In vitro activity of human ACPA on T cells

Gidi Karmon
Smadar Gertel
Eszter Szarka
Esther Houri-Levi
Yehuda Shoenfeld
Howard Amital
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
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

**TNFα 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 $\rightarrow$ 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)

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
  

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

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

- human ACPA against citrullinated vimentin induce osteoclast activity and bone loss
  
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
Specific binding of ACPA to RA ACPA$^+$ patient lymphocytes
Specific binding of ACPA to RA ACPA\(^+\) patients lymphocytes

- **RA**
  - ACPA: N=16
  - IgG: N=15
  - 16/6: N=15

- **Healthy**
  - ACPA: N=15
  - IgG: N=15
  - 16/6: N=15

Significance levels:
- RA ACPA vs Healthy: p<0.02
- RA IgG vs Healthy: N.S.
- RA 16/6 vs Healthy: p=0.05
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

PBMC → ACPA or IgG or Anti-DNA autoantibody (16/6) → 24h → Cytokines mRNA expression by real time PCR
ACPA up-regulates IL-1β 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
Cit-ME a multi-epitope citrullinated peptide could reduce cytokine up-regulation induced by ACPA.
ACPA alters pro- and anti-inflammatory cytokine expression in RA patient's lymphocytes.

ACPA

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>RA (N=10)</th>
<th>Healthy (N=5)</th>
<th>IgG</th>
<th>ACPA (N=10)</th>
<th>Healthy (N=5)</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-8</td>
<td>**</td>
<td>N=10</td>
<td>N=5</td>
<td>**</td>
<td>N=7</td>
<td>N=4</td>
</tr>
<tr>
<td>CCL-3</td>
<td>**</td>
<td>N=7</td>
<td>N=4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGF-β</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Neutralization of AntiCitrullinated Protein Antibodies in Rheumatoid Arthritis – A Way to Go?

Cátia F. Cerqueira, Lars Klareskog and Per-Johan Jakobsson
Rheumatology Unit, Department of Medicine, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
(Received 11 July 2013; Accepted 6 October 2013)

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

- ACPA
- Neutrophil
- Lymphocyte
- Oxidative burst
- Inflammatory cytokines
- T cells proliferation
- Increase tissue damage
Lab head:
Prof. Howard Amital

Lab members:
Dr. Smadar Gertel
Dr. Abdulla watad
Dr. Hussien Mahajanha

The Zabludowicz center for Autoimmune diseases members:
Head Prof. Yeduda shoenfeld
Prof. Miri Blank
Dr. Boris Gilbord