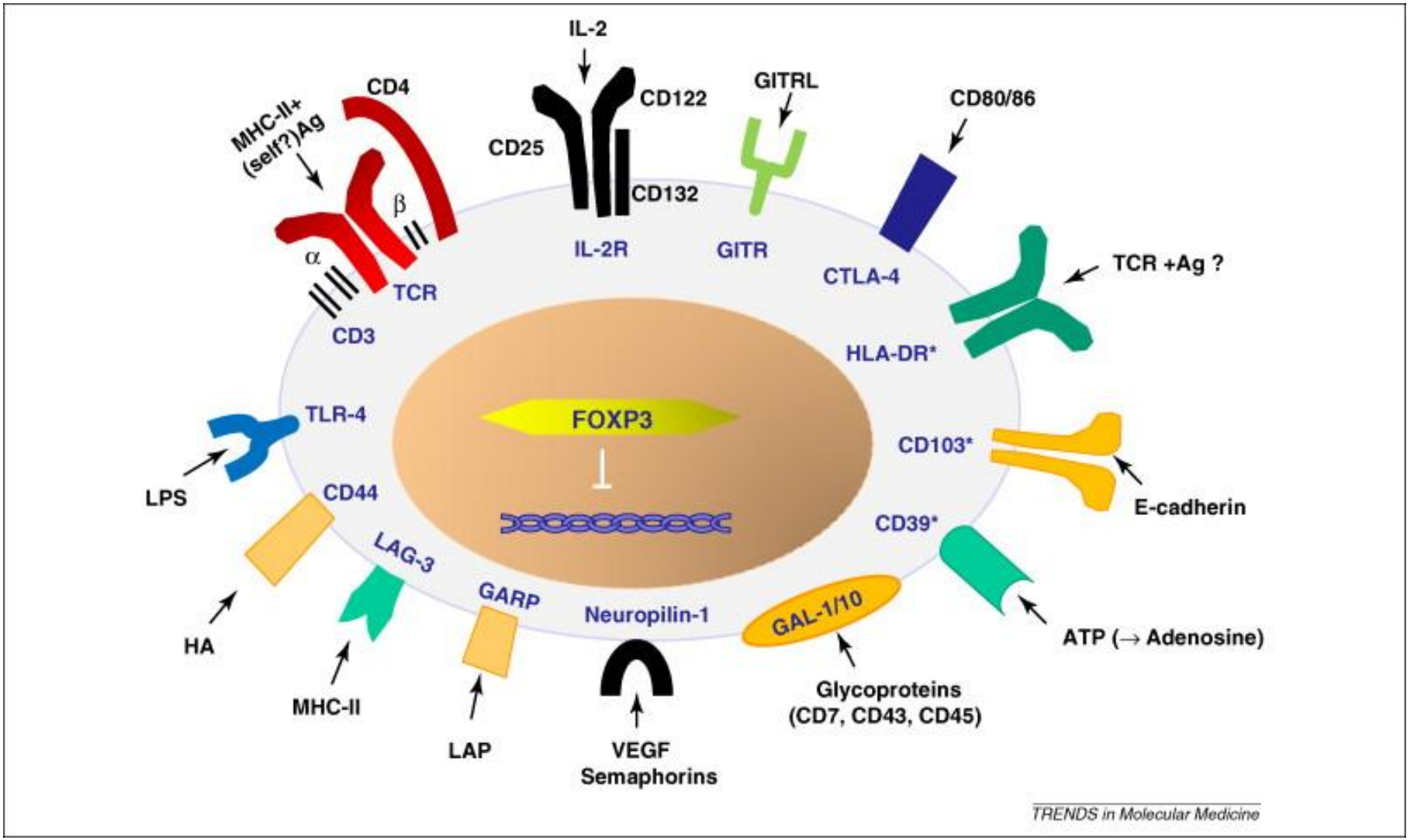
8th European Immunology Conference , Madrid, 29.6.2017.



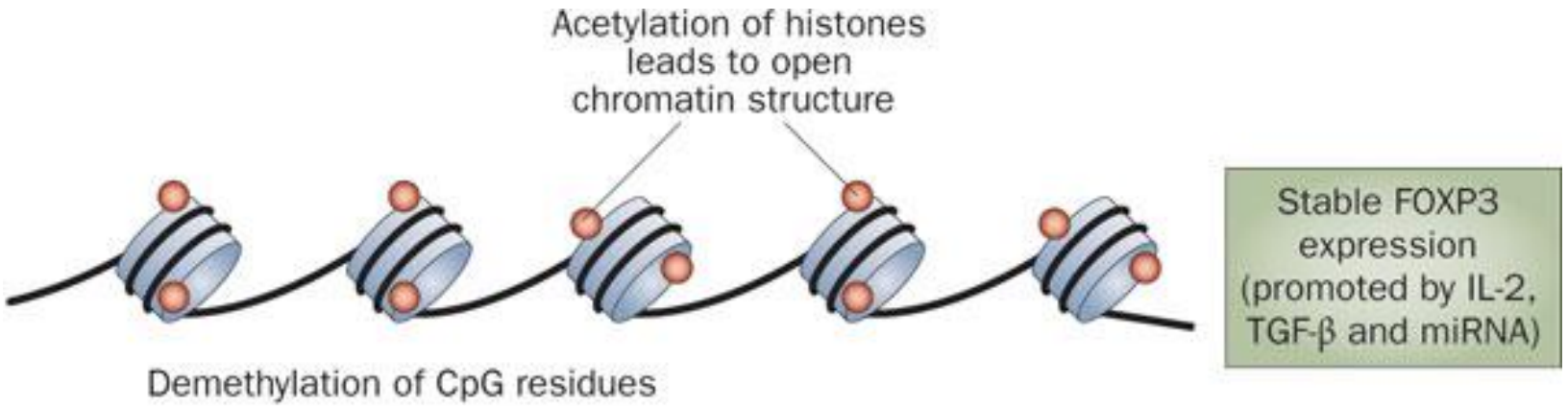
Mills KHG, Nature Reviews Immunology 2004



Vignali et al., How regulatory T cells work? 2008 Nature Reviews Immunology 8, 523-532


IL-2
CD4
MHC-II+
(self?)Ag
CD122
CD25
CD132
GITRL
CD80/86
β
α
TCR
CD3
IL-2R
GITR
CTLA-4
TCR +Ag ?
HLA-DR*
FOXP3
TLR-4
LPS
CD103*
E-cadherin
CD44
CD39*
LAG-3
GARP
Neuropilin-1
GAL-1/10
ATP (→ Adenosine)
HA
MHC-II
LAP
VEGF
Semaphorins
Glycoproteins
(CD7, CD43, CD45)
TRENDS in Molecular Medicine

Stability of FoxP3 expression



Notley & Ehrenstein, Nature Reviews Rheumatology 6, 572-577

Treg application in therapy

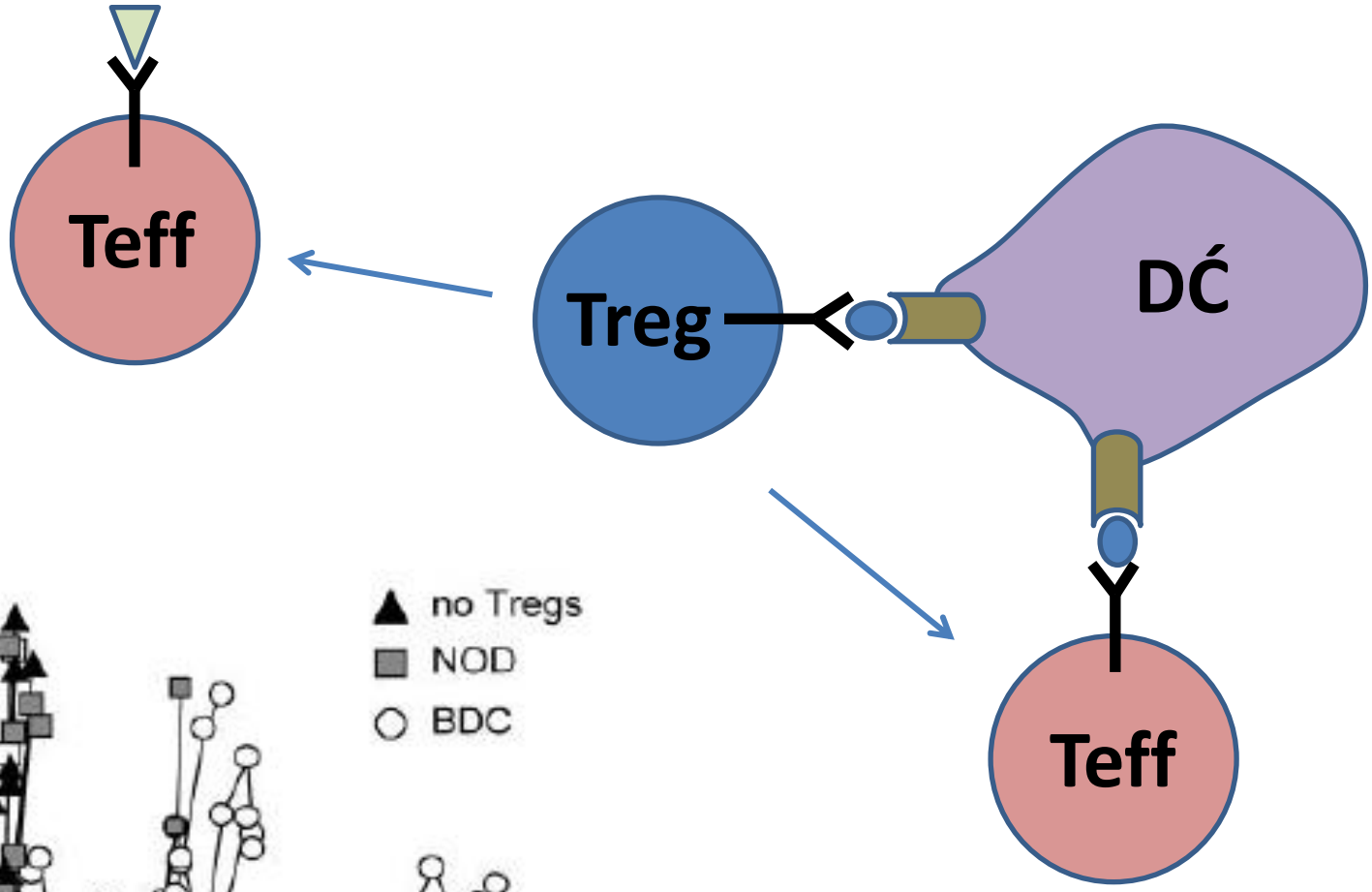
- 702 clinical studies:

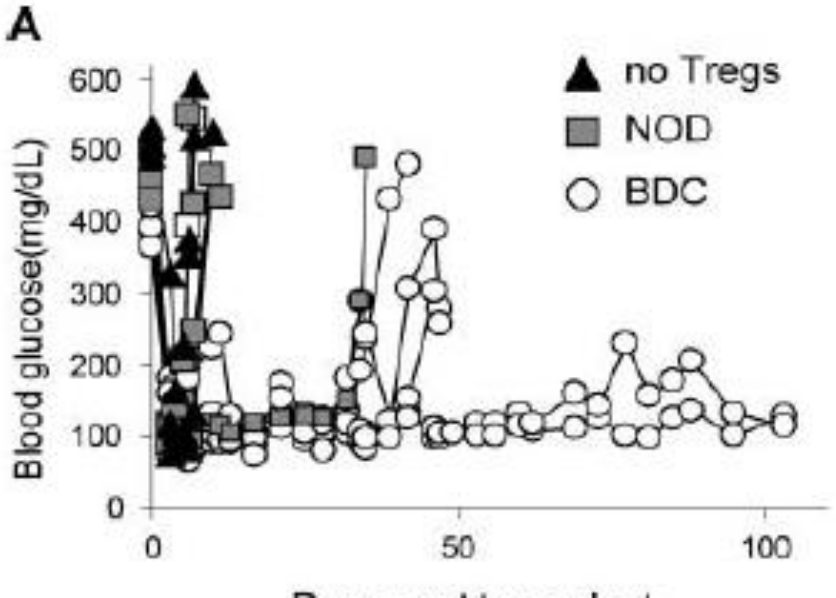
- Type 1 diabetes
- Multiple sclerosis
- Rheumatoid arthritis
- Lupus
- GVHD
- Asthma
- Kidney transplantation

- **Type 1 diabetes trials:**

- Gdansk University (Poland)
- University of California, San Francisco (USA)

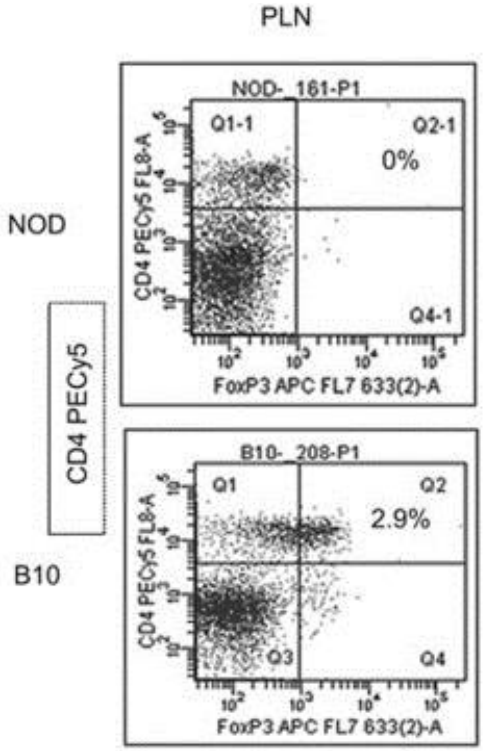
Dilema – non-specific or antigen-specific action?



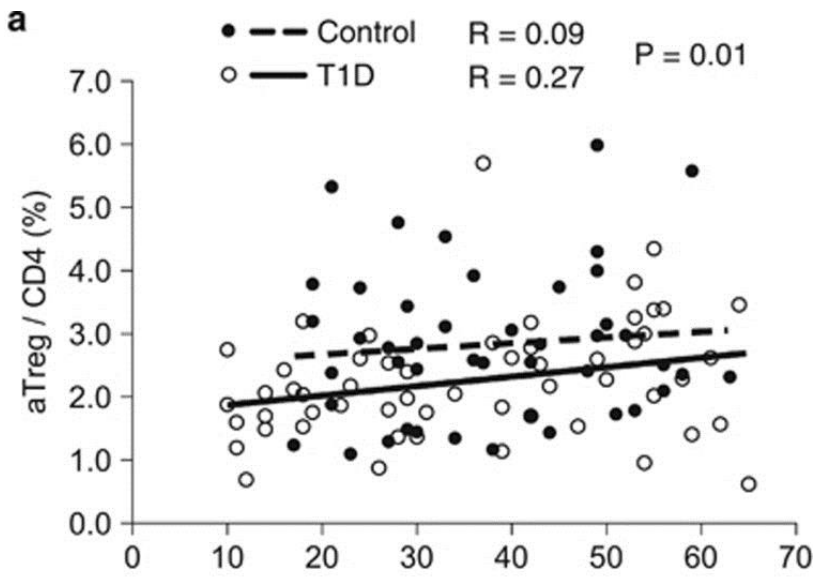


Functionality of Tregs

- Autoimmunity might arise as a consequence of Treg defects



Cellular & Molecular Immunology (2012) 9, 455–463

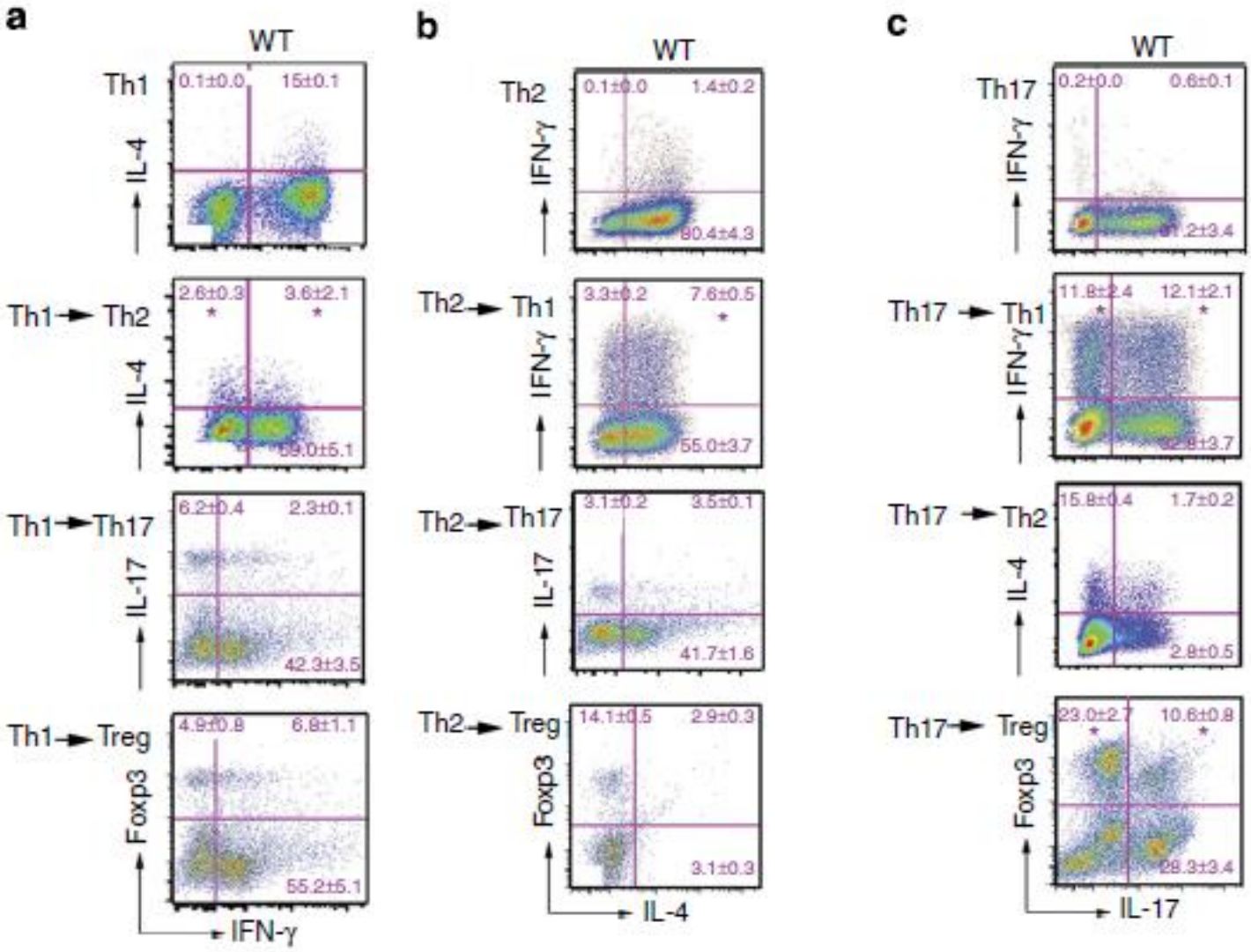


Clin Transl Immunology. 2016 Jan; 5(1): e56.

Premisses

- Autoantigen-specific Treg are more efficient than polyclonal
- Individuals with autoimmunity might have a "defect" in Treg function
- Autoantigen-specific Treg are difficult to isolate due to their low numbers
- Autoantigen-specific T effectors are more abundant
- Due to cell plasticity, it is possible to convert effector into regulatory cells

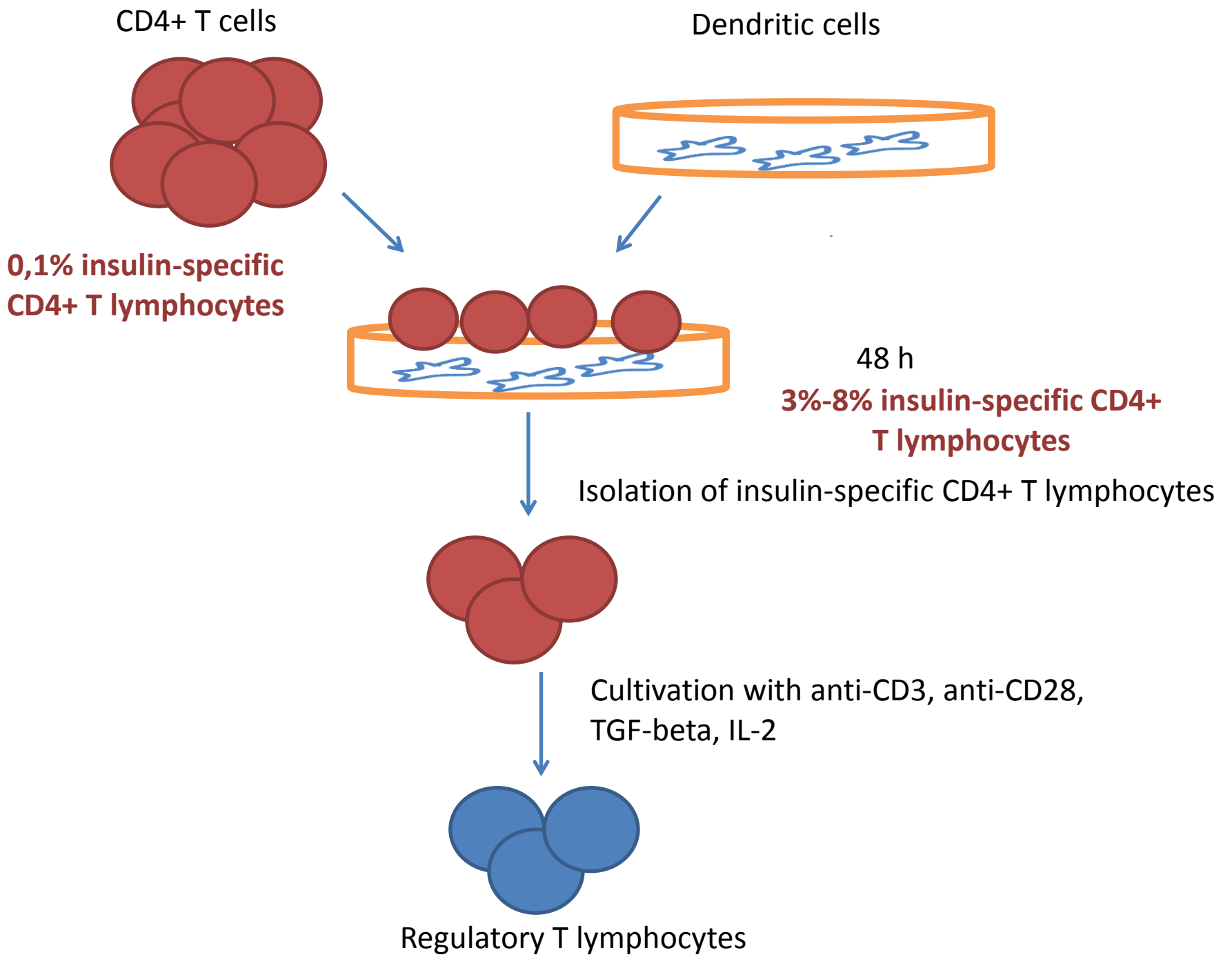
Alchemy of CD4+ cells



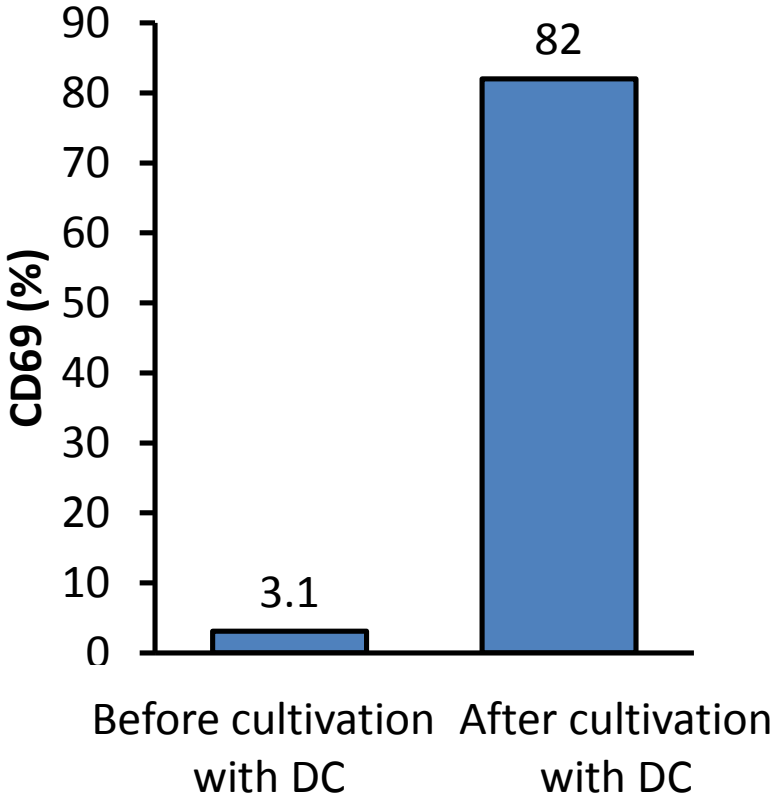
Li Q, Nature Communication 2014

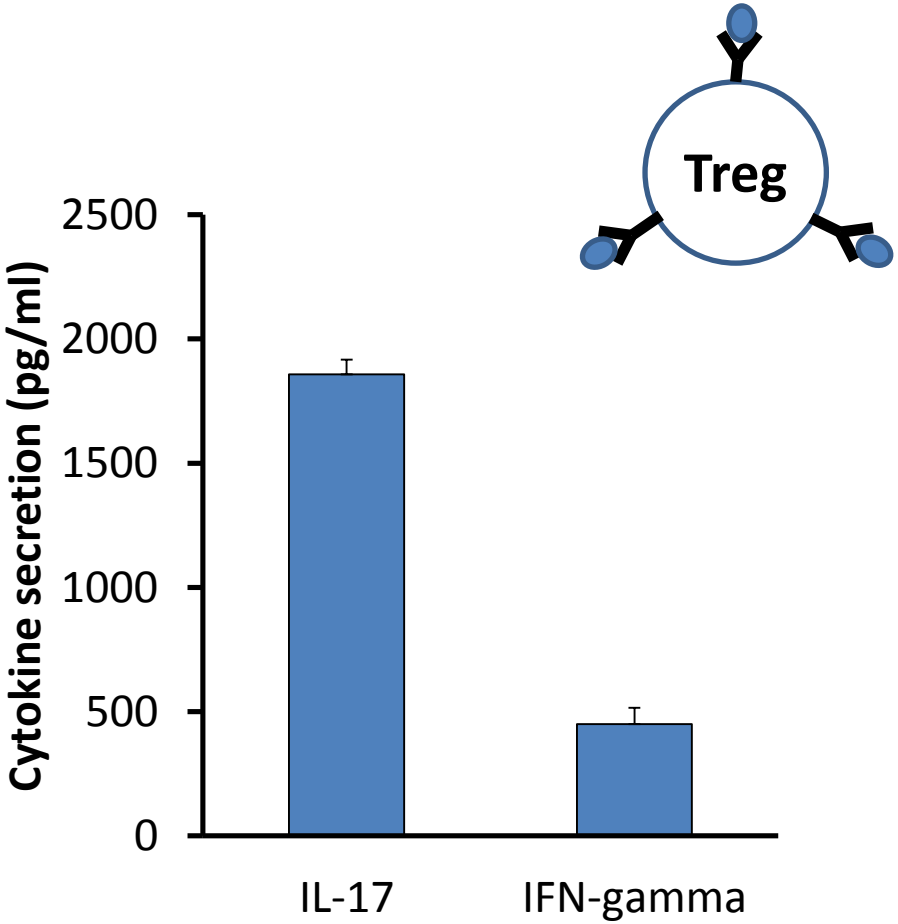
Premisses

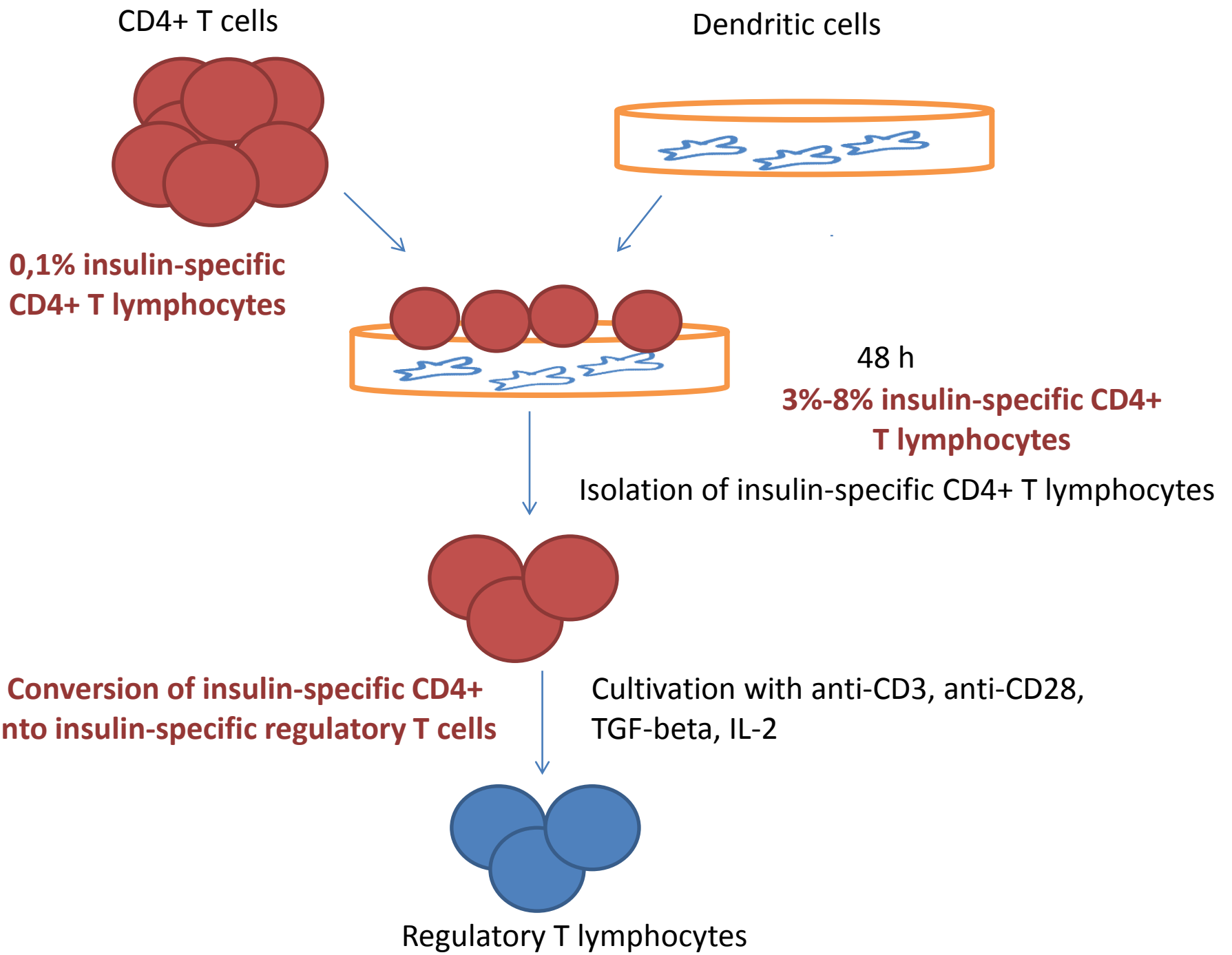
- Autoantigen-specific Treg are more efficient than polyclonal
- Individuals with autoimmunity might have a “defect” in Treg function
- Autoantigen-specific Treg are difficult to isolate due to their low numbers
- Autoantigen-specific T effectors are more abundant
- Due to cell plasticity, it is possible to convert effector into regulatory cells


CD4+ T cells
Dendritic cells
0,1% insulin-specific
CD4+ T lymphocytes
48 h
3%-8% insulin-specific CD4+
T lymphocytes
Isolation of insulin-specific CD4+ T lymphocytes
Cultivation with anti-CD3, anti-CD28,
TGF-beta, IL-2
Regulatory T lymphocytes

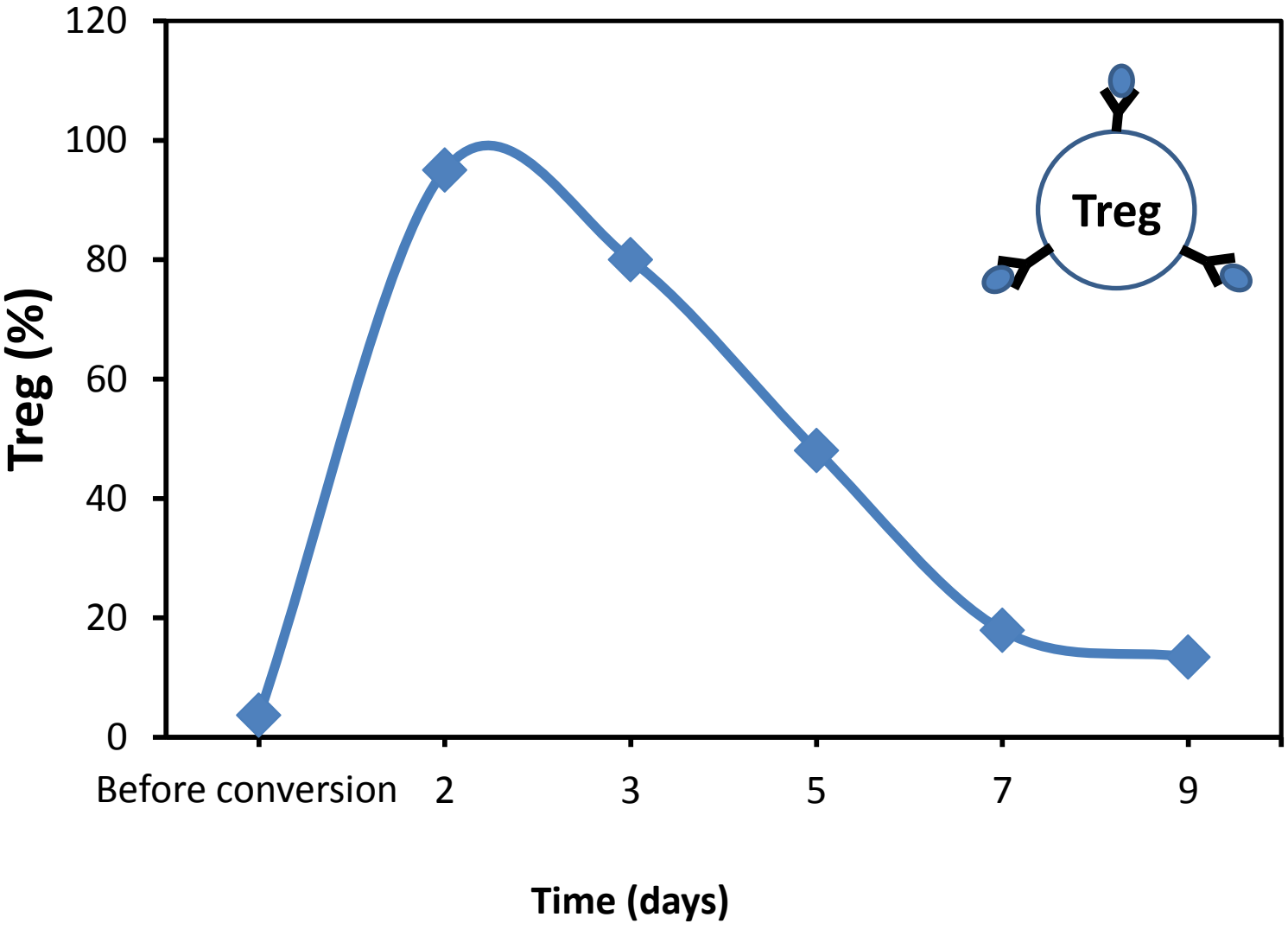
Insulin-specific CD4+ cells are effector cells



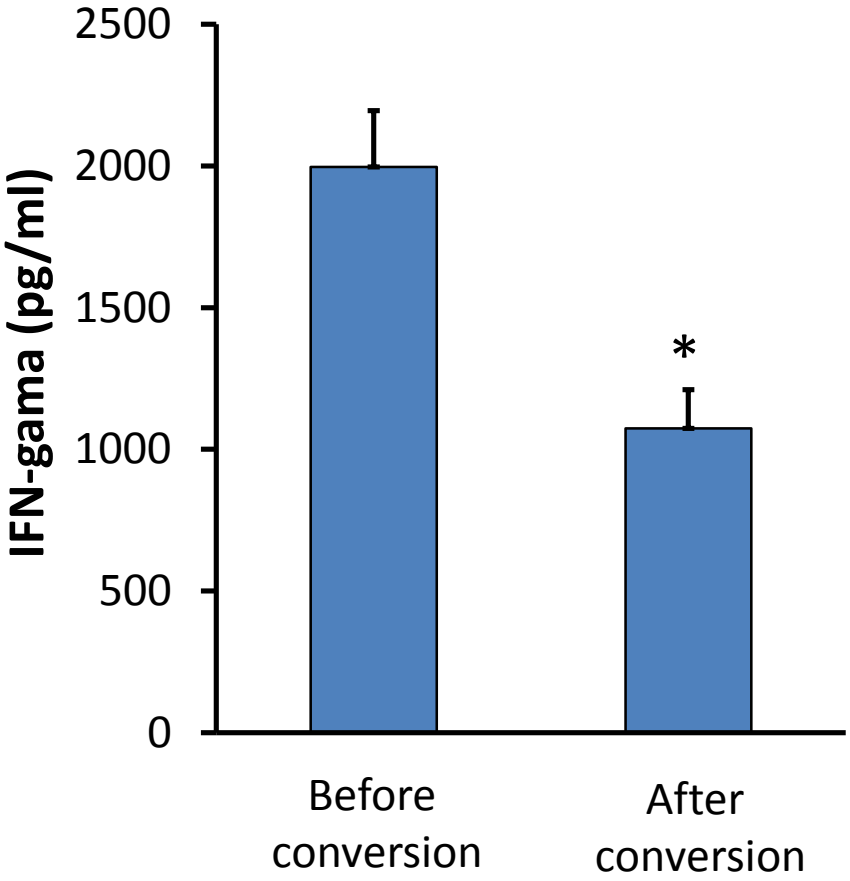


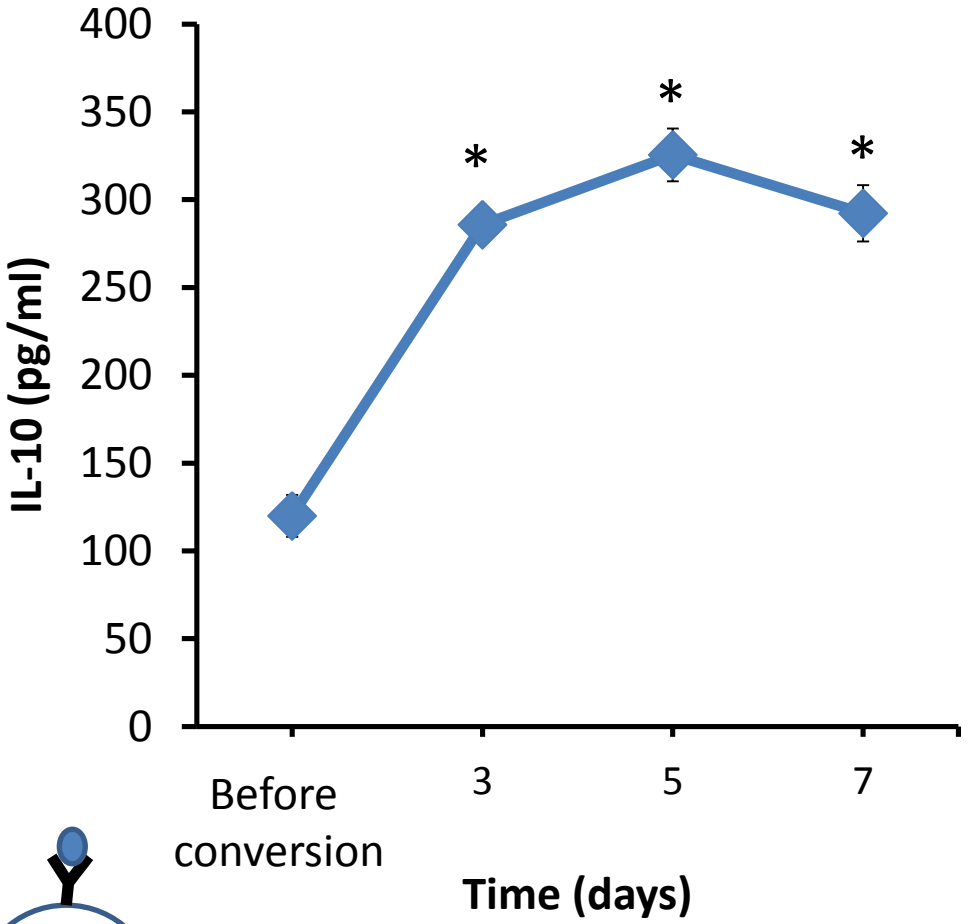

CD4+ T cells
Dendritic cells
0,1% insulin-specific CD4+ T lymphocytes
48 h
3%-8% insulin-specific CD4+ T lymphocytes
Isolation of insulin-specific CD4+ T lymphocytes
Conversion of insulin-specific CD4+ into insulin-specific regulatory T cells
Cultivation with anti-CD3, anti-CD28, TGF-beta, IL-2
Regulatory T lymphocytes

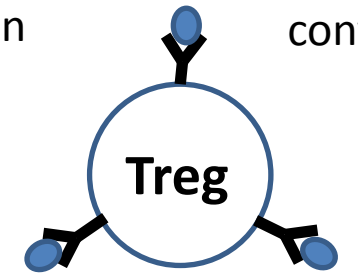
CD4+CD25highFoxP3+



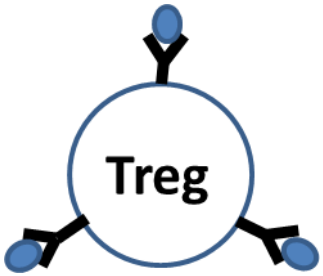
From inflammatory to anti-inflammatory phenotype

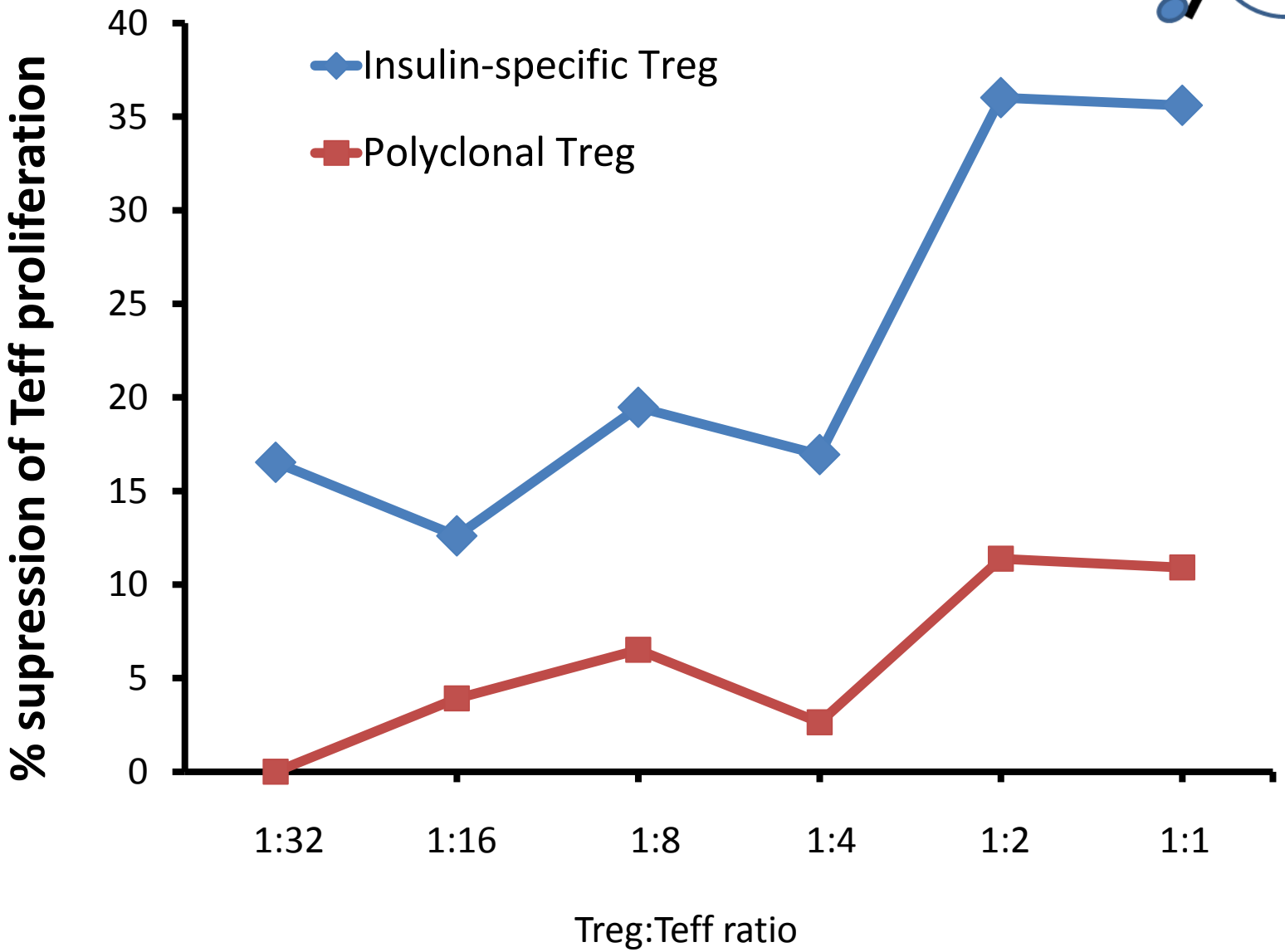




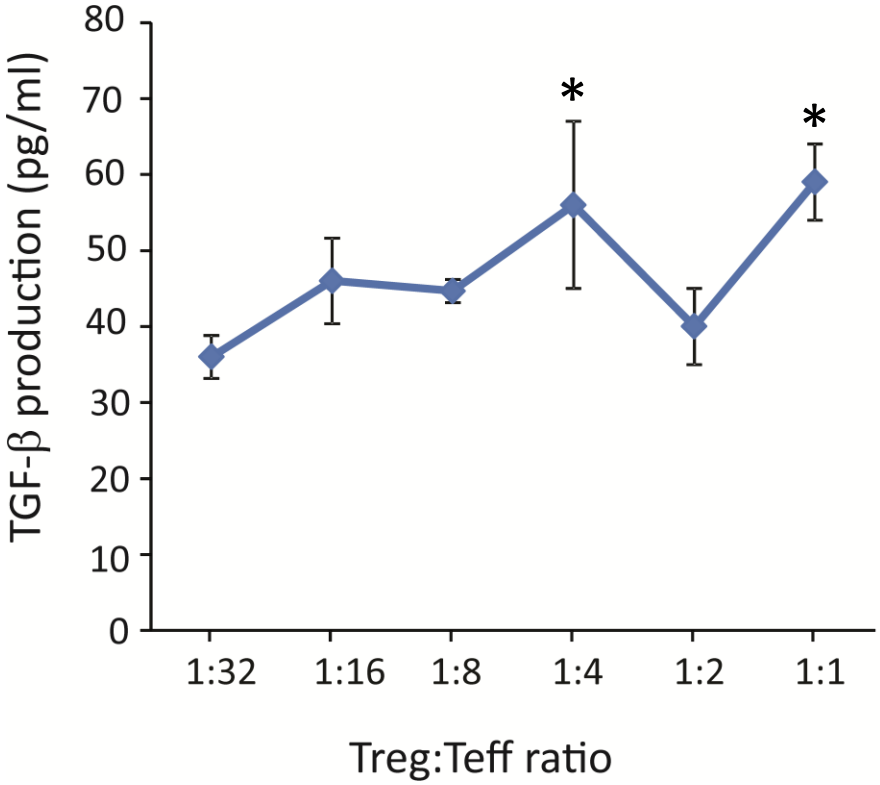


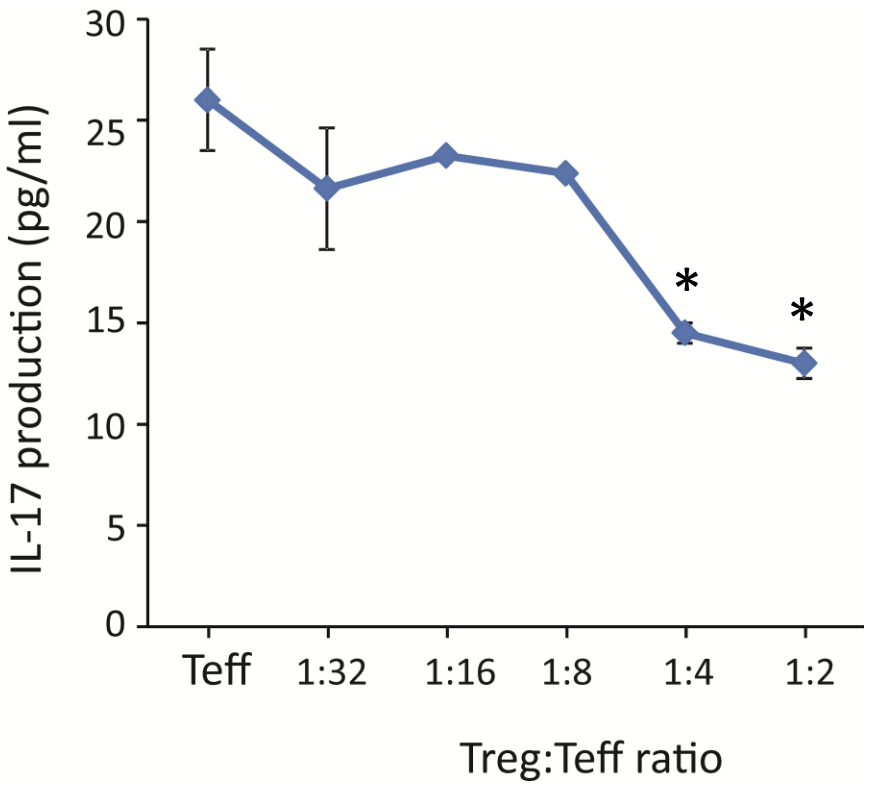
Treg supressive function





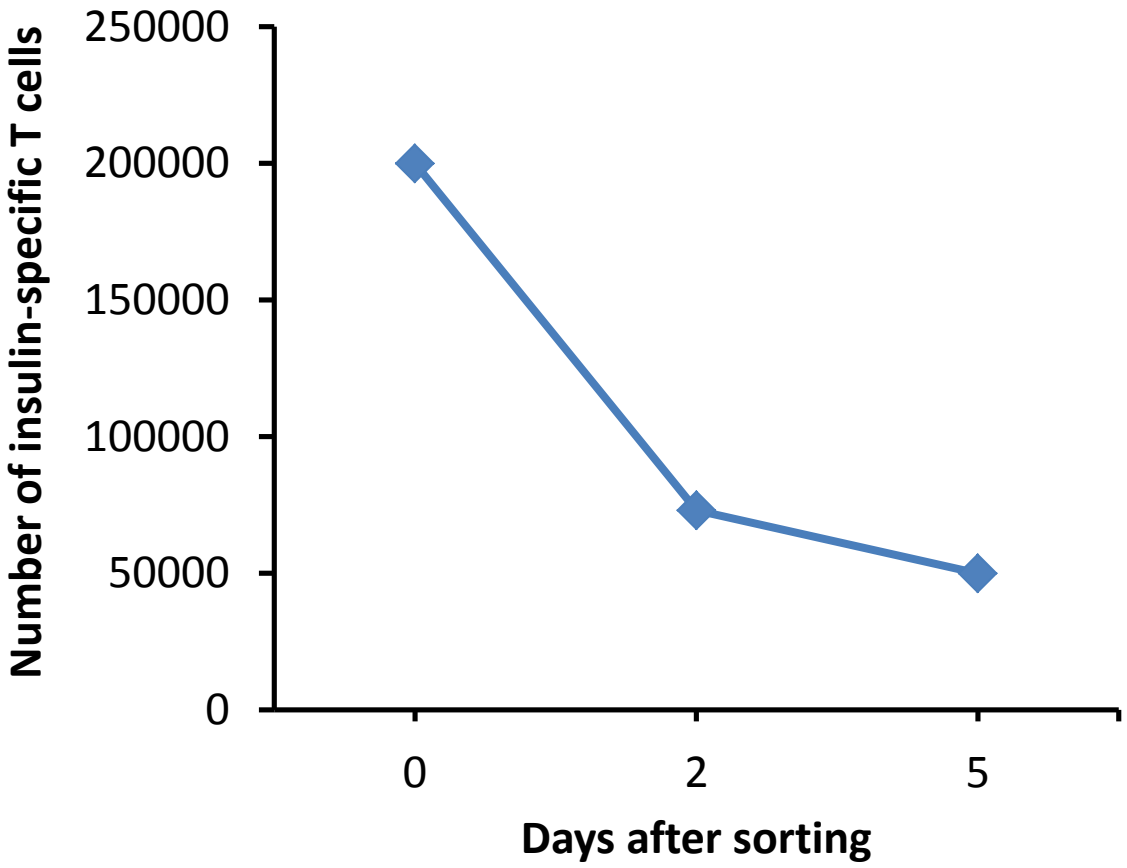
Treg supressive function





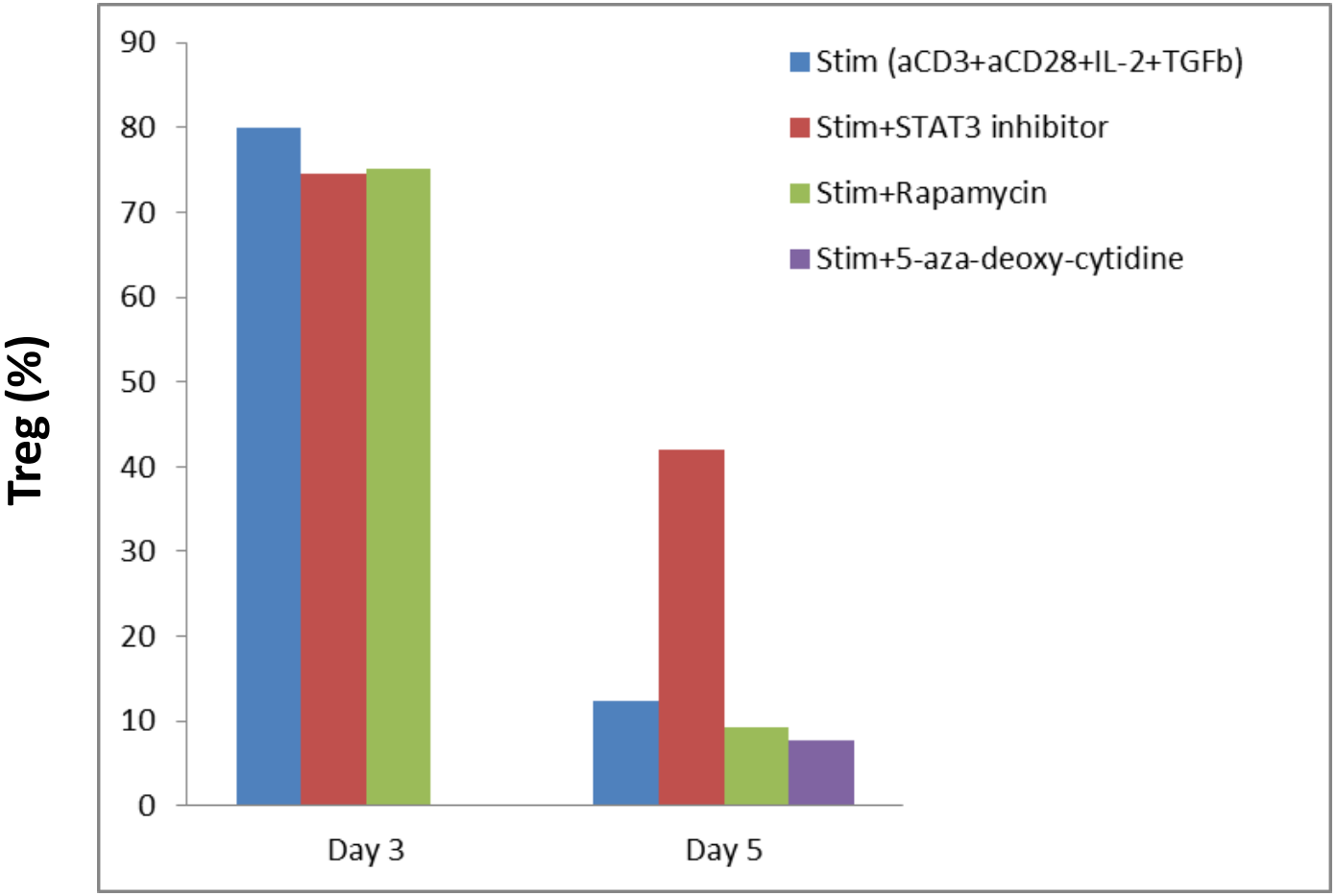
Shortcomings...

- During the conversion lot of cells dye



Shortcomings...

- % of Tregs decrease significantly over time

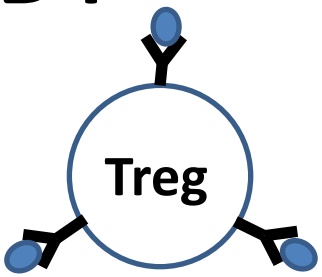


Department of Immunology, IBISS, Serbia

Iacocca Family Foundation project (2016-2018)

ivana@ibiss.bg.ac.rs

Can tolerogenic DCs direct naive CD4+ cells into Tregs in vitro?



	%
Insulin-specific CD4+	9±0,4
Treg	2,6±0,4