Rheumatoid Arthritis and Citrullinated Proteins

Anti-Cyclic Citrullinated Peptide Antibodies
(Anti-CCP)

Alina Chen
MacMillan Group Meeting
Literature Talk
March 8th, 2022
Outline

What is Rheumatoid Arthritis

Citrullination & Citrullinated Peptides

Anti-CP antibodies (ACPA’s)

Potential therapeutics
What is Rheumatoid Arthritis?
symptoms & causes

Rheumatoid Arthritis

**Early Symptoms**

- joint pain, swelling, stiffness
- morning stiffness
- fatigue
- mild fever

*Rheumatoid arthritis*

- A chronic, inflammatory, autoimmune disease
- Affects joints in hand, wrists, knee
- Common symptoms: swollen joints, weight loss, fatigue
- Specific causes unknown

**Risk factors**

- genetics
- smoking
- age
- sex
- obesity


*Deane, K.D.; et al. Arthritis & Rheumatology 2021, 73, 2, 181.*
What is Rheumatoid Arthritis?
symptoms & causes

eventually leads to cartilage damage and bone erosion
also affects internal organs: skin, eyes, heart, etc.

What is Rheumatoid Arthritis?
symptoms & causes

80% of RA patients between the ages of 35-50

Women are 3 times more likely to develop RA than men

Vaccination

Risk
Hereditity  Age  Lifestyle  Pollution

70% of RA patients have wrist and hand problems

Complications
Heart attack  Stroke

90% of RA patients have symptoms in the foot

Management
Exercise  Surgery  Dietary supplements

Antirheumatic drugs  Stop smoking  Limit alcohol

What is Rheumatoid Arthritis?

Diagnosis

- May proceed rapidly, or take months/years
- Earlier treatment prevents development of RA
- “Early” stage of RA not defined or universally agreed

What is Rheumatoid Arthritis?

**diagnosis**

2010 American College of Rheumatology/European League against Rheumatism

Patients who 1) have at least 1 joint with definite clinical synovitis (swelling), 2) with the synovitis not better explained by another disease

A score of ≥ 6/10 by scoring or with erosions typical for RA

No

Yes

Start methotrexate

**score**

<table>
<thead>
<tr>
<th>Joint involvement</th>
<th>score</th>
</tr>
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<tbody>
<tr>
<td>≥1 large joint</td>
<td>0</td>
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**Serology**

Negative RF and ACPA                    | 0     |
Low positive RF or ACPA                  | 2     |
High-positive RF or ACPA                 | 3     |

**Duration of symptoms**

< 6 weeks                                | 0     |
≥6 weeks                                 | 1     |

**acute-phase reactants**

Normal CRP and ESR                       | 0     |
Abnormal CRP or ESR                      | 1     |

A score of ≥ 6/10 is needed for classification of a patient as having definite RA

Tanaka, Y. Caspian Inflamm Regener 2020 40, 20.
What is Rheumatoid Arthritis?

Treatment

First line: relieve pain and decrease inflammation

Nonsteroidal anti-inflammatory drugs (NSAIDs)

Steroids

What is Rheumatoid Arthritis?

Treatment

Second line: promote remission

Disease-modifying anti-rheumatic drugs (DMARDs)

B-cell depletion and inhibition antibodies

Gun, Q.; et al Bone Res. 2018, 6, 15.
What is Rheumatoid Arthritis?

Treatment

Second line: promote remission

Disease-modifying anti-rheumatic drugs (DMARDs)

No cure for rheumatoid arthritis so far

B-cell depletion and inhibition antibodies

Methotrexate

B-cell depletion and inhibition antibodies

Gun, Q.; et al Bone Res. 2018, 6, 15.
Outline

What is Rheumatoid Arthritis

Citrullination & Citrullinated Peptides

Anti-CP antibodies (ACPA’s)

Potential Therapeutic Targets
What is Rheumatoid Arthritis?

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**Yes**

definite RA

Start methotrexate

**No**

non-RA

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Citrullination and Citrullinated Peptides

anti-citrullinated peptide/protein antibodies (ACPA)

- 95~98% specificity to RA patients
- precede onset of clinical symptoms by years
- related to more severe and erosive phenotype


Citrullination and Citrullinated Peptides

**history**

Citrullination and Citrullinated Peptides

Citrullination and Citrullinated Peptides

citrullinated peptides & RA

Prof. Walther J. Van Venrooij

Swiss-Model: 4PCW
Citrullination and Citrullinated Peptides
citrullinated peptides & RA

Prof. Walther J. Van Venrooij

Citrullination and Citrullinated Peptides
citrullinated peptides & RA

Prof. Walther J. Van Venrooij

Citrullination and Citrullinated Peptides
citrullinated peptides & RA

Collected sera from 288 patients
134 RA patients, 154 controls

Blot contains fliggarin

Citrullinated peptides competitively
binds to IgG over fliggarin

Citrullination and Citrullinated Peptides

\[ \text{cfc1} \rightarrow \text{Arg}_{312} \rightarrow \text{Cit} \]

Is fliggarin the only CP associated with RA?

Citrullination and Citrullinated Peptides
hypercitrullination in RA patients

Protein citrullination probed across 12 RA patients synovial tissues and SF
Hypercitrullination seen across almost all

Citrullination and Citrullinated Peptides
scope of citrullinated proteins

>100 citrullinated proteins identified

Keratin

Fibrinogen

Collagen

How does hypercitrullination occur?

RCSB PDB: 6EC0; 1LWU; 5CVA
Citrullination and Citrullinated Peptides
Citrullination by PAD

Peptidylarginine deiminase (PAD)

Citrullination and Citrullinated Peptides
Citrullination by PAD

The PAD Family

PAD2
- expressed in: skeletal muscles, brain, spleen, secretory glands, macrophages
- Regulated by Ca^{2+} and partially regulated by hormones

PAD4/5
- expressed in: white blood cells
- resides in cell nucleus
- citrullinates histones

Citrullination and Citrullinated Peptides
Citrullination by PAD

**positively charged**

\[
\begin{align*}
\text{HN} & \quad \text{H}_2\text{N} \\
\text{HN} & \quad \text{O}
\end{align*}
\]

**neutral**

\[
\begin{align*}
\text{HN} & \quad \text{H}_2\text{N} \\
\text{HN} & \quad \text{O}
\end{align*}
\]

Irreversible?

Ca\(^{2+}\), reductive environment

Peptide arginine

PAD

Peptide citrulline

Citrullination and Citrullinated Peptides
Citrullination by PAD

Citrullination and Citrullinated Peptides
Citrullination by PAD

positively charged

\[
\begin{array}{c}
\text{HN} \\
\text{H} \\
\text{N} \\
\text{NH}_2
\end{array}
\]

neutral

\[
\begin{array}{c}
\text{HN} \\
\text{H} \\
\text{N} \\
\text{O}
\end{array}
\]

irreversible?

Ca\(^{2+}\), reductive environment

PAD activity Ca\(^{2+}\) threshold: 10 μM

cytosolic Ca\(^{2+}\): 0.1 μM

peptide arginine

peptide citrulline

Citrullination and Citrullinated Peptides
Citrullination by PAD

positively charged

irreversible?

Ca²⁺, reductive environment

PAD

neutral

peptide arginine

peptide citrulline

How can PAD be activated?
Why do we find PAD extracellularly?

Citrullination and Citrullinated Peptides
hypercitrullination in RA patients

Protein citrullination probed across 12 RA patients synovial tissues and SF
Hypercitrullination seen across almost all
Citrullination and Citrullinated Peptides
Citrullination by PAD

PR3: neutrophil marker

CD14: monocyte marker

BID: marker for apoptosis activated through extracellular activation

Caspase-3 cleavage: indication of apoptosis

Citrullination and Citrullinated Peptides
Citrullination by PAD

Different apoptotic pathways

Granzyme B/perforin  Spontaneous  UVR  NETosis

Hypercitrullination

Citrullination and Citrullinated Peptides
Citrullination by PAD

Different apoptotic pathways

- Granzyme B/perforin
- Spontaneous
- UVR
- NETosis

\[ \text{Ca}^{2+} \]

Hypercitrullination

Citrullination and Citrullinated Peptides
Citrullination by PAD

Different apoptotic pathways

Granzyme B/perforin  Spontaneous  UVR  NETosis

Ca^{2+}

PAD

Hypercitrullination

Citrullination and Citrullinated Peptides
Citrullination by PAD

Different apoptotic pathways

Granzyme B/perforin

Spontaneous

UVR

NETosis

$\text{Ca}^{2+}$

knockout

$\text{PAD}$

Hypercitrullination

Citrullination and Citrullinated Peptides
Citrullination by PAD

Granzyme B/perforin

$\text{Ca}^{2+}$

PAD

$\text{knockout}$

Hypercitrullination

Citrullination and Citrullinated Peptides
Citrullination by PAD

serine protease

Granzyme B/perforin

Ca^{2+}

PAD

pore forming

knockout

Hypercitrullination

Citrullination and Citrullinated Peptides
Citrullination by PAD

perforin

Ca$^{2+}$

PAD

Hypercitrullination

Citrullination and Citrullinated Peptides
Citrullination by PAD

Membrane lesion induced Ca\(^{2+}\) influx is most responsible for hypercitrullination

Anti-Citrullinated Peptide/Protein Antibodies

ACPA

- 95~98% specificity to RA patients
- precede onset of clinical symptoms by years
- related to more severe and erosive phenotype

Anti-Citrullinated Peptide/Protein Antibodies

ACPA evolves overtime to become less specific

**promiscuous ACPA**
- No specific function
- Recognize different CP’s

**private ACPA**
- Target specific CP’s
- May be arthritogenic

ACPA recognizes >100 citrullinate proteins

Anti-Citrullinated Peptide/Protein Antibodies

general immune response pathways

Inducers
- Infection
- Tissue damage

Sensors
- Macrophage
- Dendritic cell
- Mast cell
- Neutrophil

How to restrain the excessive activation of immune cells?

How to relieve necro-inflammation and promote tissue repair?

How to clear the pro-inflammatory mediators and inhibit their signal transduction?

Targeting tissues

Mediators
- Cytokines: e.g., TNF, IL-6, IL-1
- Chemokines: e.g., CCL-2, CXCL8
- Autacoids: e.g., histamine, eicosanoids, bradykinin
Anti-Citrullinated Peptide/Protein Antibodies
macrophage activation

- Binds to citrullinated proteins cell surface
- Activates macrophages, monocytes, lymphocytes
  - Increases TNFα (cytokine) production
  - Alternatively, it activates TLR4

Liu, M.; et al. Arthritis & Rheumatism 2010 62, 1213
Anti-Citrullinated Peptide/Protein Antibodies

**neutrophil activation**

- Immune complex binds to Fcγ receptors
- Activates neutrophils
- Neutrophile degranulation, ROS release
- Initiate cytokine & chemokine cascade

Anti-Citrullinated Peptide/Protein Antibodies
Interaction with osteoclast

osteoclast

• Large multinucleated cell
• Causes bone dissolution and absorption
• ACPA promotes osteoclast activation & deffrentiation
• May be independent of antigen binding

Anti-Citrullinated Peptide/Protein Antibodies

Outline

What is Rheumatoid Arthritis

Citrullination & Citrullinated Peptides

Anti-CP antibodies (ACPA’s)

Potential Therapeutic Targets
Potential Therapeutics
peptide inhibition of ACPA

- Tested against sera from 927 RA patients and 461 healthy controls
  - 98% specificity was aimed for
- ACPA were isolated from patients and pre-incubated with fibrinogen
  - Performed anti-CCP2 competitive ELISA
Potential Therapeutics
peptide inhibition of ACPA

a  ACPA pool I

Fibrinogen α chain 573 peptide

84% inhibition
IC$_{50}$ : 59 μM ± 8

b  ACPA pool II

Fibrinogen α chain 573 peptide

50% inhibition
IC$_{50}$ : 548 μM ± 100
Potential Therapeutics
peptide inhibition of ACPA

92% inhibition
$IC_{50}: 28 \mu M \pm 5$

Cyclic citrullinated peptide showed enhanced reactivity
Potential Therapeutics

peptide inhibition of ACPA

[Cit573]fibrinogen(563–583)

SFTI-1
sunflower trypsin inhibitor

ACPA binding epitope

enhanced stability & structural rigidity

fibrinogen

aCCP2 IgG

Potential Therapeutics
peptide inhibition of ACPA

ACPA pool I: 79% inhibition
IC$_{50}$: 20μM

SFTI-1
sunflower trypsin inhibitor

ACPA binding epitope

ACPA pool II: 61% inhibition
IC$_{50}$: 87μM

enhanced stability & structural rigidity

Rheumatoid Arthritis and Citrullinated Proteins

outlook

hypercitrullination

• Is neutrophil apoptosis the cause of hypercitrullination?
• What caused massive apoptosis?

formation of IC

downstream activation of inflammation pathway

ACPA generation
Rheumatoid Arthritis and Citrullinated Proteins

Outlook

• Origin of ACPA?
• Pathogenic?
• Why preced RA?
Rheumatoid Arthritis and Citrullinated Proteins

outlook

hypercitrullination

ACPA generation

formation of IC

downstream activation of inflammation pathway

• More activation pathway?
• Therapeutic target?
Rheumatoid Arthritis and Citrullinated Proteins

outlook

• ACPA is only present in 2/3 of patients
• Biomarkers that predict patients’ response to treatments?

formation of IC

downstream activation of inflammation pathway

ACPA generation

hypercitrullination
