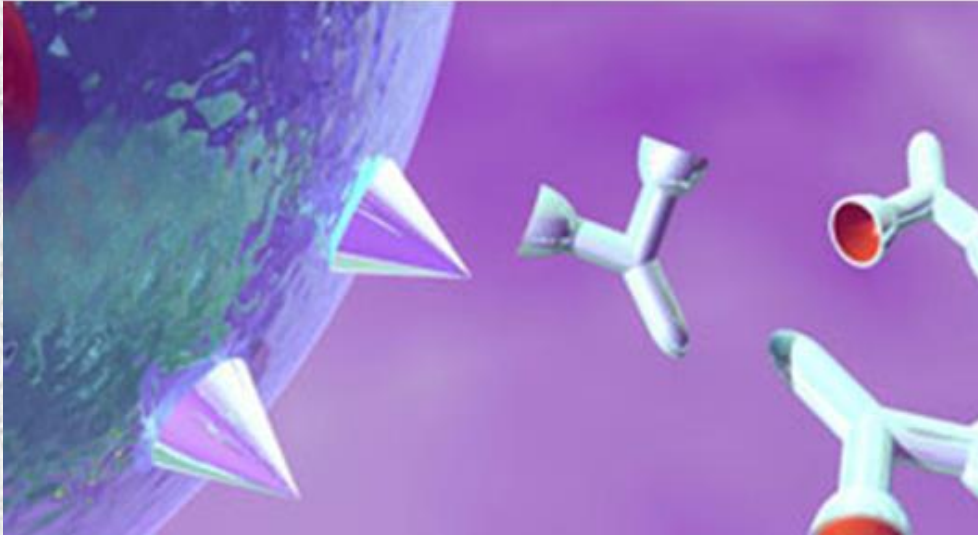


***In vitro* activity of human ACPA on T cells**



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Rheumatoid Arthritis (RA)

Definition:

Autoimmune disease characterized by progressive synovial inflammation resulting in irreversible joint erosions, cartilage and bone destruction .

RA is a multisystem disease with extra-articular manifestations such as: atherosclerosis, respiratory illness and cardiovascular disease

Prevalence:

1% of the population

Gender ratio – Women: Men – 3:1



Figure 11-12 The Immune System, 2/e (© Garland Science 2005)

Current drugs in the treatment of RA

Disease-modifying anti-rheumatic drugs

(DMARDs):

MTX (methotrexate), SSZ (sulfasalazine), HCQ (hydroxychloroquine), CQ (chloroquine), AZA (azathioprine) Leflunomide (Arava)

Limitations of DMARDs therapies

- **Significant failure rates (lack of efficacy in some patients)**
- **Not specific and toxic**

Biological agents in RA

TNF α antagonists

Etanercept (Enbrel)
Infliximab (Remicade)
Adalimumab (Humira)
Golimumab (Simponi)
Certolizumab Pegol (Cimzia)

Interleukin-1 antagonist

Anakinra (Kineret)

Anti B-cell monoclonal antibody

Rituximab (Rituxan)

Suppressors of T-cell activation

Abatacept (Orencia)

monoclonal antibody against the Interleukin-6 receptor

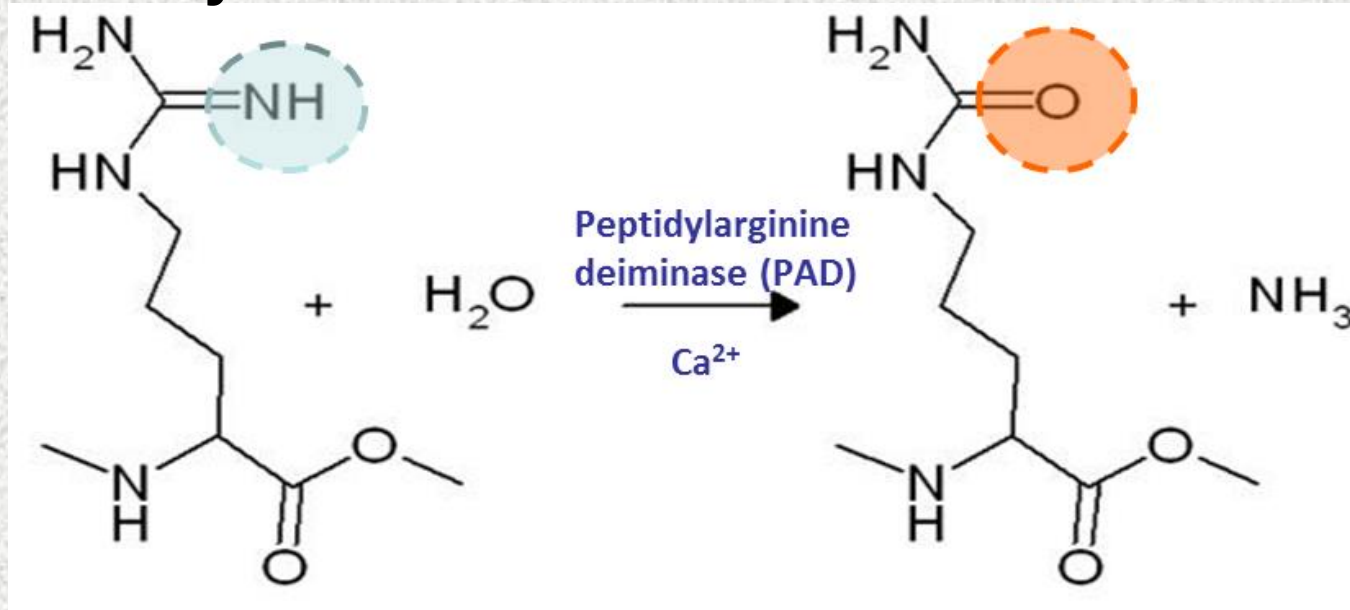
Tocilizumab (Actemra)

Limitations of therapies

- **May cause serious infections, opportunistic infections, malignancies/lymphoma**
- **Demyelination**
- **Hematologic abnormalities**
- **Progressive multifocal leukoencephalopathy**

Citrullinated peptides in RA

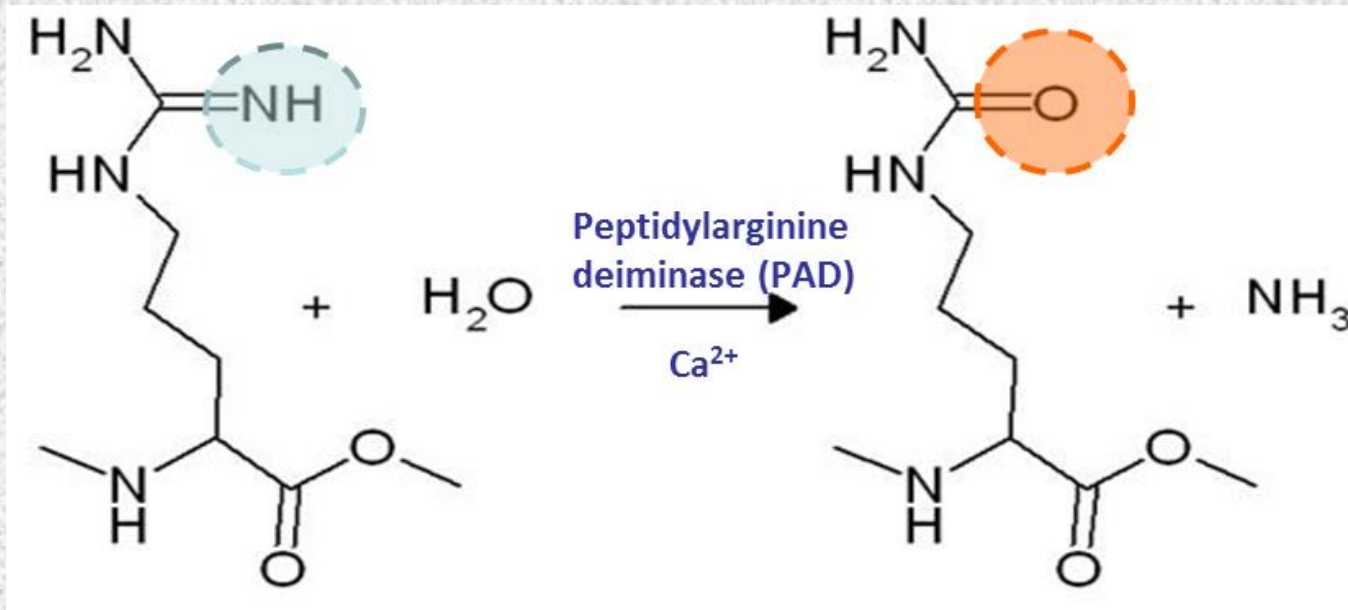
Citrullination, arginine → citrulline, an unnatural amino acid, not encoded by the DNA



The citrullinated autoantigens are neo-epitopes can lead to autoimmunity since they are not expressed in the thymus during lymphocyte selection

Citrullinated peptides in RA

Common proteins that undergo Citrullination are: Keratin, Filagrin, Fibrinogen, Vimentin and type II collagen

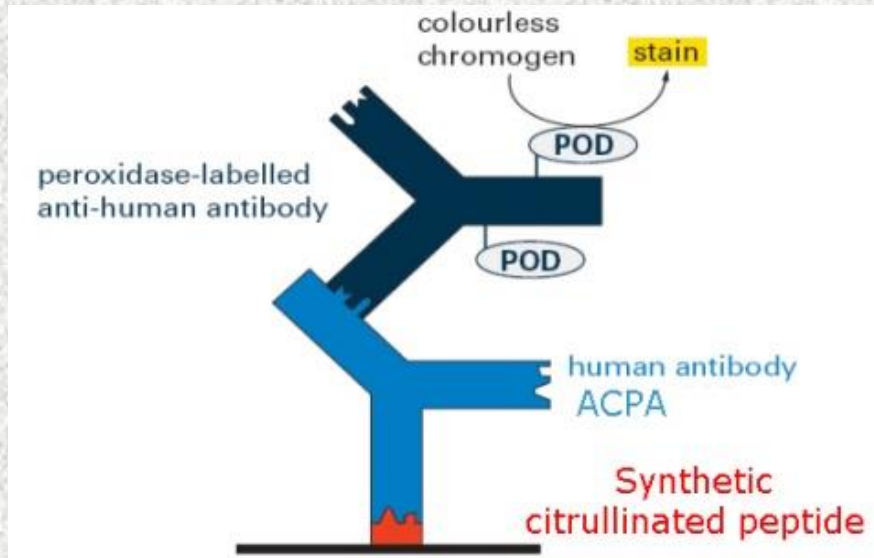


The presence of ACPA is a bad prognostic factor

Citrullinated peptides used for diagnosis

ACPA are very specific for RA (95% specificity)

Serre G. Arthritis Rheum 2002;46(8):2051-8.



ACPAs likely play a role in the pathogenesis of RA.

Kuhn KA, Mol Immunol 2008;45:2808-19

Role of ACPA pathogenesis in RA

- ACPA enhance **tissue injury** in mice induce for arthritis

Kuhn KA et al J Clin Invest 2006 Apr;116(4):961-73.

- ACPA induces production of **TNF- α** in monocyte/macrophages via binding to surface-expressed citrullinated glucose-regulated protein 78 (cit-GRP78)

Lu MC et al. Arthritis Rheum 2010 May;62(5):1213-23.

- Intracellularly, ACPA **activate ERK1/2 and JNK signaling** pathways and lead to activation of NF- κ B and production of TNF- α

Lu MC et al. J Clin Immunol. 2013 Apr;33(3):558-66.

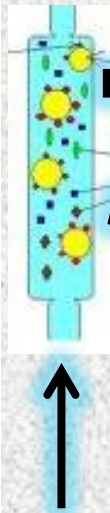
- human ACPA against citrullinated vimentin **induce osteoclast activity** and bone loss

Harre U, et al. J Clin Invest 2012;122:1791-802.

Human ACPA isolation for *in vitro* assays

Affinity purified **ACPA**

Highly ACPA+ RA



Effect on **RA patients** and **Normal controls** lymphocytes

Effect on **RA patients** and **Normal controls** Neutrophils

Citrullinated peptides bounds:

Cit-Collagen type II

Cit- Vimentin

Cit- β -fibrinogen

Cit-filaggrin

Can ACPA binds lymphocytes of RA patients or normal controls ?

Lymphocytes were blocked with Fc blocker

↓
ACPA

or

IgG

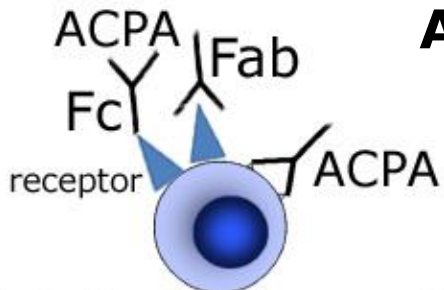
or

Anti-DNA autoantibody (16/6)

1 hour

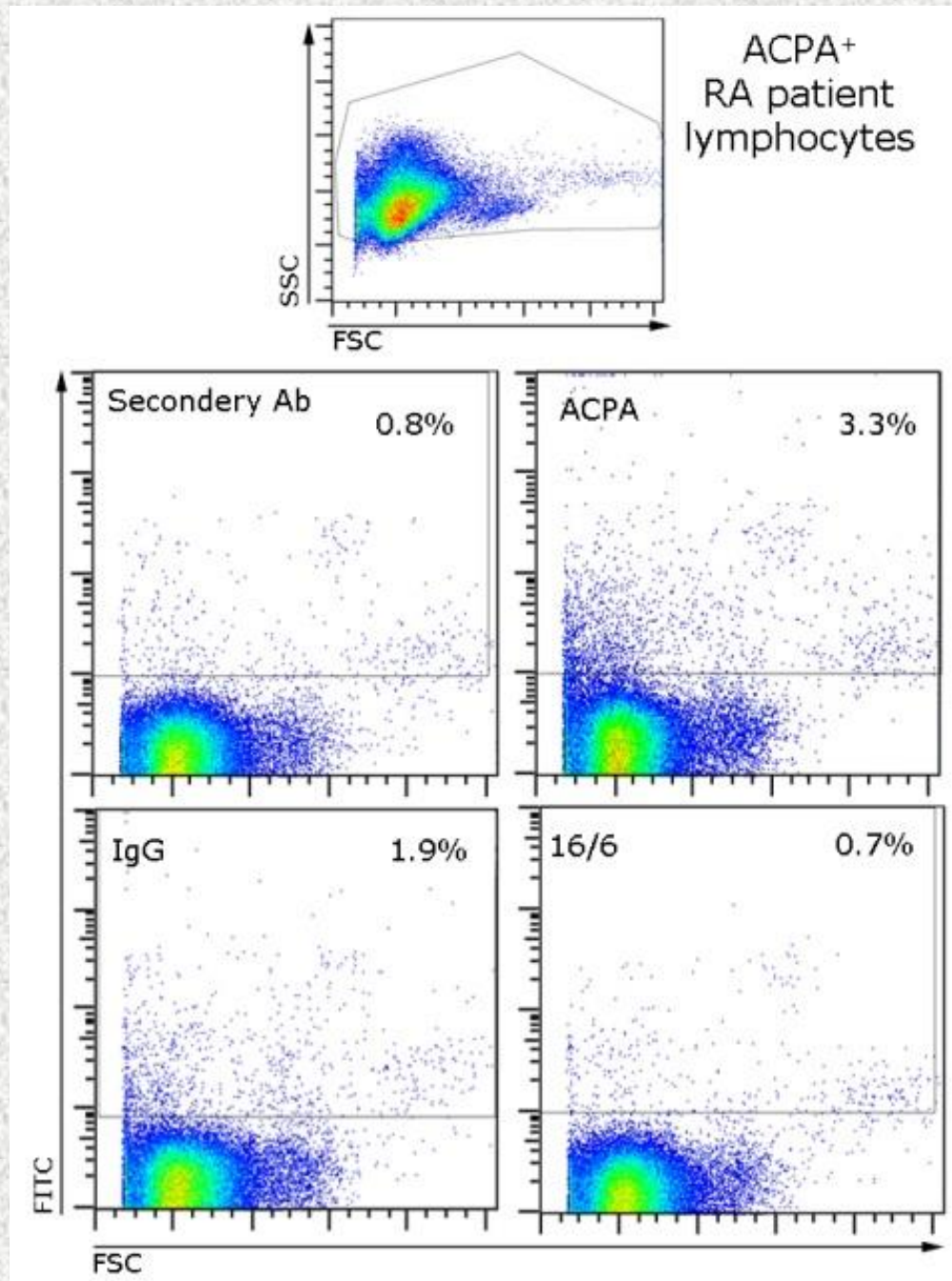


↓
Anti-Human FITC

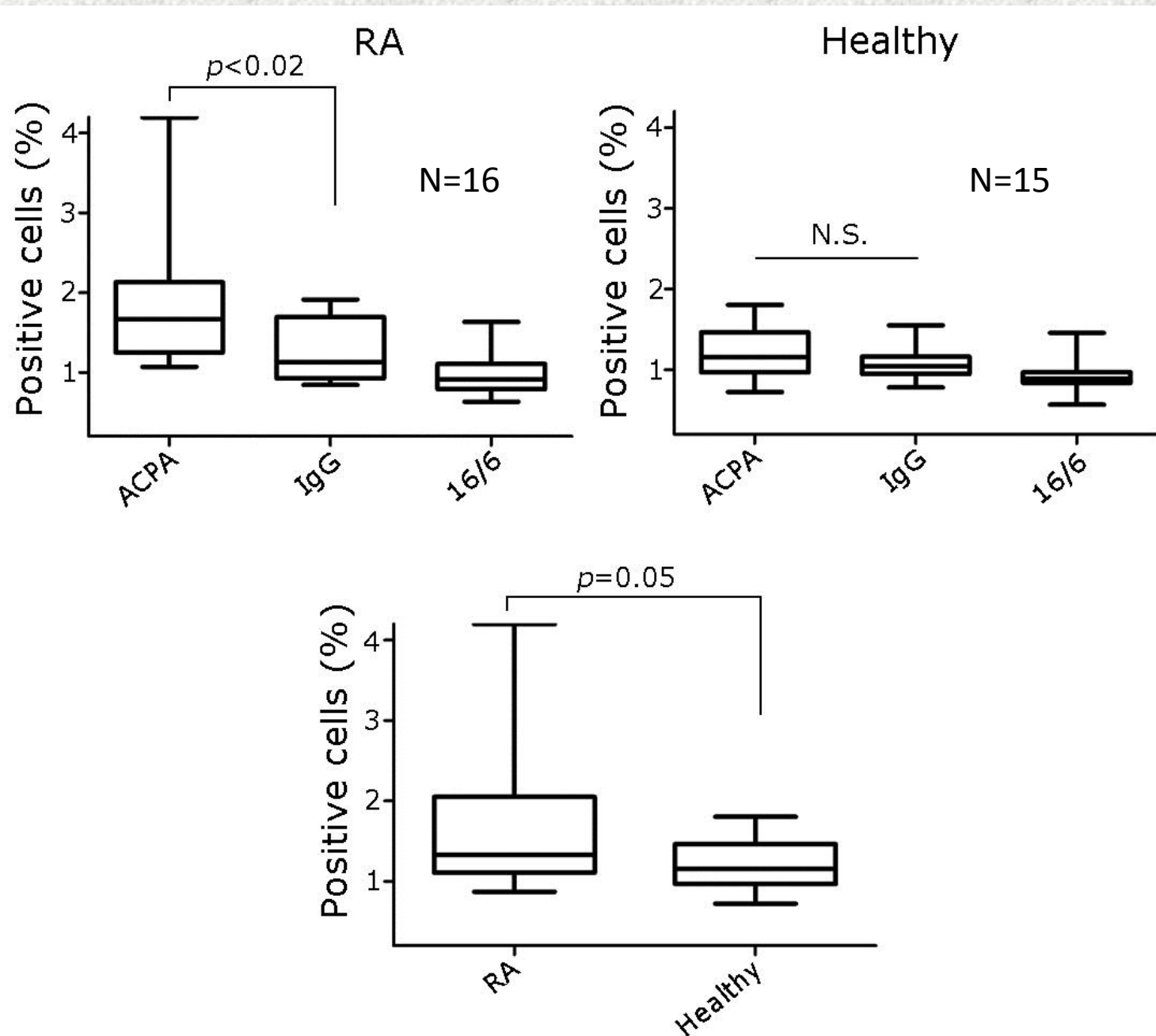


RA derived immune cells

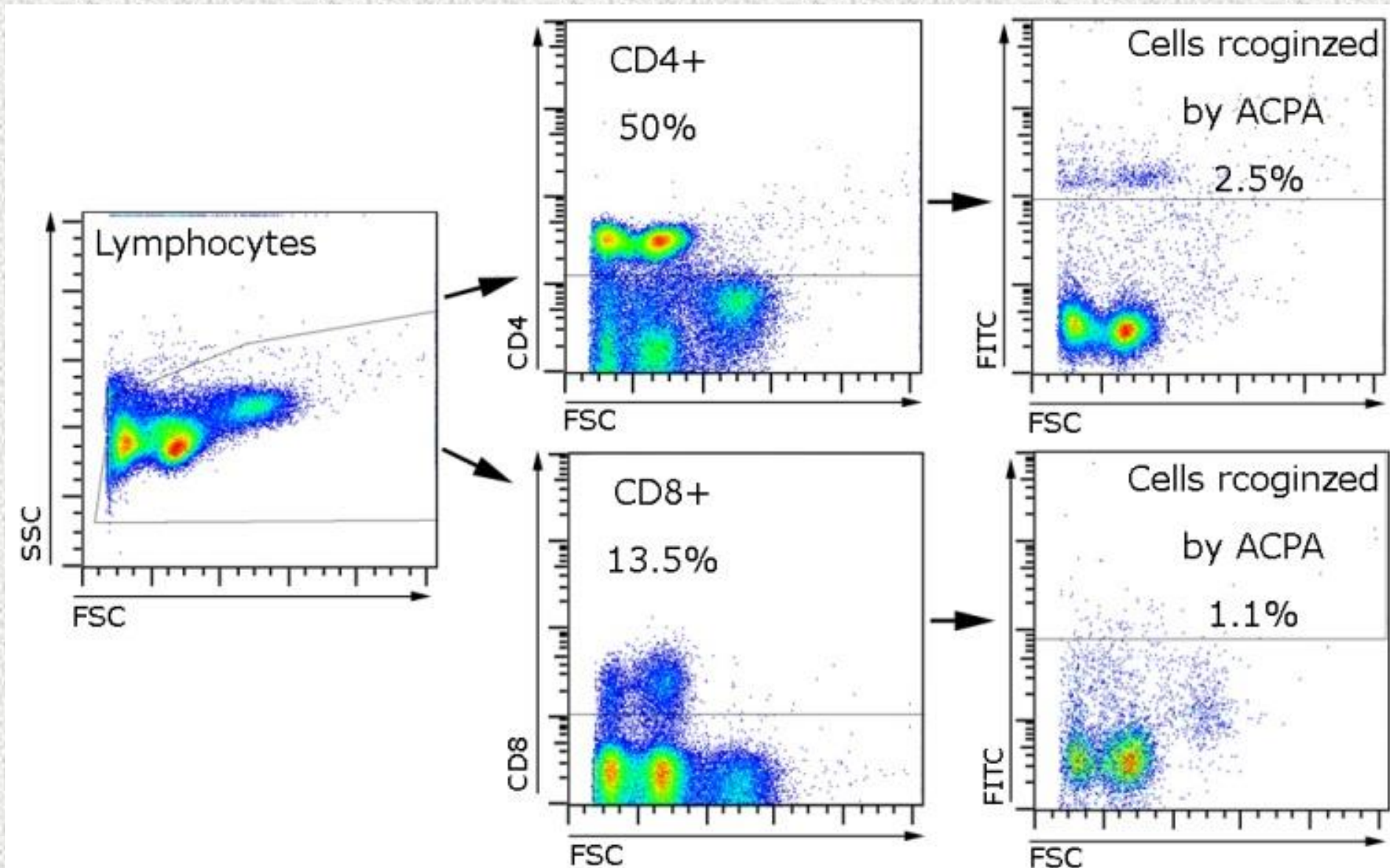
Specific binding of ACPA to RA ACPA⁺ patient lymphocytes



Specific binding of ACPA to RA ACPA⁺ patients lymphocytes

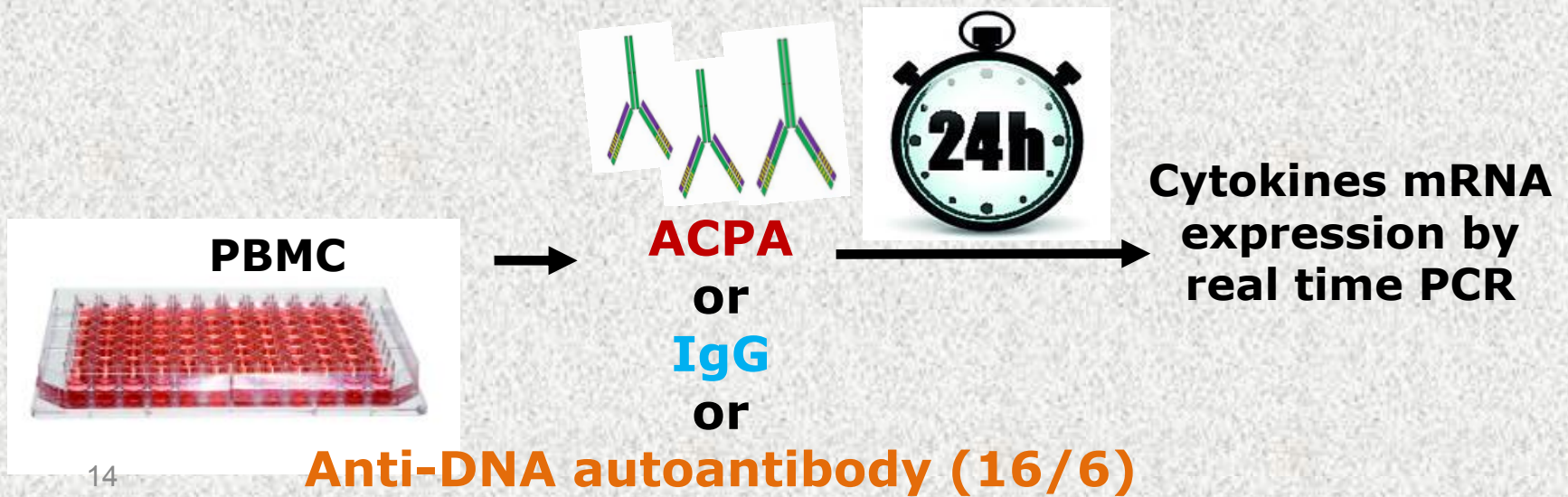


At least part of lymphocytes ACPA recognized are CD4 T cells

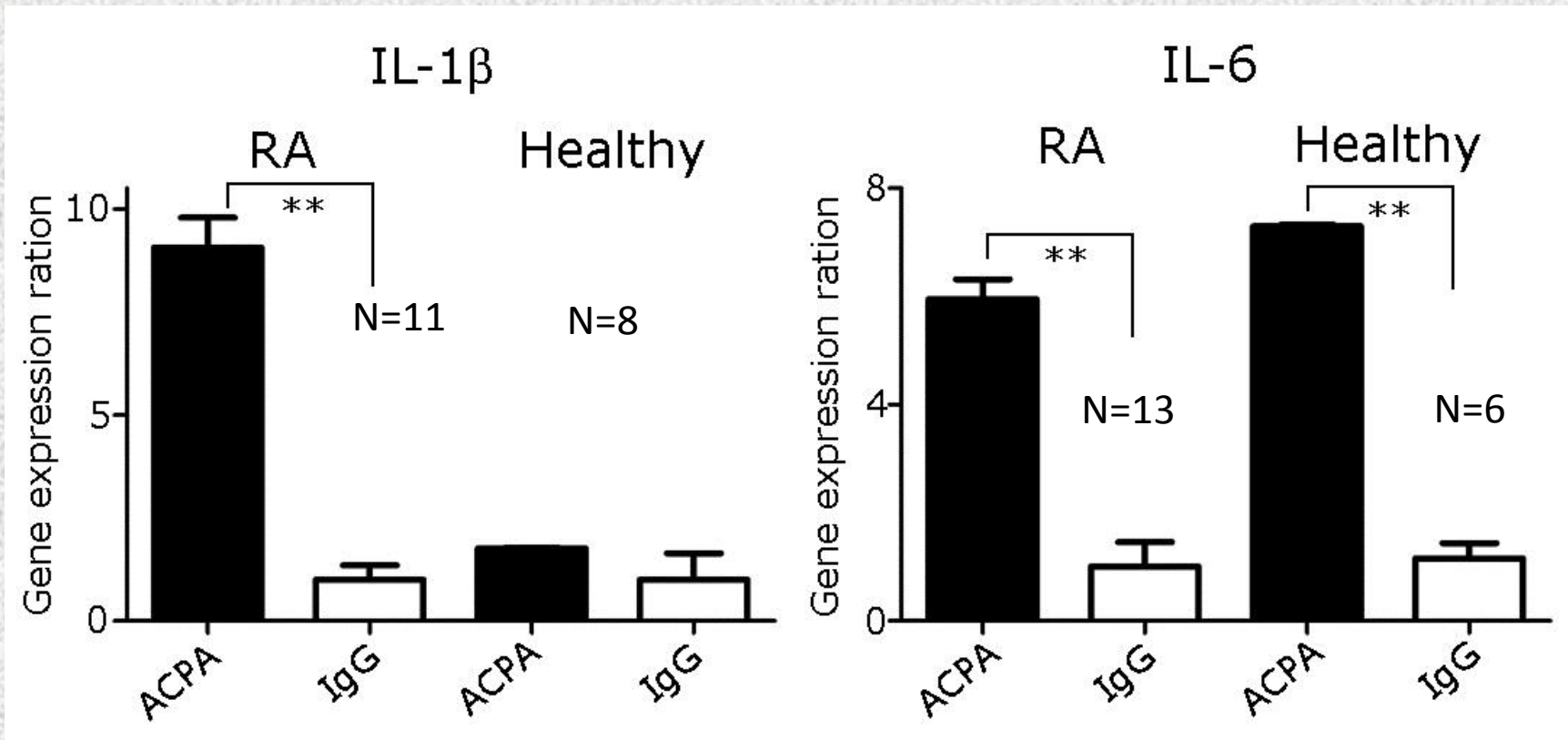


Can ACPA effect cytokines expression of RA patients or Normal controls lymphocytes ?

Immunomodulation of cytokine gene expression in PBMC



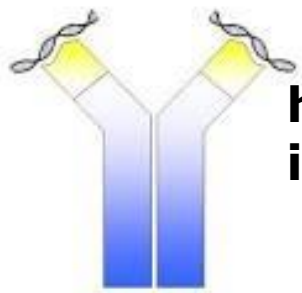
ACPA up-regulates IL-1 β and IL-6 cytokine expression in RA patient's lymphocytes.



RA ACPA Vs RA IgG $p < 0.0001$
H ACPA Vs H IgG $p = 0.0104$
RA ACPA Vs H ACPA $p = 0.0003$

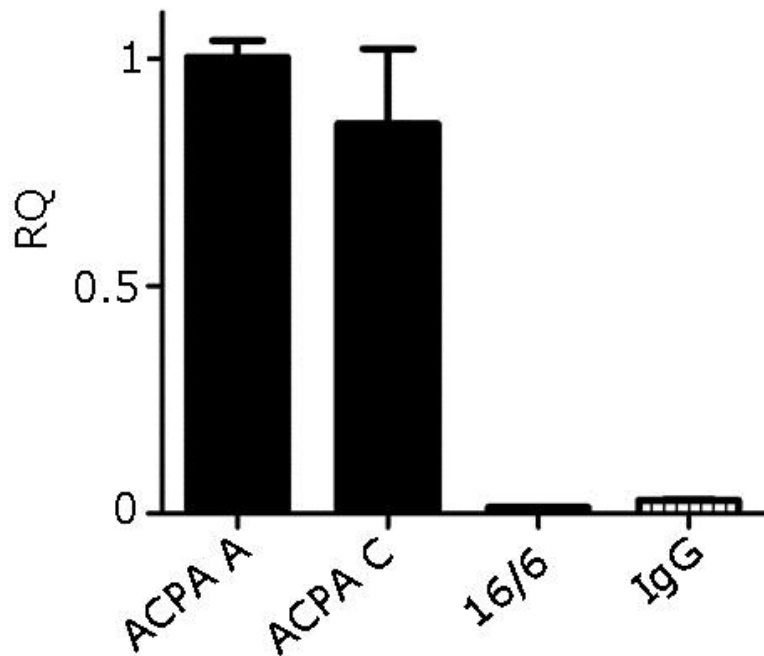
RA ACPA Vs RA IgG $p < 0.0001$
H ACPA Vs H IgG $p = 0.022$
RA ACPA Vs H ACPA $p = 0.044$

Pathogenic cytokines up-regulation in RA patient's lymphocytes is ACPA-specific

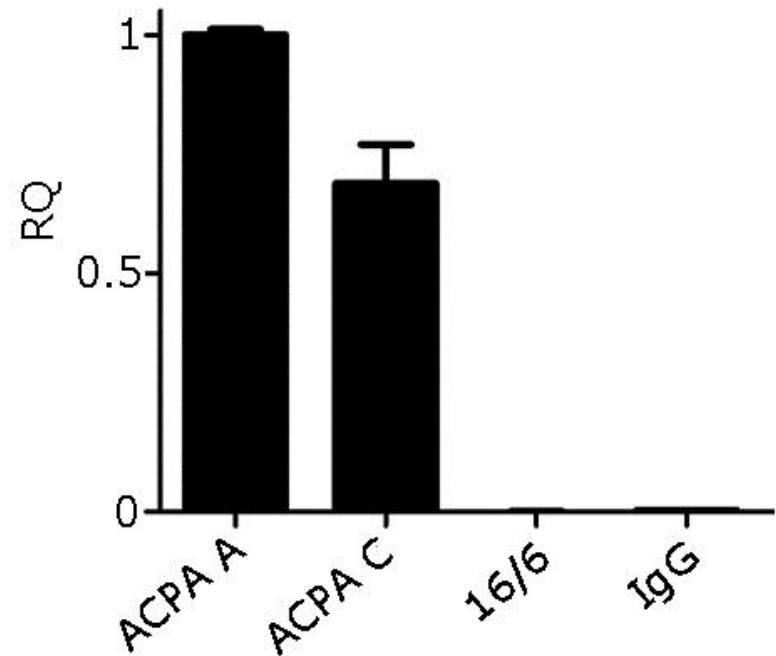


human16/6 idiotype - anti-DNA antibody idiotype involved in the pathogenesis of experimental lupus

IL-1 β



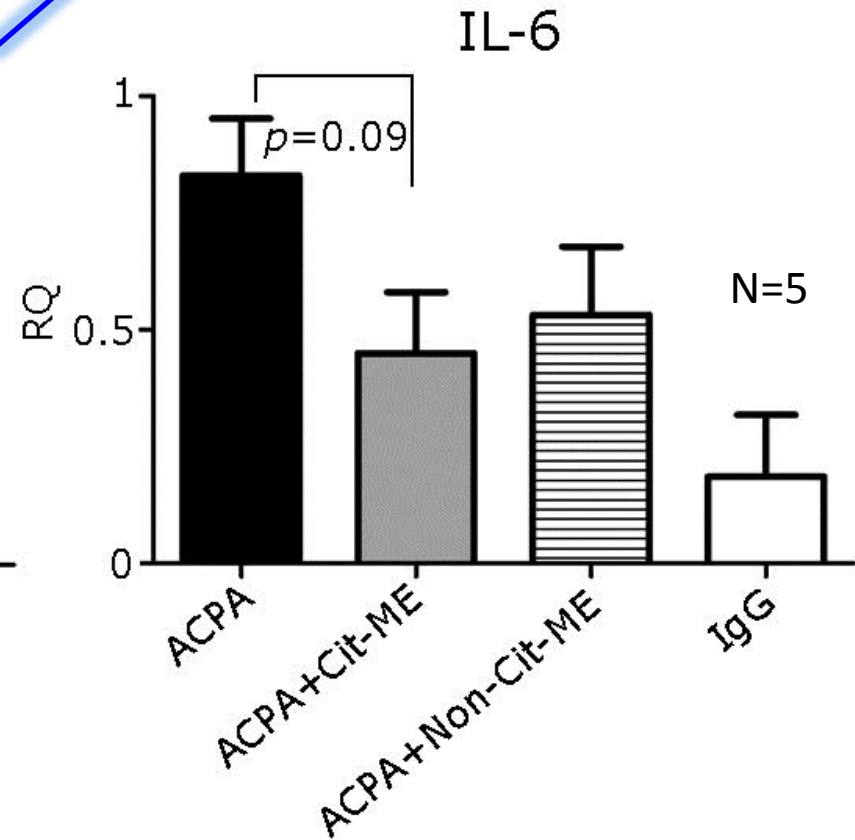
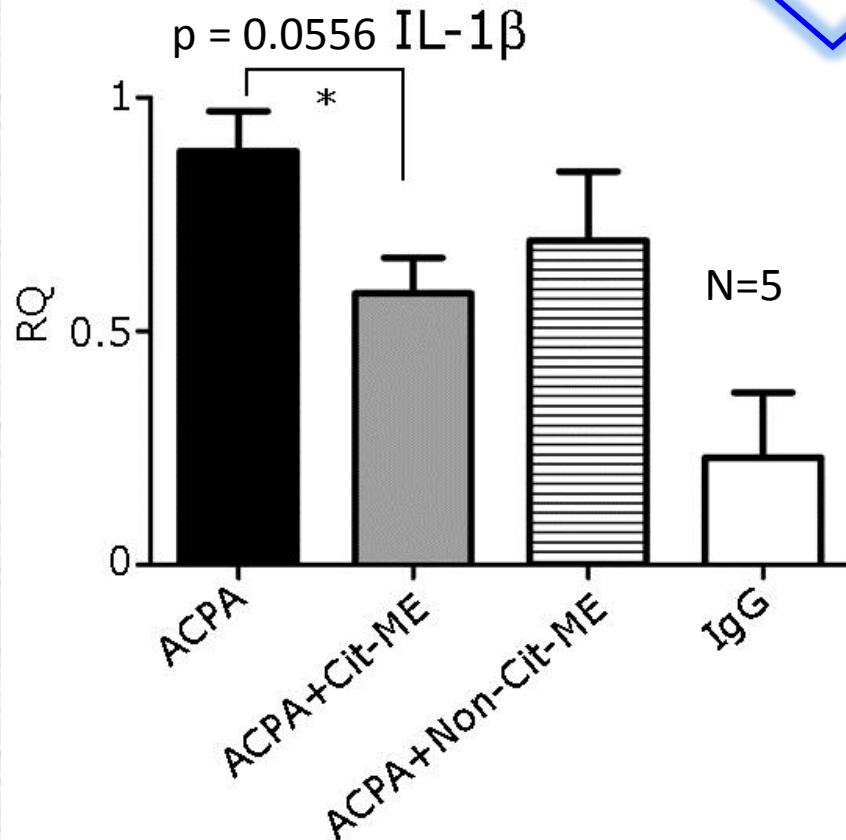
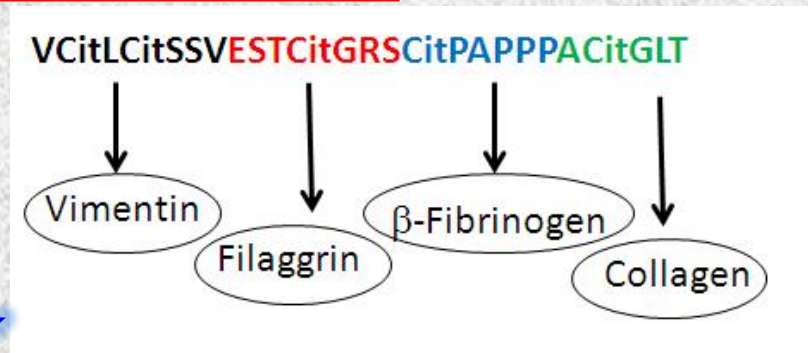
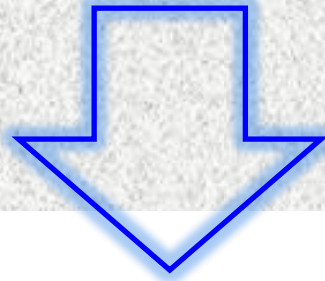
IL-6



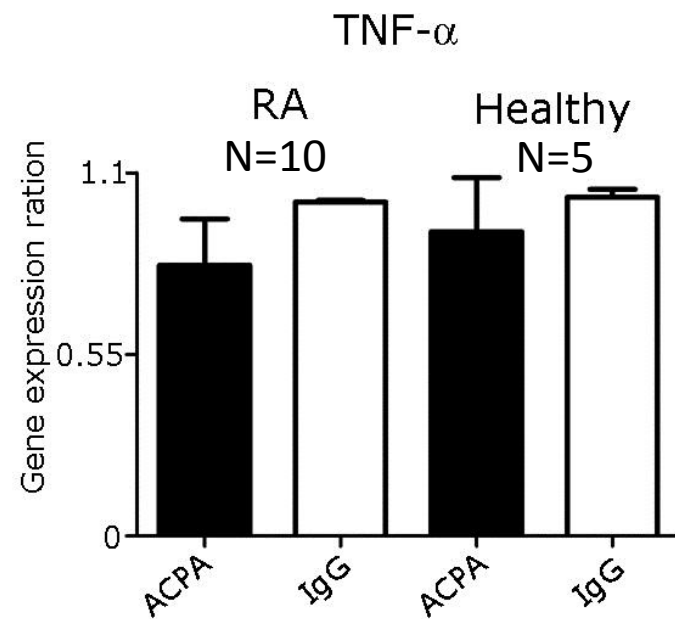
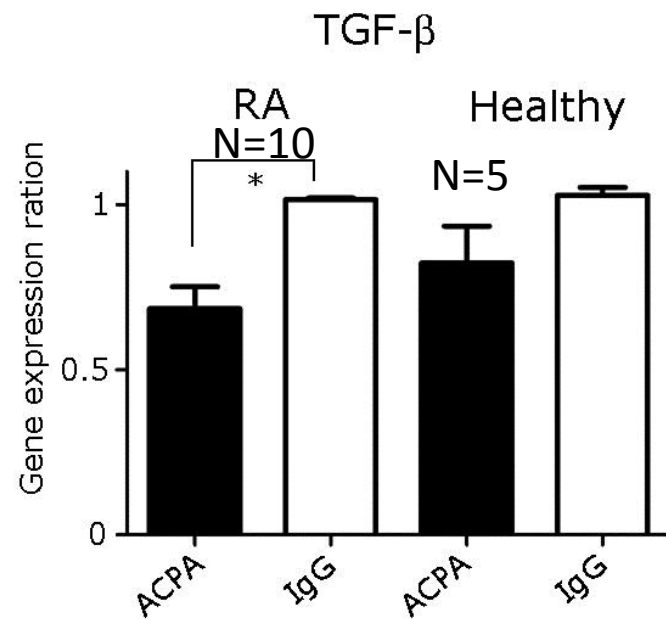
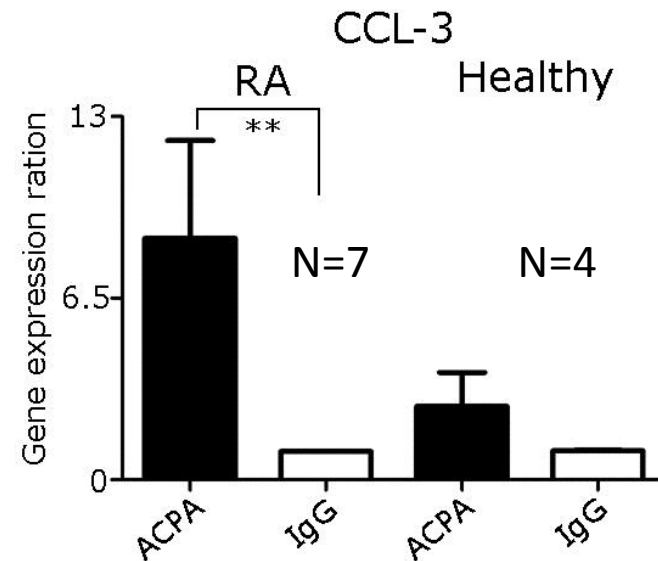
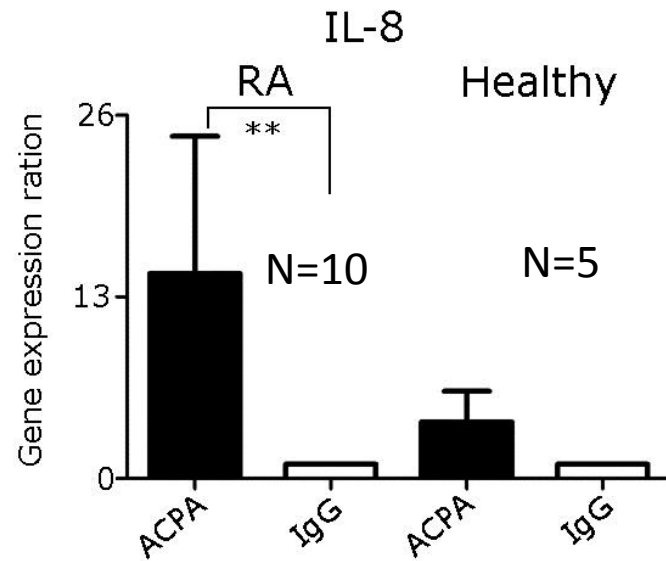
Cit-ME a multi-epitope citrullinated peptide could reduces cytokines up-regulation induced by ACPA



ACPA+Cit-ME



ACPA alters pro- and anti-inflammatory cytokine expression in RA patient's lymphocytes.



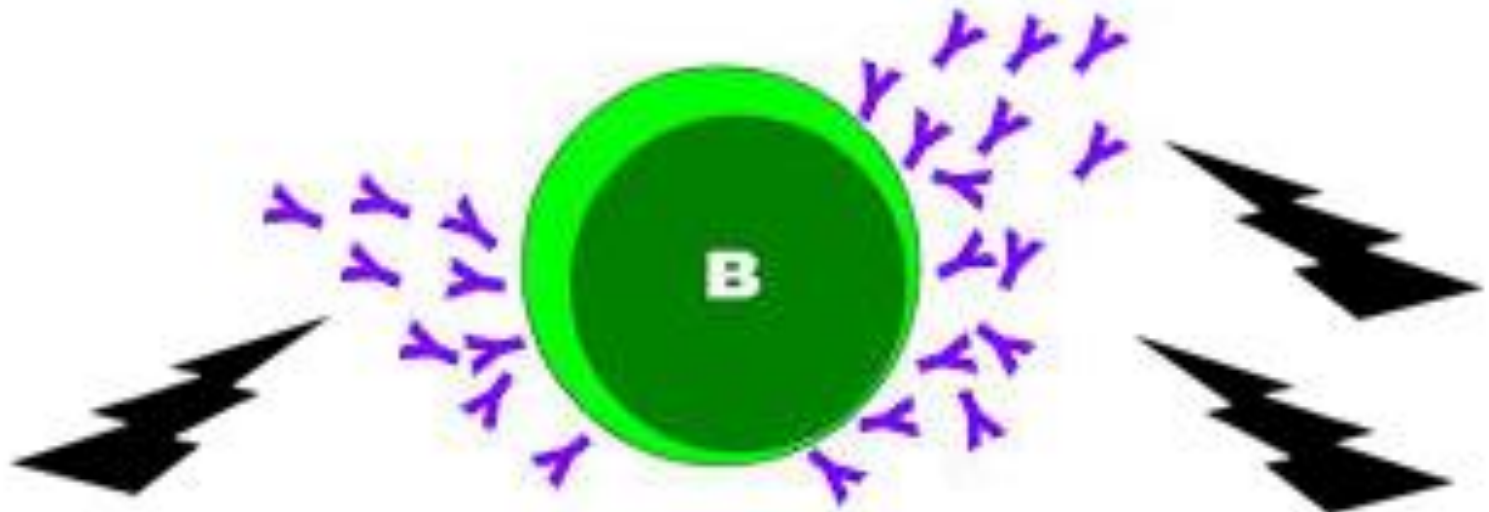
Neutralization of ACPA will improve arthritis ?

Neutralization of AntiCitrullinated Protein Antibodies in Rheumatoid Arthritis – A Way to Go?

Cátia F. Cerqueira, Lars Klareskog and Per-Johan Jakobsson

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(Received 11 July 2013; Accepted 6 October 2013)



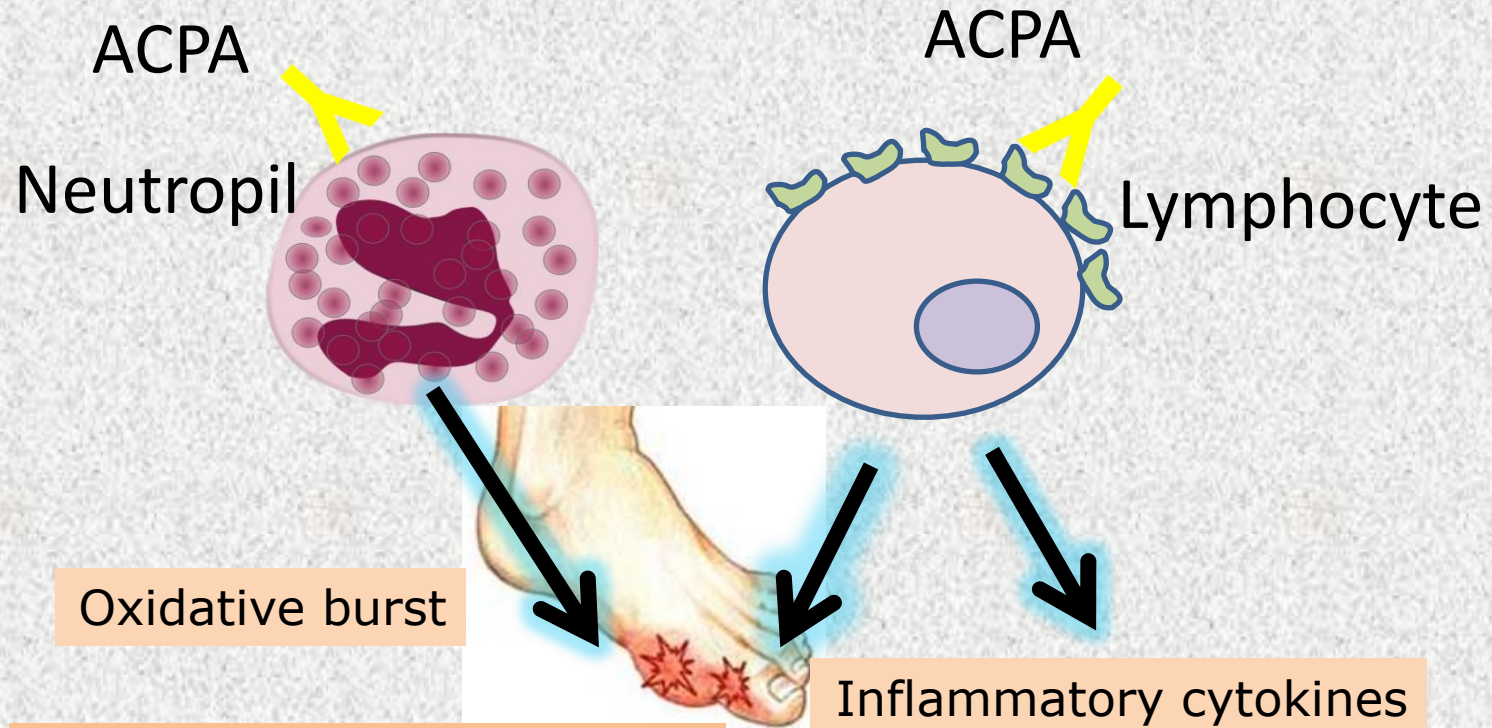
Neutralization of anticitrullinated protein antibodies in rheumatoid arthritis - a way to go?

Cerqueira CF¹, Klareskog L, Jakobsson PJ. Basic Clin Pharmacol Toxicol. 2014;114:13-7.

Conclusions

- **ACPA binds only part of the ACPA⁺ RA patients T lymphocytes.**
- **However, ACPA significantly up-regulates inflammatory cytokines expression in ACPA⁺ RA patients**
- **ACPA also down regulates a major anti inflammatory cytokine**

Mechanisms of ACPA involvement in arthritis



Increase tissue damage

Inflammatory cytokines

T cells proliferation



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