Update on the Pathogenesis of Rheumatoid Arthritis

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Cytokines promote inflammatory synovitis

**Diagram**

- **DC**
  - IL-12, IL-23
  - Chemokines, ECM, co-stimulation
- **Th1/Th17**
  - IL-17, IL-22
  - Cell contact, co-stimulation
- **Macrophage**
  - IL-17, IL-22
  - IFNγ
- **B cell**
  - TNFα
  - IL-1
  - IL-15
  - IL-18
  - IL-6
  - IL-20
  - IL-32
  - IL-33
  - RANKL
  - GM-CSF
  - IL-10
  - IL-1Ra
  - IL-18BP
  - sIL-1R
  - sTNFR
  - IL-27
  - IL-35
  - TGFβ

**Text**

Cytokines are implicated in each phase of RA pathogenesis

**Cellular recruitment**
- TNF, IL-1
- IL-6, IL-18
- VEGF
- Chemokines

**Immunologic activation and organization**
- IL-23, IL-27
- IL-12, IL-15, IL-18
- Chemokines, LTα

**Cellular retention and survival**
- IFNα/β, IL-15
- TNF

**Tissue response**
- IL-17, BMPs
- RANKL, TGFβ
- TNF, IL-1

PsA and RA were originally treated as the same disease

- Not until the 1950s were the typical features of PsA described\(^1\)
- Before Moll and Wright produced the first classification of PsA, disease descriptions included:\(^2\)
  - Arthritis confined to the DIP joints with psoriasis
  - Atypical arthritis with atypical psoriasis
  - Arthritis following prolonged, uncontrollable psoriasis
  - Coincident psoriasis and RA – there being no distinct entity
- Classification criteria have evolved to address the difficulty in PsA diagnosis\(^1, 3\)
- Still, joint involvement in psoriasis/PsA is often treated in the same way as RA, with drugs known to be ineffective against some PsA symptoms\(^4\)

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Class II genes account for about 30% of genetic susceptibility to RA

<table>
<thead>
<tr>
<th>HLA-DR associations with rheumatoid arthritis defined by DR $\beta_1$ sequence position 70–74</th>
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</thead>
<tbody>
<tr>
<td><strong>DR type</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>DR4 -W4</td>
</tr>
<tr>
<td>-W14</td>
</tr>
<tr>
<td>-W15</td>
</tr>
<tr>
<td>DR1</td>
</tr>
<tr>
<td>DR4 -W10</td>
</tr>
<tr>
<td>-W13</td>
</tr>
</tbody>
</table>

Q=glutamine; K=lysine; R=arginine; A=alanine; D=aspartic acid; E=glutamic acid
Current RA genetic risk loci from GWAS
Citrullination is a process of protein modification.


PAD, peptidyl arginine deiminase
PAD expression in the lung

- PAD2 and PAD4 are present in all lung tissues

COPD, chronic obstructive pulmonary disease; PAD, peptidyl arginine deiminase.

Citrullination improved peptide binding to come HLA class II alleles and leads to T-cell activation

ACPA and RF precede RA

- ACPAs and RFs in patients appear many years prior to RA onset\(^1\)
- IgA RFs also appear in patients years prior to clinical symptoms\(^2\)

ACPA, anti-citrullinated protein antibody; Ig, immunoglobulin; RA, rheumatoid arthritis; RF, rheumatoid factor.

Evolution of rheumatoid arthritis

Immune response develops

Pathologic inflammatory response

Genetic and environmental factors

Time

ACPA=anti-citrullinated protein antibodies; mφ=macrophage; MHC=major histocompatibility complex; RF=rheumatoid factor; TCR=T-cell receptor.

Prognostic value of ACPA in patients with recent-onset RA

- Major advance in standardized diagnostic testing
- In ~70% of RA patients, ACPA is present at the early stages of disease
- ACPA (+) patients develop significantly more severe structural damage than those who are ACPA(-)

ACPA, anti-ckrullinated protein antibody; RA, rheumatoid arthritis.

# ACPA or CCP?

- ACPA are autoantibodies detected using ELISAs containing CCP\(^1\)

- The anti-CCP antibodies detected this way are a surrogate for ACPA

<table>
<thead>
<tr>
<th>CCP(^1)</th>
<th>ACPA</th>
</tr>
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| Artificial antigen in commercial, diagnostic ELISA tests used for clinical decision making  
  - FDA 510(k) approved  
  - Sensitivity and specificity of tests differ  
  - Proprietary synthetic antigens  
  - CCP1=first-generation test\(^1\)  
  - CCP2=second-generation test\(^1\)  
  - Anti-CCP2 refers to the diagnostic laboratory result | Antibody response in the patient to multiple citrullinated protein antigens  
  - Originally detected in tests developed in academic research laboratories  
  - Common ACPA targets include citrullinated forms of fibrinogen, vimentin, Type II collagen (CII), and α-enolase\(^2\)  
  - **Note:** ACPA is sometimes used colloquially to describe the results of the anti-CCP2 assay |

ACPA, anti-citrullinated protein antibody; CCP, cyclic citrullinated peptide; ELISA, enzyme-linked immunosorbent assay; FDA, Food and Drug Administration.

Several epitopes are recognised by ACPAs

**Filaggrin 48–65**
- Fibrinogen B 246–267
- Fibrinogen
- Fibrinogen A 211–230
- Fibrinogen A 582–599
- Fibrinogen A 556–575
- Fibrinogen A 616–635
  - Vimentin
  - H2B/a 62–81
  - H2A/a 1–20
  - Histones 2A
  - Histones 2B
  - Clusterin 221–240
  - Clusterin 231–250
  - Biglycan 247–266
  - Enolase 1A 5–21
  - Vimentin 58–77
  - Apolipoprotein E
  - Apolipoprotein E 277–296

**Anti-CCP**

Anti-CCP: A screening test covering several different ACPA responses

ACPA, anti-citrullinated protein antibody; CCP, cyclic citrullinated peptide.
Poor prognostic factors in RA

EULAR 2013 recommendations

Autoantibody (ACPA/RF)-positive

Early joint damage

High disease activity

ACPA, anti-citrullinated protein antibody; EULAR, European League Against Rheumatism; RA, rheumatoid arthritis; RF, rheumatoid arthritis.
ACPAs form immune complexes to activate macrophages

Self-antigen (citrullinated protein) → Immune complexes bind to Fc receptor → Macrophage activation and cytokine secretion

ACPA → Immune complexes → Activated macrophage → Proinflammatory cytokines

ACPA, anti-citrullinated protein antibody.

Adapted from Nimmerjahn F and Ravetch JV. Nat Rev Immunol 2008;8:34–47
Percentage of early RA with erosions by serology

Percentage of Patients with Erosions

<table>
<thead>
<tr>
<th>RF+/ACPA+</th>
<th>RF+/ACPA-</th>
<th>RF-/ACPA+</th>
<th>RF-/ACPA-</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>80</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

Kachamart W et al. Rheumatol Int. 2015;35:1693-1699
Percentage of early RA with erosions by serology after 1 year

Katchamart W et al. Rheumatol Int. 2015, 35:1693-1699
Changes in cortical bone structure

Bone structure is altered in ACPA(+) non-arthritic individuals compared with ACPA(-) controls.

Arrows show cortical thinning, cortical fenestration as well as small bone erosions. ACPA, anti-citrullinated protein antibody.

ACPA and osteoclast differentiation

ACPA 0 ng/mL  ACPA 1 ng/mL

ACPA 10 ng/mL  ACPA 100 ng/mL

Osteoclastogenesis**

Bone resorption**

* P<0.05; ** Resorption pit assay (n = 3) with different concentrations of MCV-ACPAs and of IgG fractions deprived of ACPA.
ACPA, anti-citrullinated protein antibody; Ig, immunoglobulin; MCV, mutated citrullinated vimentin.

Interaction between ACPA and RF in RA-mediated bone loss

ACPA, anti-citrullinated protein antibody; RA, rheumatoid arthritis; RF, rheumatoid factor.

Moving from citrullination to ACPA


ACPA, anti-citrullinated protein antibody; CCP, cyclic citrullinated peptide; CPA, citrullinated protein antibody; DRB, D related beta; HLA, human leukocyte antigen; PAD, peptidyl arginine deiminase; PTPN22, Protein tyrosine phosphatase, non-receptor type 22; RA, rheumatoid arthritis; SE, shared epitope; TNF, tumour necrosis factor.
Sputum ACPAs and NETs in FDRs of RA patients
Sputum ACPAs and NETs in FDRs of RA patients

Demoruelle MK et al Arthritis Rheumatol; 2017; 69:1165-75
Porphyrymonas gingivalis citrullinates bacterial and human proteins

- This organism and several other common infectious agents have been suggested to trigger RA
- Generally via molecular mimicry

Classification and disease duration

The cells and cytokines involved in the pathogenesis of psoriasis, PsA, and RA

Clinical features of PsA and RA

**Typical features of RA**

- Rheumatoid nodules
- Rheumatoid vasculitis
- Serum rheumatoid factor
- Symmetric joint involvement
- Primarily hand and wrist involvement
- Pannus

**Typical features of PsA**

- Dactylitis
- Enthesitis
- Spondylitis
- Asymmetric joint involvement
- DIP involvement
- Psoriasis
- Nail disease
- Radiographically evident enthesopathic changes


DIP, distal interphalangeal; RA, rheumatoid arthritis.
Genomic analysis highlights differences between PsA and RA

OR plots for eight SNPs demonstrating evidence for association to PsA susceptibility, highlighting the opposing direction of effects for REL, PLCL2 and KIF5A.


OR, odds ratio; RA, rheumatoid arthritis; SNP, single nucleotide polymorphism.
MHC-1-opathy versus autoimmunity

**Comparison of the features of MHC-1-opathies and autoimmune diseases**

<table>
<thead>
<tr>
<th>Feature</th>
<th>MHC-1-opathy</th>
<th>Autoimmune disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>No female predominance</td>
<td>Female predominance</td>
</tr>
<tr>
<td><strong>Age of onset</strong></td>
<td>Generally young</td>
<td>Wide age range</td>
</tr>
<tr>
<td><strong>Eye disease</strong></td>
<td>Anterior or posterior uveitis, or both</td>
<td>Can include scleritis</td>
</tr>
<tr>
<td><strong>Injury</strong></td>
<td>Linked to tissue-specific injury</td>
<td>No link</td>
</tr>
<tr>
<td><strong>Barrier function perturbation</strong></td>
<td>Skin, mouth, gut</td>
<td>Absent</td>
</tr>
<tr>
<td><strong>Course without therapy</strong></td>
<td>Waxing and waning</td>
<td>Progressive</td>
</tr>
<tr>
<td><strong>Genetics</strong></td>
<td>MHC-1, ERAP1/2, <strong>IL-23/IL-17 axis</strong></td>
<td>MHC-II</td>
</tr>
<tr>
<td><strong>Gut involvement</strong></td>
<td>Clinical or subclinical gut disease is common</td>
<td>No link to gut disease (except coeliac disease)</td>
</tr>
<tr>
<td><strong>Therapy</strong></td>
<td>Respond to anticytokine therapy, but not B-cell depletion</td>
<td>May respond to anticytokine therapy and B-cell depletion</td>
</tr>
<tr>
<td><strong>Joint disease</strong></td>
<td>Sites of mechanical stress (entheses and lower limbs)</td>
<td>Polyarticular and synovial</td>
</tr>
<tr>
<td><strong>Underpinning theory</strong></td>
<td>Danger as immunological driver</td>
<td>Self versus non-self discrimination</td>
</tr>
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</table>

ERAP, endoplasmic reticulum aminopeptidase; IL, interleukin; MHC, major immunohistocompatibility complex.
Differentiation of RA and the diffuse inflammation in PsA

- Early in RA joint disease localisation is to the synovium
  - Synovium the primary target organ
- In early PsA the inflammatory changes have a widespread distribution
  - Appear to relate to patterns of joint stressing around ligaments, adjacent bone and soft tissues
  - As opposed to a specific antigen territory

PsA: DIP joint with extensive inflammatory changes in all tissues
The physiology of entheses – the enthesis organ (more than an insertion)

- Neighbouring tissues are also involved in the dissipation of stress\(^1\)
  - Periosteal and sesamoid fibrocartilage, bone, soft tissues, synovium
- Widespread stress dissipation may explain the diffuse tissue swelling observed in SpAs\(^2\)

- The synovio-entheseal complex (SEC) can form part of the enthesis organ\(^3\)
- Damage to the enthesis by micro-damage or other mechanisms can lead to inflammation of the SEC due to their close association\(^3\)

Model for how enthesitis leads to joint and bone damage

Mechanical stressing can lead to micro-damage and micro-inflammation at normal entheses\(^1,2\)

In psoriasis/PsA patients, a dysregulated inflammatory response occurs at the enthesis\(^3\)

Diffuse inflammation occurs across all structures of the enthesal organ and SEC\(^4\)

Osteolysis occurs from persistent inflammation\(^5\)

IL-23 induces spondyloarthropathy by acting on ROR-γt+ CD3+CD4−CD8− enthesial resident T cells

IL-23 drives entheseseal-resident T cells in the pathogenesis of spondyloarthritis

Lories RJ and McInnes IB Nat Med 2012;18:1018-9
# Cytokine targets in inflammatory arthritis

<table>
<thead>
<tr>
<th></th>
<th>RA</th>
<th>PsA</th>
<th>AS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>IL-6</td>
<td>+++</td>
<td>+1</td>
<td>-2</td>
</tr>
<tr>
<td>IL-17</td>
<td>+3</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>IL12/23</td>
<td>-4</td>
<td>+++</td>
<td>+5</td>
</tr>
<tr>
<td>JAK</td>
<td>+++</td>
<td>+++</td>
<td>+6</td>
</tr>
</tbody>
</table>

+++: approved treatment or positive phase III trial  
++: not approved but positive phase III trial  
+: not approved but limited efficacy from phase II or III clinical trials  
-: negative clinical trial

Regulation of autoantibody activity by the IL-23-Th17 axis determines the onset of autoimmune disease

Collagen induced arthritis

IL-23−/−

WT

Pfeifle R et al. Nat Immunol 2017;18:104-113
Regulation of autoantibody activity by the IL-23-Th17 axis determines the onset of autoimmune disease

Pfeifle R et al. Nat Immunol 2017;18:104-113
TNF

T cells

B cells

IL-6

TNF

IL-6
Ideally, biomarkers would help clinicians select an optimal therapeutic strategy for patients.

Analysis of predictive biomarkers

Prediction algorithm used to assess treatment response

Patient presents with inflammatory arthritis or new-onset RA

Allocation of ideal treatment: Treat right the first time

Therapy 1

Therapy 2

Therapy 3

RF and/or anti-CCP seropositivity enriches response to rituximab

**CERERRA**¹

<table>
<thead>
<tr>
<th>RF status</th>
<th>Anti-CCP status</th>
<th>RF and anti-CCP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>1.6 2*</td>
<td>1.1 2**</td>
</tr>
<tr>
<td>Negative</td>
<td>1.4 2.1**</td>
<td>1.1 2**</td>
</tr>
</tbody>
</table>

**RABBIT registry**²

<table>
<thead>
<tr>
<th>RF status</th>
<th>Anti-CCP status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>64.4</td>
</tr>
<tr>
<td>Negative</td>
<td>54.9</td>
</tr>
</tbody>
</table>

Mean change in DAS28 at 6 months

Patients with EULAR Good/Moderate response (%)

* *p = 0.009; **p = 0.002 vs. seronegative patients; ***p = 0.001 vs. RF–.

Boolean remission to abatacept in biologic-naïve RA patients at 6 months (ACTION study)

Error bars represent 95% CI.

Alten R, et al. Arthritis Rheumatol 2015; 67(Suppl. 10); Abstract 551
Pathogenesis of RA is driven by complex interactions of immune cells and cytokines

Conclusion

• Pathogenesis of RA and PsA are different

• Different cytokines have different pathogenic roles in inflammatory rheumatic diseases

• Cytokines have different roles during different stages of inflammatory arthritis

• In RA, immune activation as evident by ACPA serpositivity, is present before symptoms

• In HLA-DR4 positive individual, citrullinated peptide are more potent antigens

• Infection can leads to development and amplification of ACPA response leading to disease

• RA are heterogenous: ACPA +ve and ACPA-ve diseases may be fundamentally different and associated with difference in response to rituximab and abatacept