

Neutrophils and NETs: A double edged sword

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NET: neutrophil extracellular traps

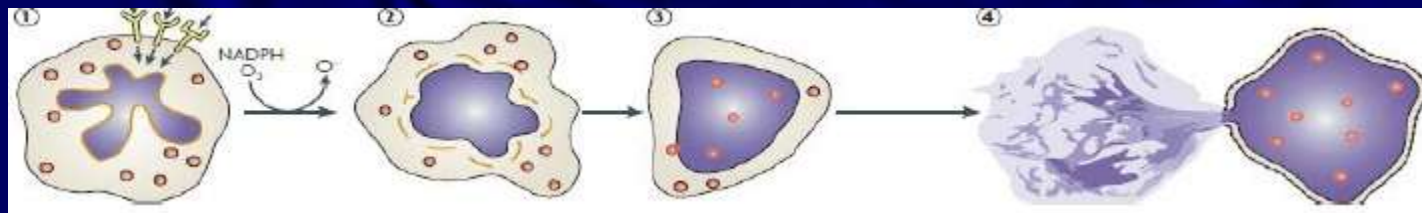
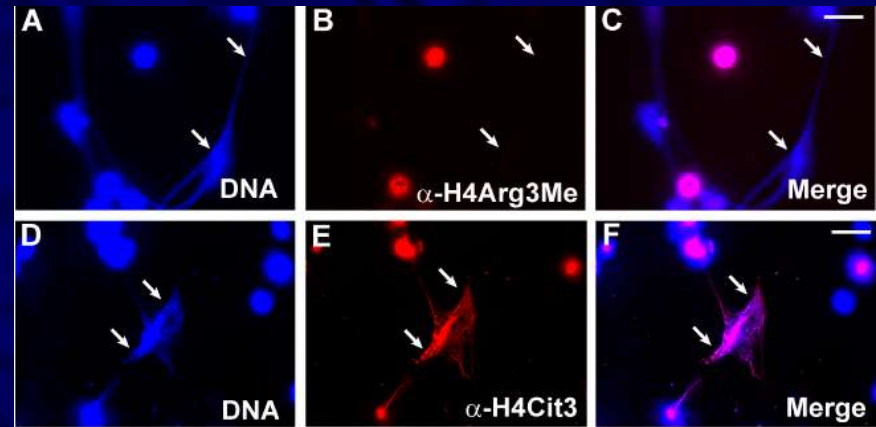
- In neutrophils (also in eosinophils and mast cells) different inflammatory stimuli induce:

Nuclear translocation of PAD4

Histone deimination

Chromatin unfolding

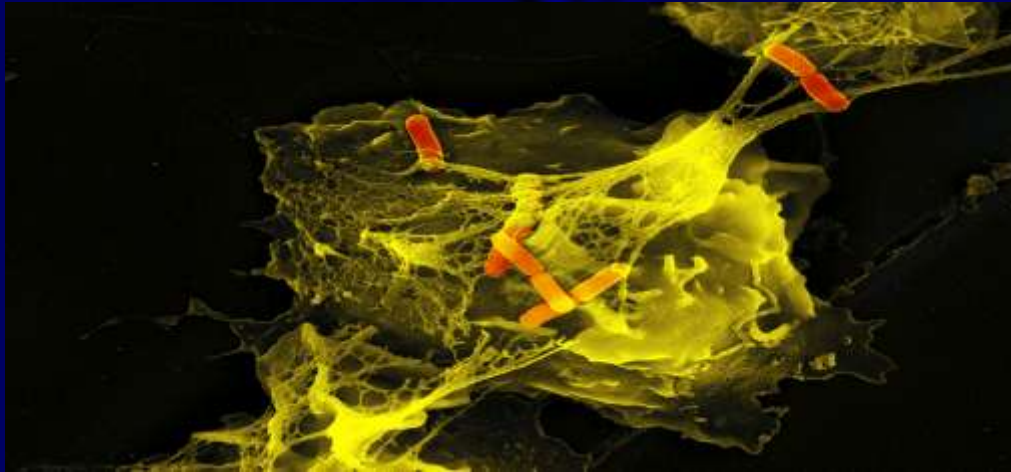
NET formation



- NETs contain DNA, deiminated core histones, anti-microbial peptides and cytoplasmic enzymes

NETs and infections

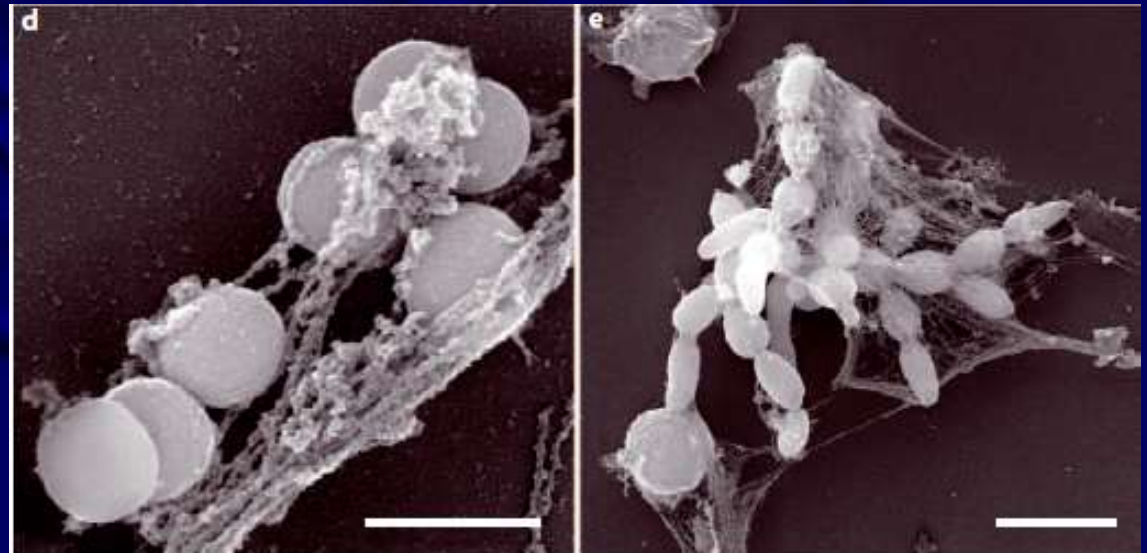
NETs are web like structures that trap and kill pathogens



- NETosis is critical to control infections caused by GRAM+, GRAM- and fungi
- Effector molecules are histones, anti-microbial peptides and proteases

Immunodeficit and NET

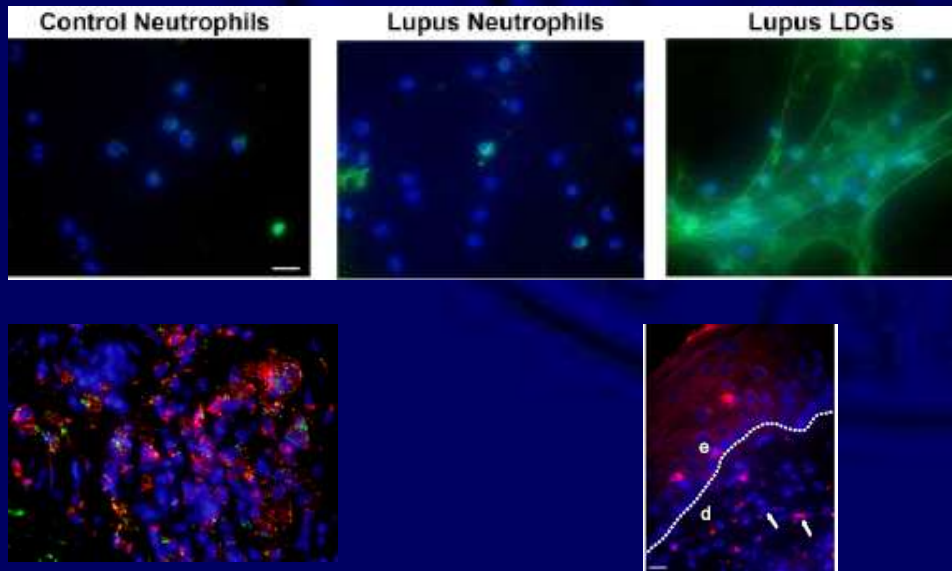
Chronic granulomatous disease: defect in NET formation caused by MPO deficit



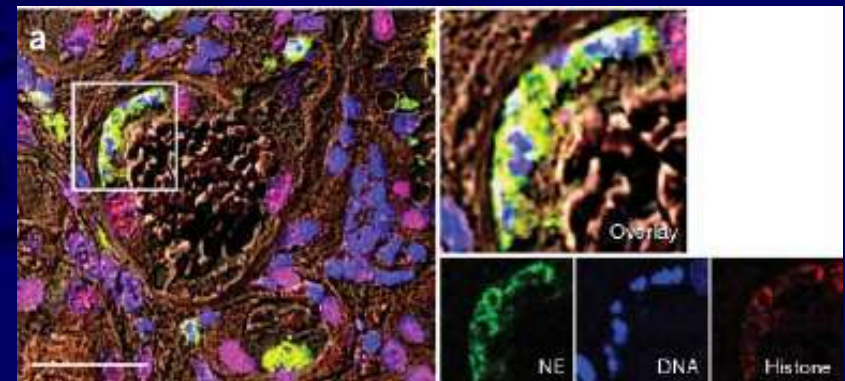
NETs and autoimmunity

- NETs expose autoantigens together with danger signals
- NETs are involved in eliciting an immune response to self antigens
- NETs are responsible for tissue damage in autoimmune disorders

Systemic lupus

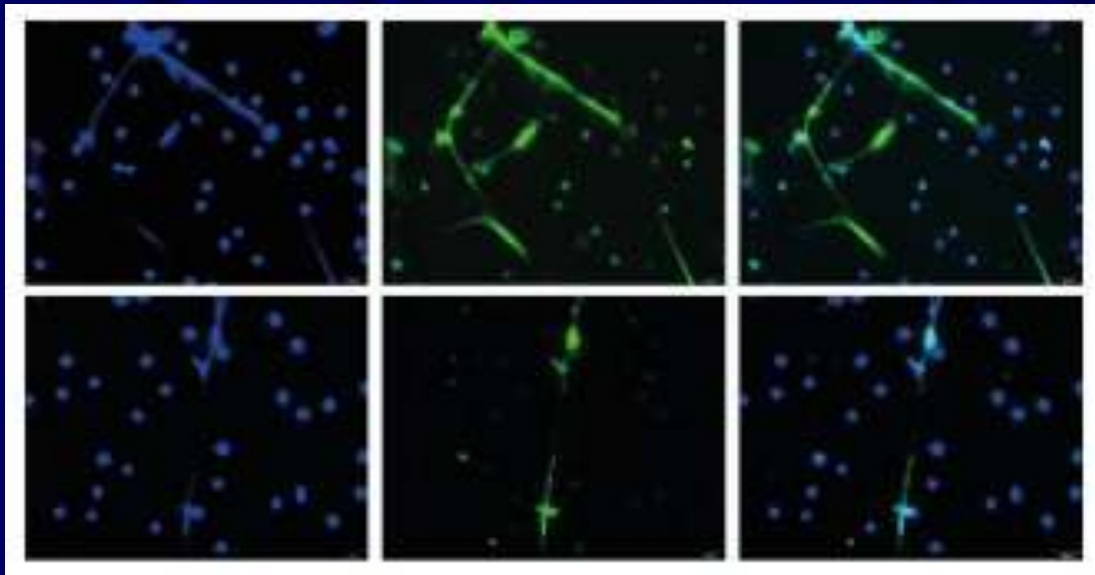


Systemic vasculitis



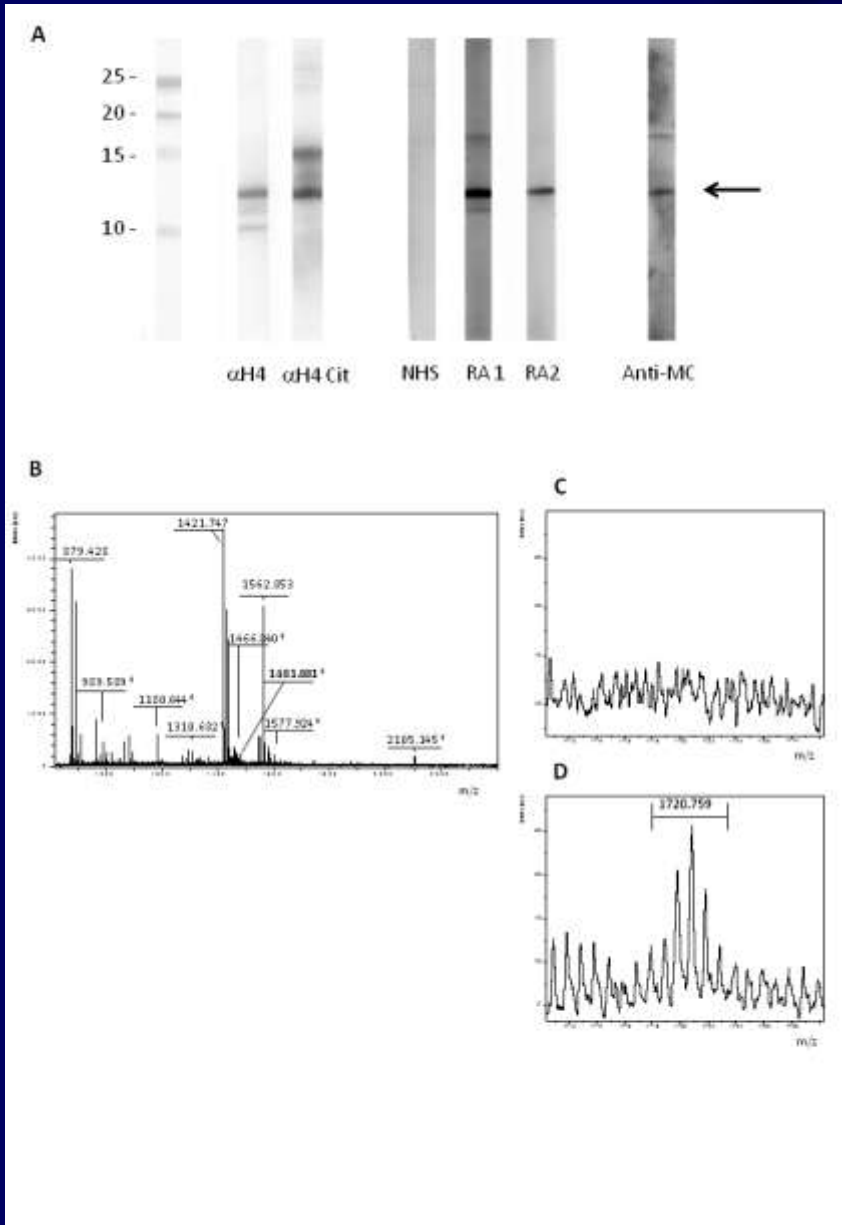
NET and rheumatoid arthritis

- ACPA (anti-citrullinated protein/peptide antibodies) are marker autoantibodies of rheumatoid arthritis, being detected exclusively in RA
- ACPA react with eiminated proteins where arginine has been substituted by citrulline
- Deiminated histones are main constituents of NETs



Corsiero et al, submitted

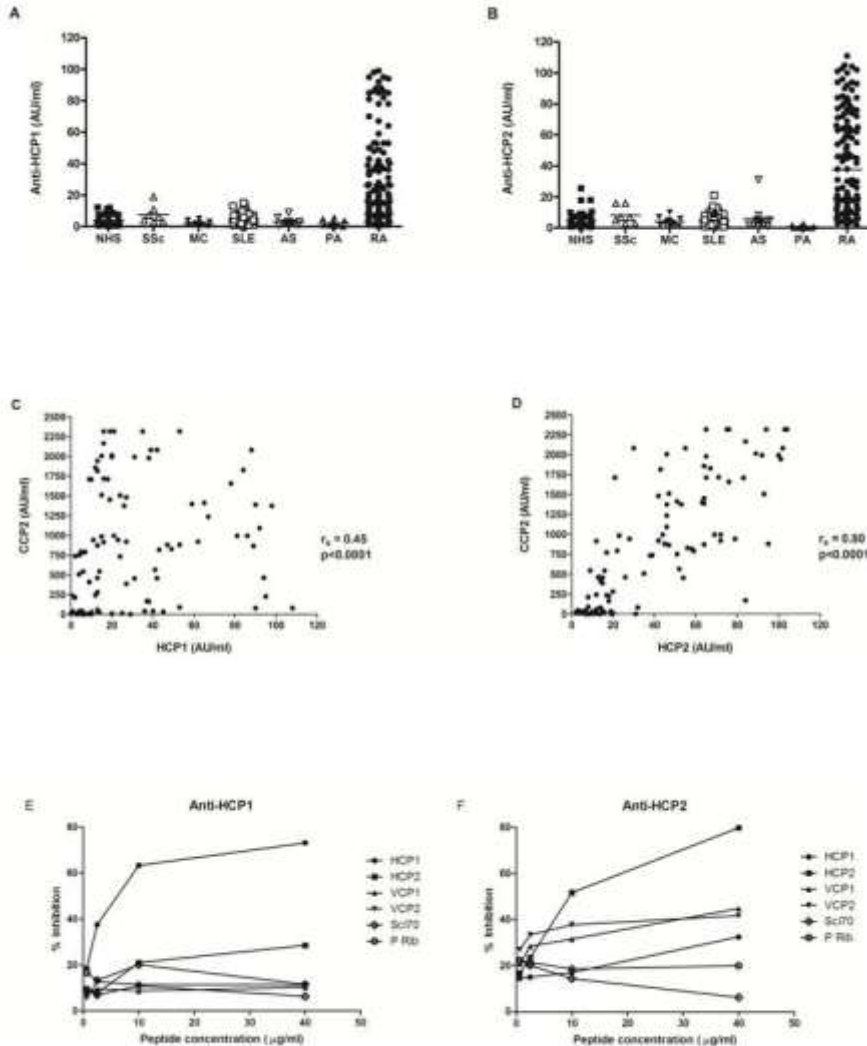
Reactivity with nuclei of activated neutrophils



- Activated neutrophils were lysed in acid; acid precipitated proteins were fractionated on gel, transferred to PVDF and probed with sera
- RA sera react with a band identified by antisera and direct sequencing as H4
- Tryptic digest was treated with antipyrine and butanedione that create an adduct of increased mass on deiminated arginine
- MALDI TOF analysis after derivatization of the digest indicates that H 4 is deiminated (Arg 23 \rightarrow Cit23)

Pratesi, ARD, 2013

Reactivity with H4 deiminated peptides

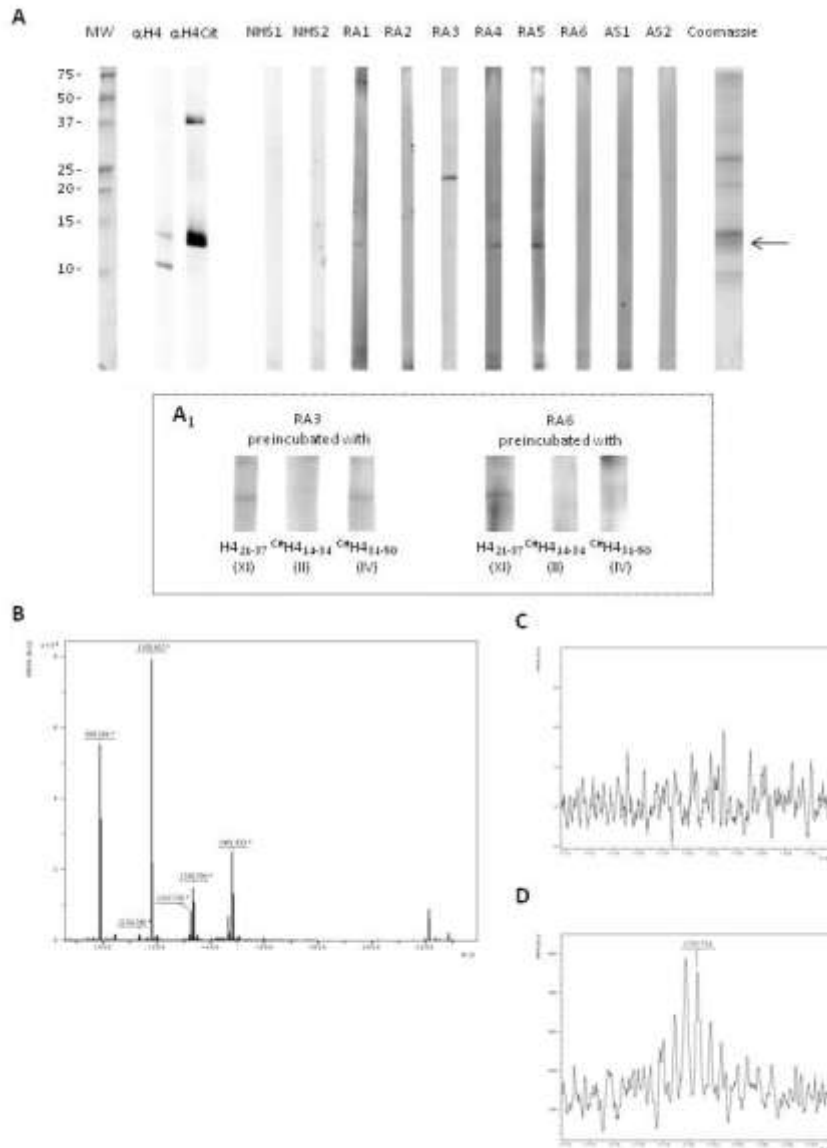


- Deiminated H4 peptides (14-34 and 31-50) are recognised by RA sera and not by disease controls
- Levels of anti-H4 peptide antibodies are correlated with anti-CCP antibodies
- Affinity purified anti-H4 peptide antibodies crossreact with other deiminated peptides

Pratesi, ARD, 2013
Patents EP 10 168 270.6,
EP11167420.6

Reactivity with NET proteins

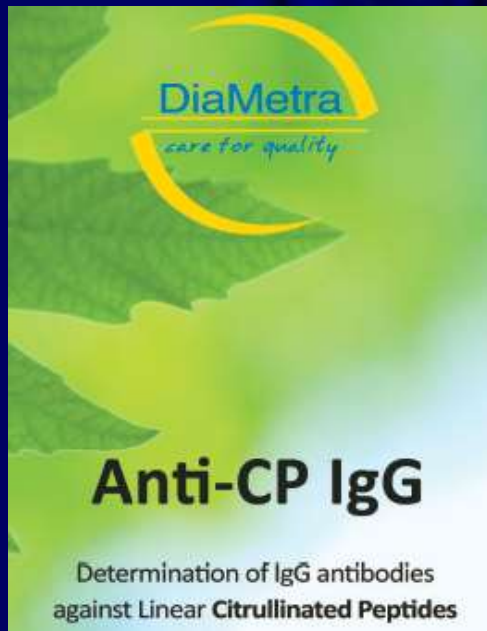
- NET proteins were acid precipitated, fractionated on gel, transferred to PVDF and probed with patient sera
- RA sera bind a protein identified as H4
- The binding is inhibited by preincubation with deiminated H4 peptides
- MALDI TOF analysis after derivatization of the digest indicates that H 4 is deiminated



Pratesi, ARD, 2013

Conclusions

- Autoantibodies from RA patients react with deiminated H4 from activated neutrophils and NETs
- In RA, NETosis exposes autoantigens and may contribute to the induction of ACPA
- Synthetic deiminated H4-derived peptides are a new substrate for ACPA detection



Aknowledgements

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