Rheum Renal Conference: Pathogenesis of ANCA
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**Old Classification**

- **Renal limited vasculitis**: glomerulonephritis with no involvement of other organs

- **Microscopic polyangiitis (MPA)**: injury to blood vessels in multiple tissues at the same time, such as kidneys, skin, nerves, and lungs

- **Granulomatosis with polyangiitis (GPA)**: vasculitis accompanied by granulomatous inflammation → often affects the lung, sinuses, nose, eyes or ears

- **Eosinophilic granulomatosis with polyangiitis (EGPA)**: granulomatous polyangiitis and asthma + eosinophilia
Current Classification

- Renal-limited MPO-ANCA vasculitis
- PR3-ANCA granulomatous vasculitis with lung and renal involvement
- MPO-ANCA necrotizing vasculitis with multi-organ involvement
- Pauci-immune, necrotizing glomerulonephritis in setting of (+)MPO-ANCA

Pathogenesis

- How are ANCAs generated
- How do ANCAs cause disease
Natural vs Pathogenic ANCAs

Natural or Non-Pathogenic ANCAs

– Circulating autoantibodies against MPO and PR3 present in healthy individuals

– May have beneficial homeostatic functions

– Pathogenic autoimmunity might arise from dysregulation of homeostatic autoimmunity

Rao et al. World J Cardiol 2015 December 26; 7(12): 829-842
Natural vs Pathogenic ANCAs

- Quantitative or qualitative differences determine AutoAb pathogenicity

- Natural MPO-ANCA vs Pathogenic MPO-ANCA
  - lower titres
  - lower avidity
  - less subclass diversity
  - less capability to activate neutrophils in vitro

- Roth et al. identified > 20 MPO epitopes recognized by MPO-ANCA in patients with ANCA-associated disease

Pathogenic ANCA- Genesis

Putative Immunogenic Mechanisms
- Autoantigen Complementarity
- Molecular Mimicry

Genetic susceptibility: HLA specificities

HLA Specificities

- GWAS: revealed HLA associations for both PR3-ANCA and MPO-ANCA disease
- Strongest genetic associations were with the antigentic specificity of ANCA for PR3 versus MPO
  - Anti-proteinase 3 ANCA a/w HLA-DP and the genes encoding α1 - antitrypsin (SERPINA1) and proteinase 3 (PRTN3)
  - Antimyeloperoxidase ANCA a/w HLA-DQ
- HLA DRB1*15 allele is over-represented in African American patients with PR3-ANCA-associated disease (odds ratio 73.3)

Lyons et al NEJM 2012. 367:214–223
Auto-Ag Complementarity

Figure 4 | Diagram of the induction of an ANCA-mediated autoimmune response by an endogenous source of an ANCA antigen peptide. The diagram illustrates the process where sense transcription leads to the production of a sense DNA strand, which in turn leads to the production of an ANCA antigen peptide. Idiotypic antibody and idiootype mimicking the autoantigen are also depicted. Exogenous sources, such as microbes, can also mimic an antisense peptide and interact with T cells and neutrophils, contributing to the autoimmune response.
Auto-Ag Complementarity

1) **Antigen #1** is the antisense peptide (or its mimic) that is complementary to the autoantigen (Antigen #2).

2) **B Cell #1** has specificity for the antisense or complementary **Antigen #1**.

3) **Antibodies #1** are specific for the autoantigen antisense or complementary peptides with idiotopes that are **Antigen #2.1**.

4) **B Cell #2** has specificity for idiotopes on antisense or anti-complementary antibodies (Antibodies #1).

5) **Antibodies #2** are anti-idiotypic antibodies that crossreact with the autoantigen (Antigen #2).

6) **Antigen #2.2** is the autoantigen sense peptide that is complementary to **Antigen #1**.

How do ANCAs cause disease

- **Primed neutrophils (TNF α, Bacterial LPS, C5a)**

- Neutrophils release ANCA target Ag at the cell surface and microenvironment

- **Activation by MPO-ANCA and PR3-ANCA IgG**

- ANCA-activated neutrophils generate a respiratory burst

- **Toxic oxygen radicals**

- Release destructive enzymes through degranulation

- Extrude NETs that have proinflammatory properties
Neutrophil ExtraCellular Traps release (NETosis)

- Active form of neutrophil death that releases a ‘sticky mesh’ of extruded chromatin, nucleic acid and a range of cytoplasmic proteins
- First described by Brinkmann et al in 2004
- Core of NETs are chromatin fibers (≈17 nm in dia) composed of DNA and histones
- Lined by granule-derived antimicrobial proteins viz neutrophil elastase, MPO, cathepsin G, proteinase 3 (PR3), defensins, and cathelicidin LL-37
- NETs target pathogens by a combination of sequestration and highly localized microbicidal activity
Netting neutrophils in autoimmune small-vessel vasculitis

Kai Kessenbrock¹,², Markus Krumbholz¹,³, Ulf Schönermarck⁴, Walter Back⁵, Wolfgang L Gross⁶, Zena Werb², Hermann-Josef Gröne⁷, Volker Brinkmann⁸ & Dieter E Jenne¹
ANCA-induced formation of NETs containing autoantigens PR3 and MPO
In vivo evidence for NET formation in individuals with SVV
Role of NETs in generation of ANCA

Autoreactive T-cells recognise MPO/PR3 with costimulatory signals induced by NETs

Activated T-cells license autoreactive B cells to produce ANCA

ANCA induces further NETosis

APCs pick up MPO and PR3 from NETs

Excessive NET formation in AAV

Neutrophils undergo NETosis and form NETs

Other stimuli of NET formation, i.e. S. aureus

Anti-NET antibodies?

Degradation by DNAses

NETS and Alternate Complement Pathway
Alternative pathway

Factor B
*Glomerular and urine levels of Bb associated with activity [48]*

C3F variant more frequent in AAV in candidate gene studies [43–45]

C3b

C5 convertase

C5

Eculizumab

C5a + C5b

Increased plasma levels of activated components C3a, C5a, Bb in AAV [49]

Further activation of complement by neutrophils *(feedback loop)*

C5aR

*Downstream effects*
- Degranulation
- Oxidative burst
- Enhanced phagocytosis
- Neutrophil chemoattractant
Neutrophil extracellular traps can activate alternative complement pathways
Detection of deposition of components of alternative complement on NETs in vitro
Immunofluorescence detection for alternative complement pathway activation on NETs
IF detection for alternative complement pathway activation on NETs
NETs induced by ANCA could activate the complement cascade in the serum
Conclusion

NETs released from ANCA-activated neutrophils could activate the alternative complement pathway, and might thus participate in the pathogenesis of ANCA associated vasculitis.
Putative sequence of pathogenic events in ANCA-mediated vasculitis