Neutrophil chromatin deposition in septic lesions

S. Hurtley., *Science* 26 January 2007

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Overview

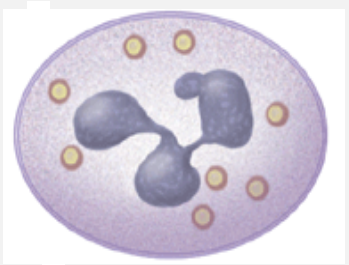
Antimicrobial functions of neutrophils

Neutrophil extracellular traps (NETs) and NETOSIS

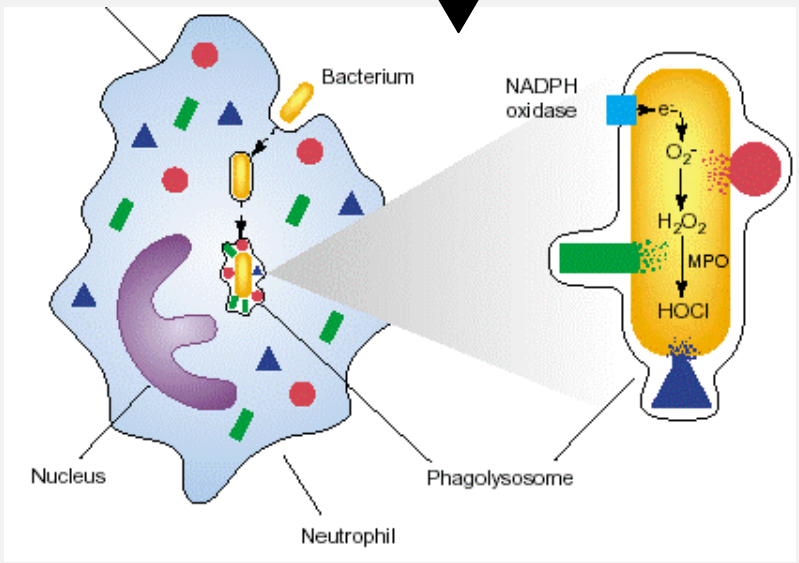
Chromatin deposition in various lesions

Clinical significance of NETs

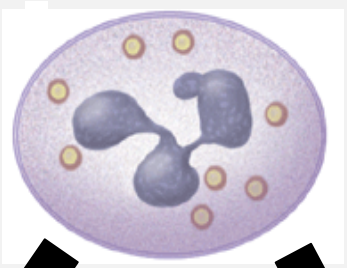
Antimicrobial functions of neutrophils



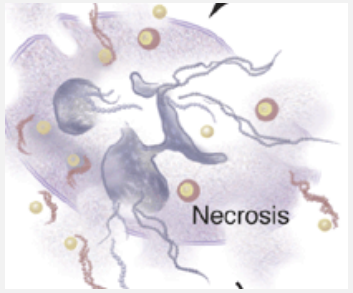
IL-8, LPS



http://edoc.hu-berlin.de/dissertationen/averhoff-petra-2006-05-24/HTML/image002.gif

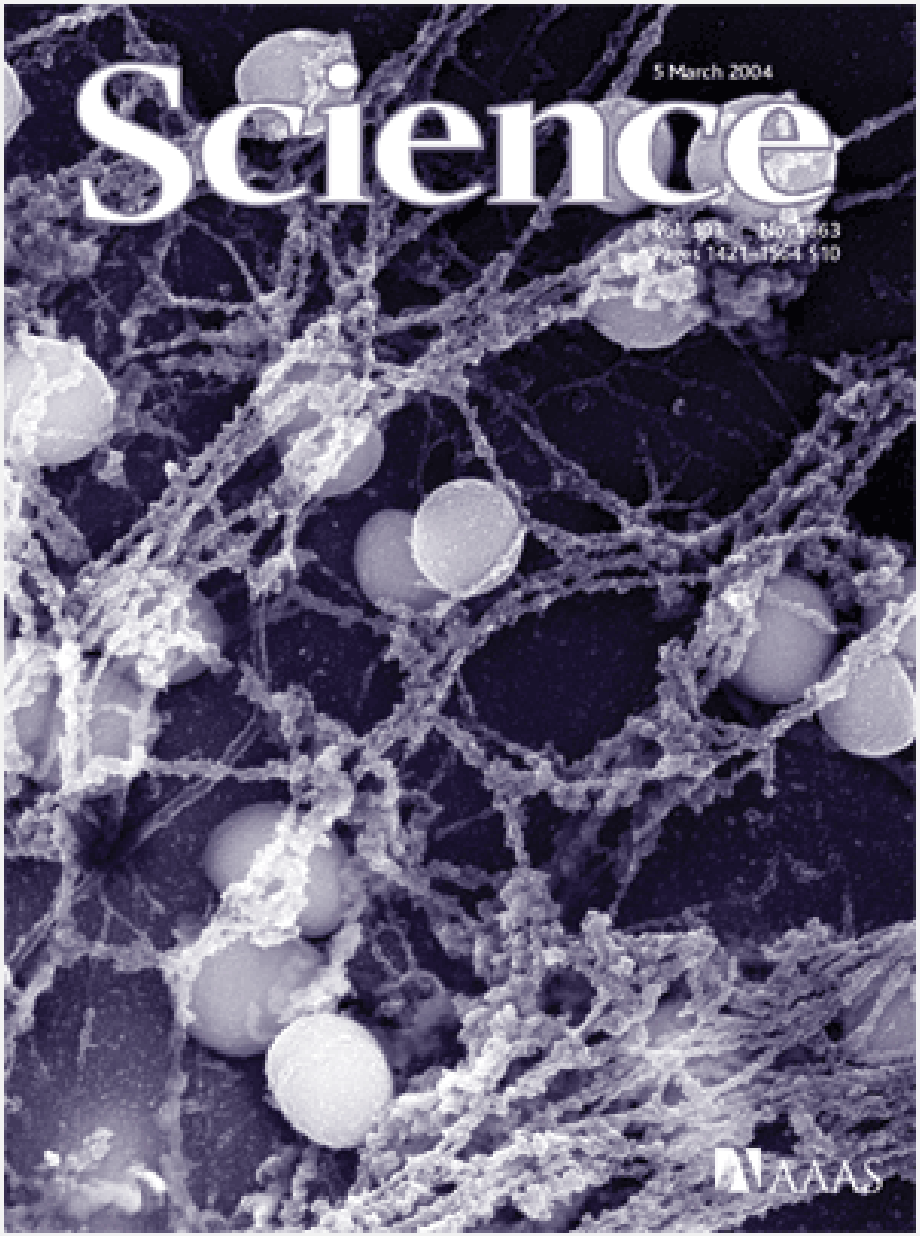


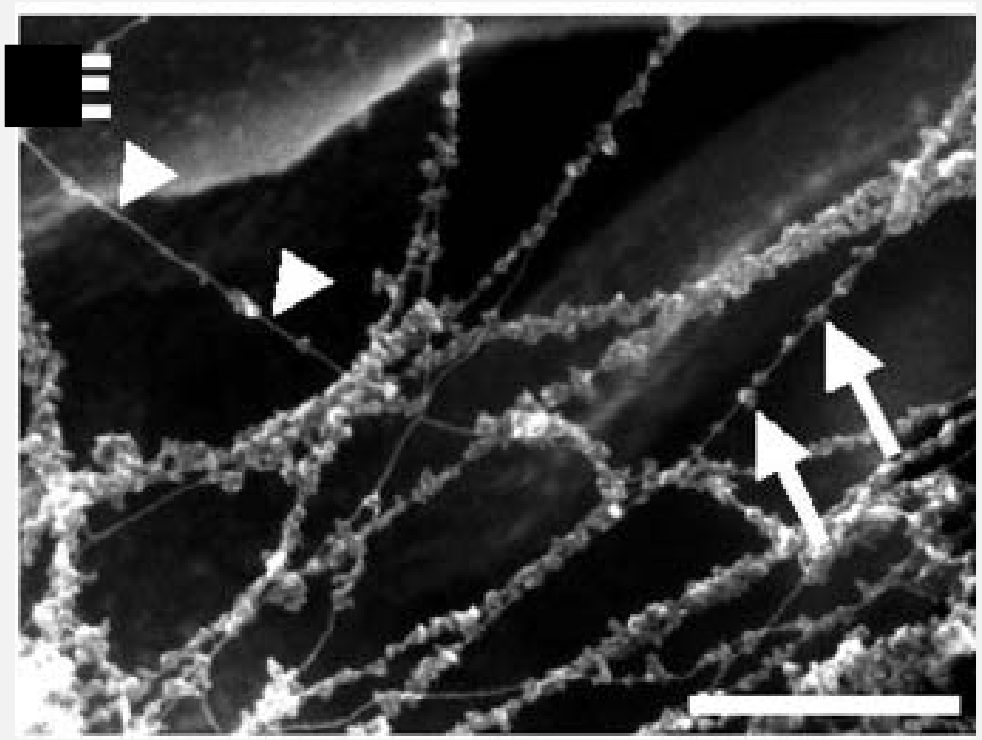
Exocytosis



NETs

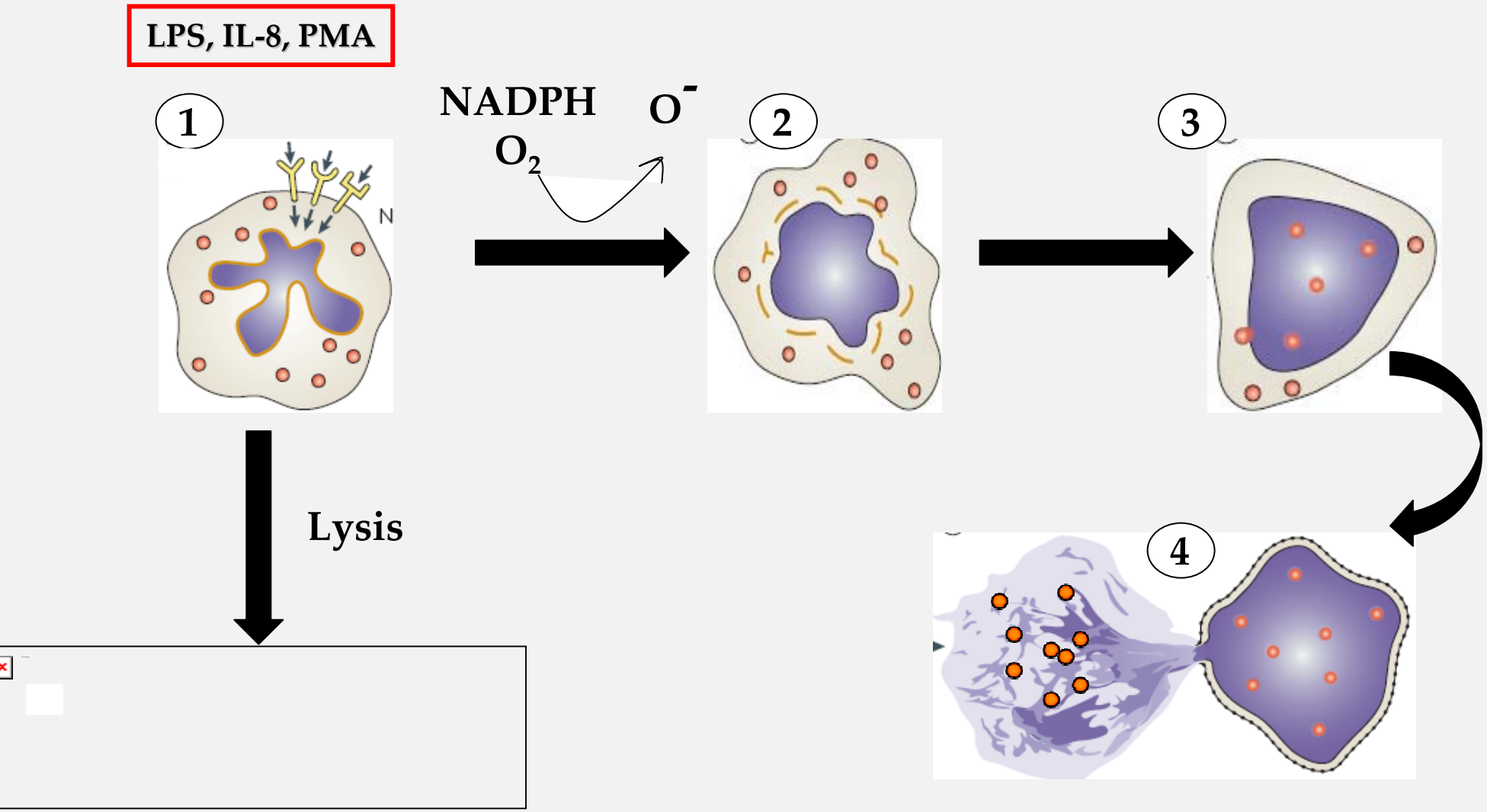
Lee et al., Science 5 March 2004:
Vol. 303. no. 5663, pp. 1477 - 1478





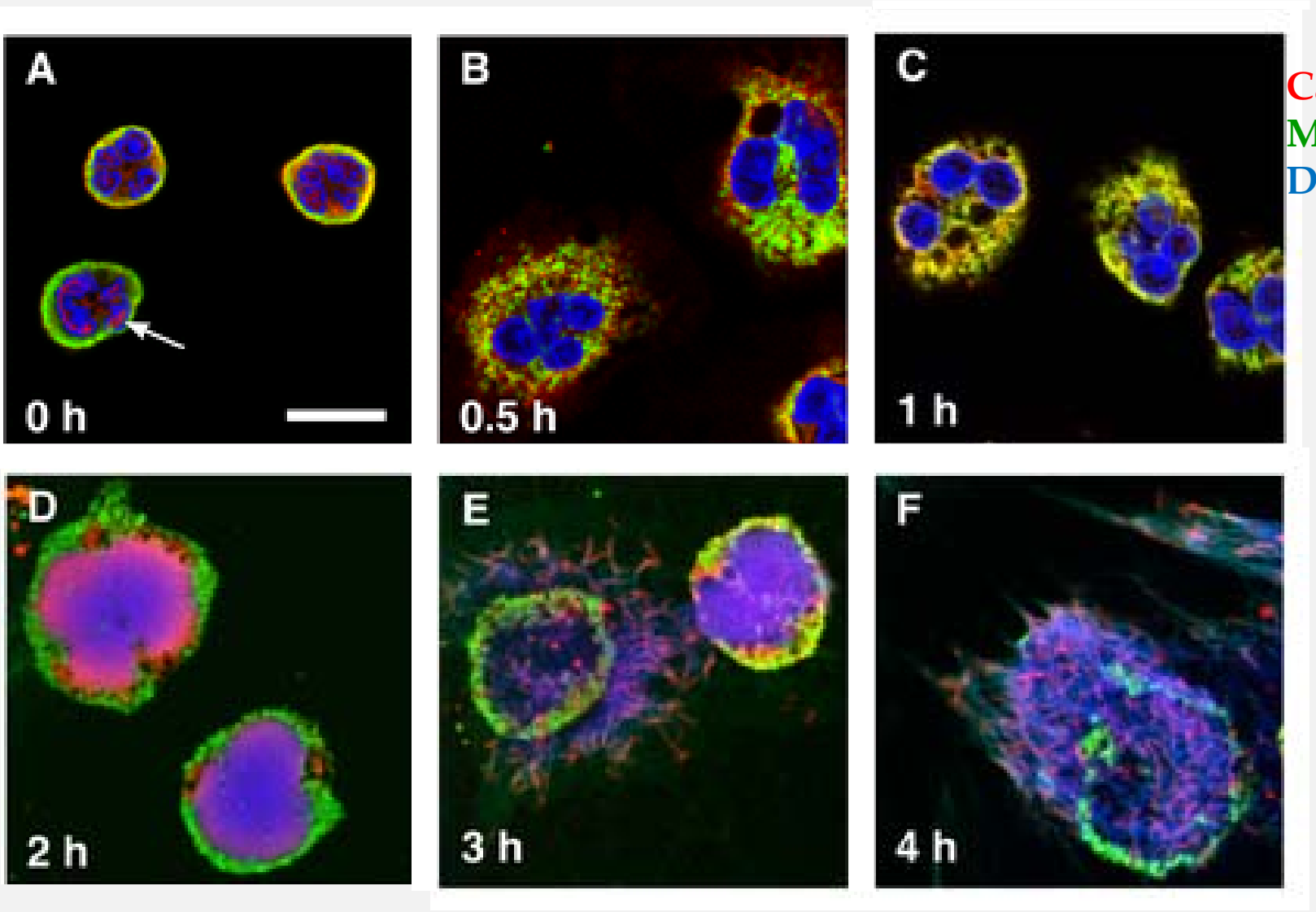
Brickmann et al, Science; Vol. 303. 1532 – 1535-2004

Mechanism of NET formation



Volker et al., Nature Reviews-2007

Visualization ofNETs by confocal microscopy

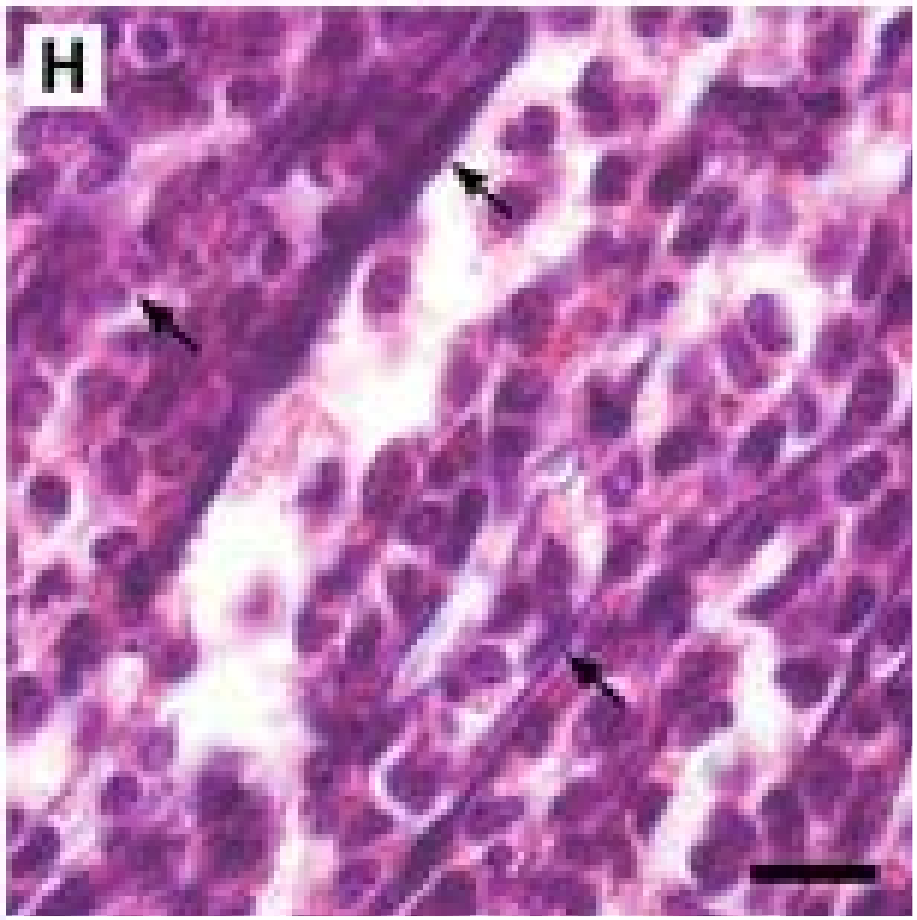


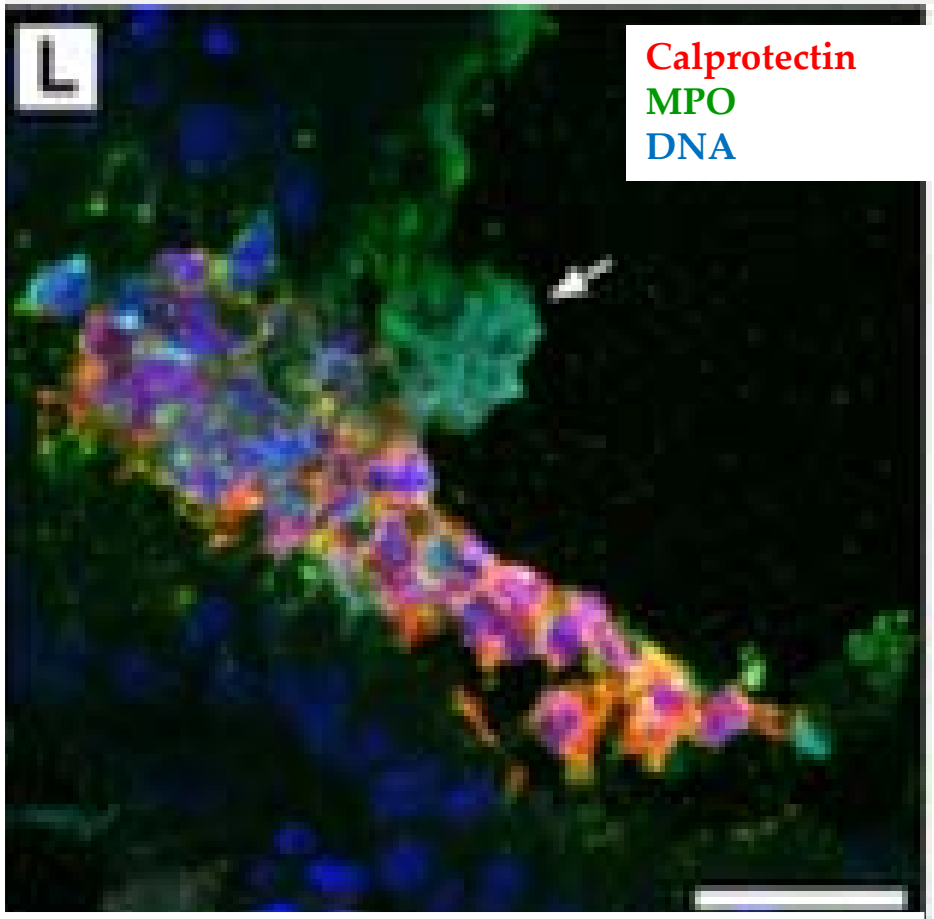
Urban et al., Plos pathology: 2009 Oct;5(10)

Pathological conditions describing NET formation

Condition	Human	Mouse	Cow	Horse	Pig
Appendicitis	+				
Streptococcus pyogenes infection	+				
Fasciitis	+				
Preeclamsia	+				
Small vessel vasculitis	+				
Tuberculosis	+				
Systemic lupus nephritis	+	+			
Pneumonia		+			
Aspergillus infection			+		
Mastitis			+		
Mannheimia infection			+		

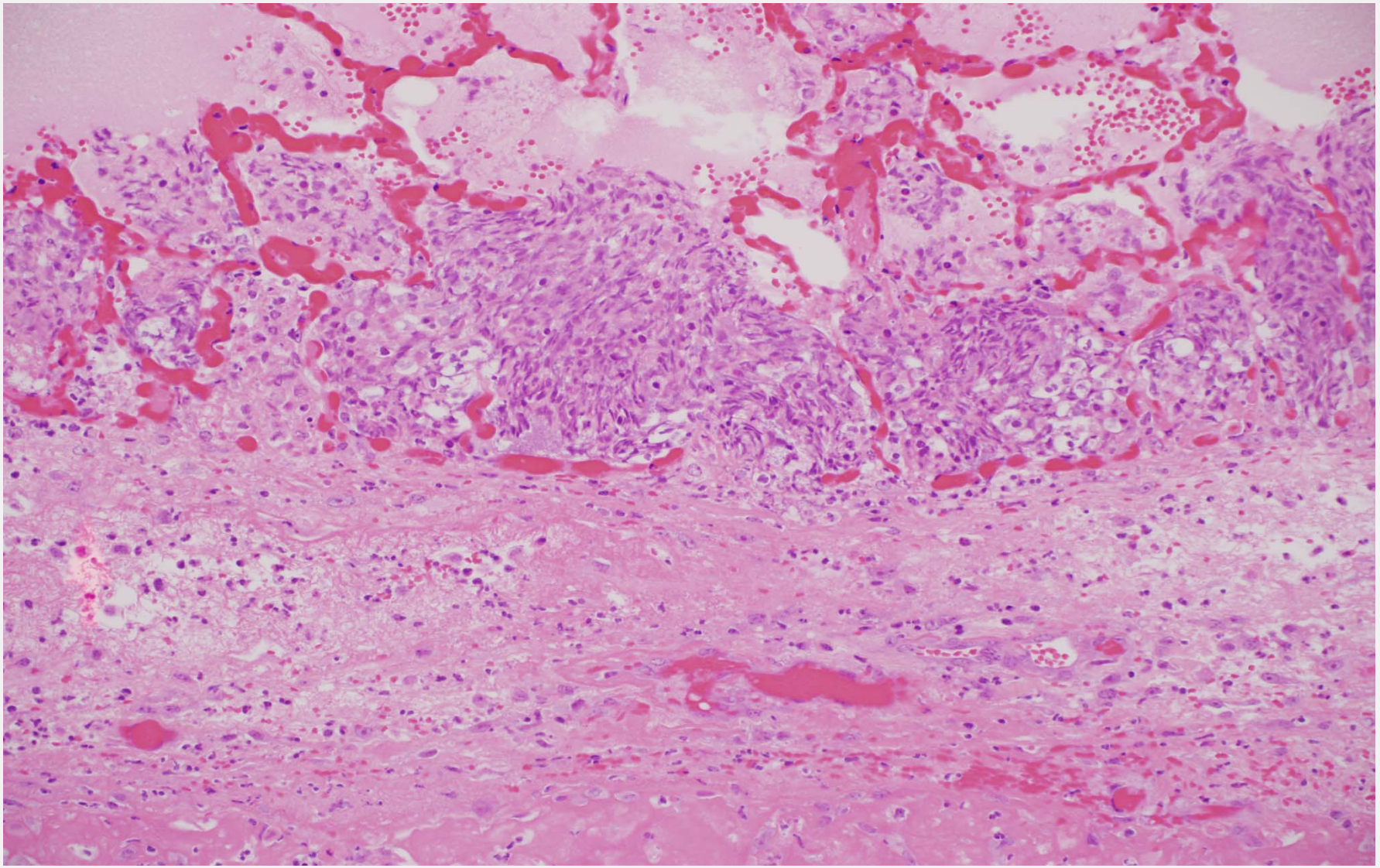
Calprotectin is present in *C. albicans* induced NETs in mouse lungs





Urban et al., Plos pathology: 2009 Oct;5(10)

Chromatin deposition in *Mannheimia haemolytica* infected bovine lung



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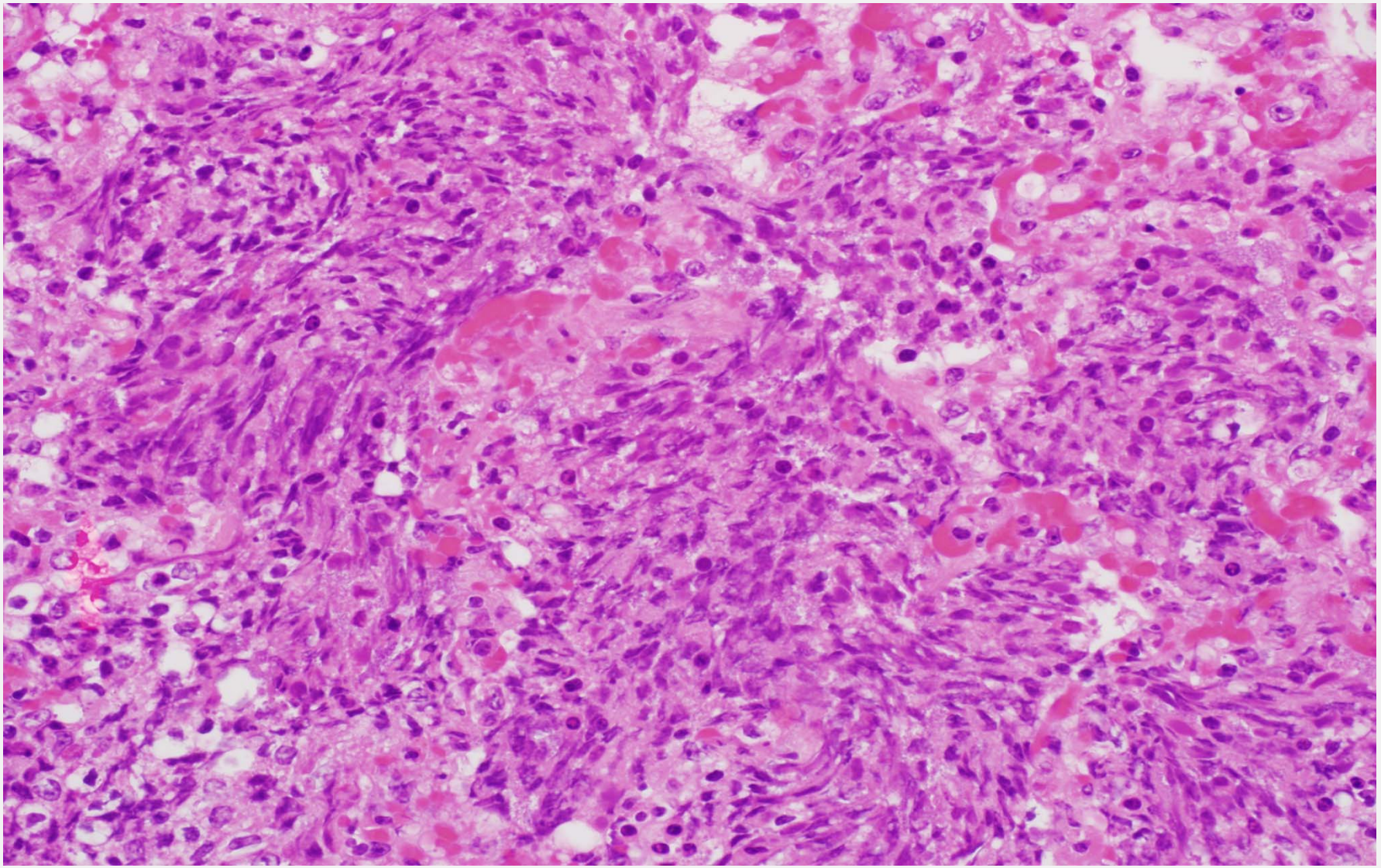
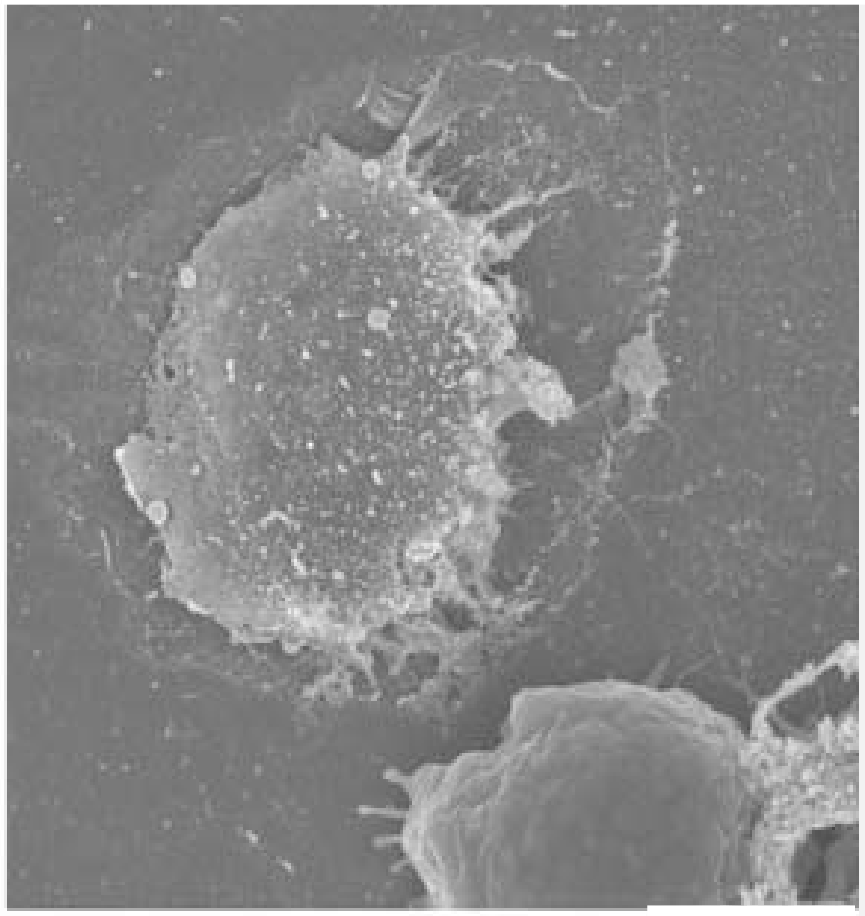
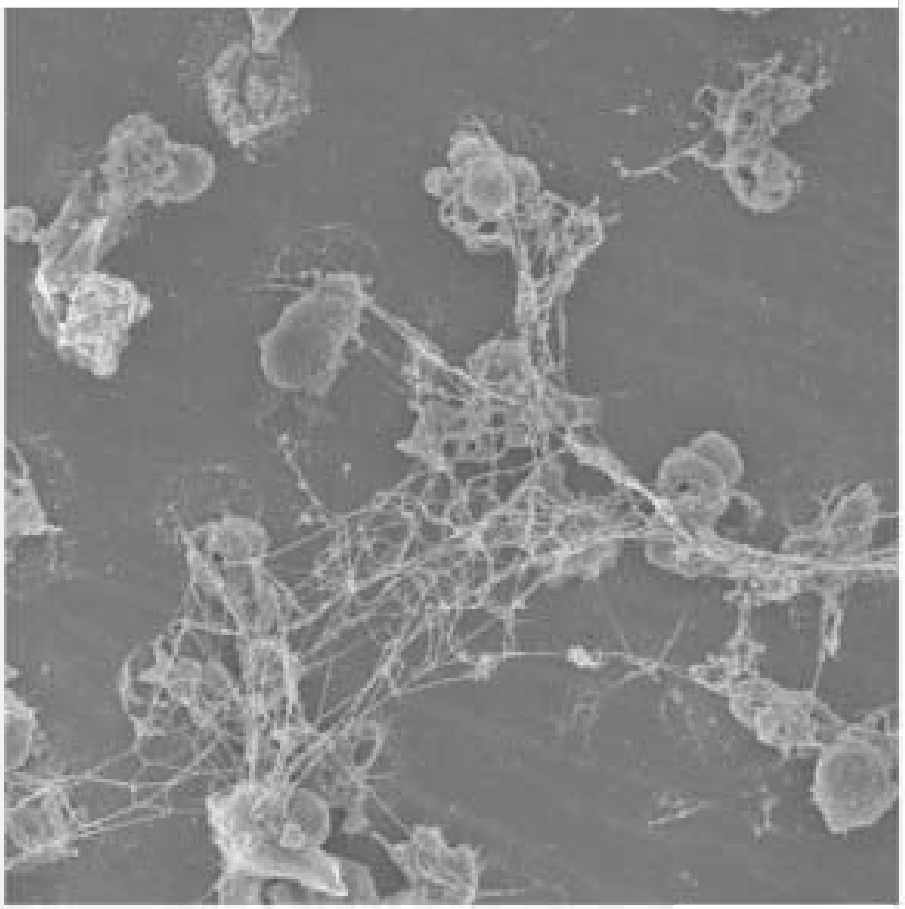


Mannheimia leukotoxin induces NET formation in bovine neutrophils

Aulik, N. A. et al. 2010. Infect. Immun. 78(11):4454-4466

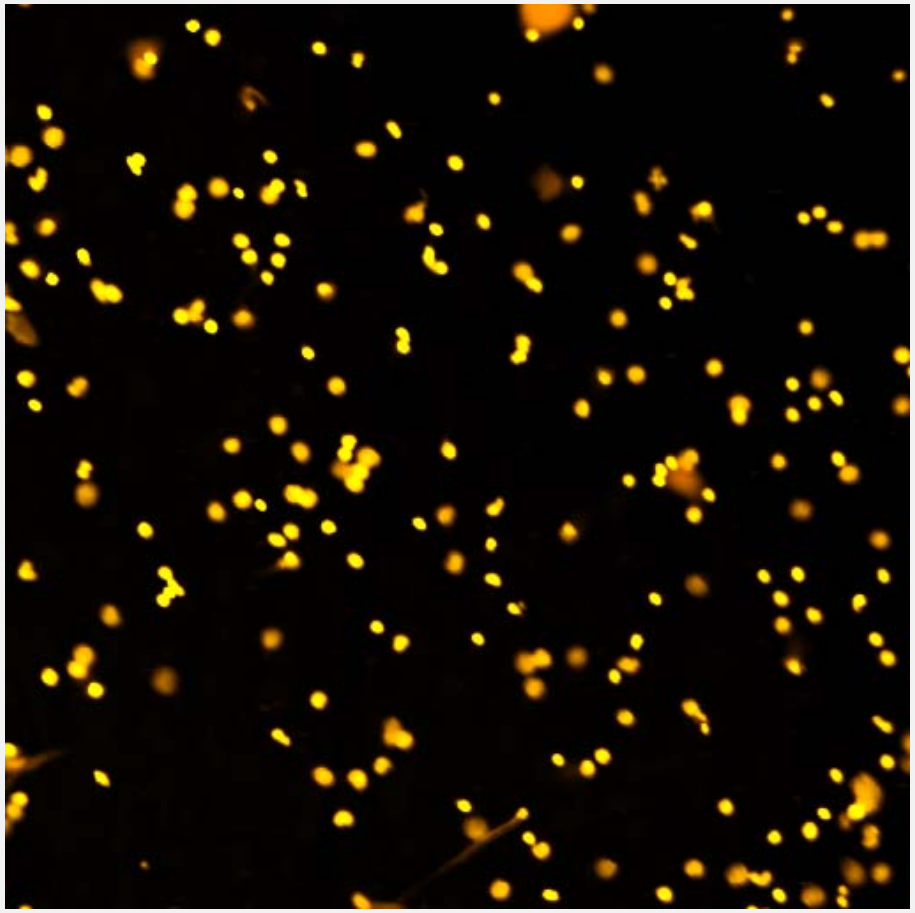


Control



LKT treated

Activated bovine neutrophils form NETs



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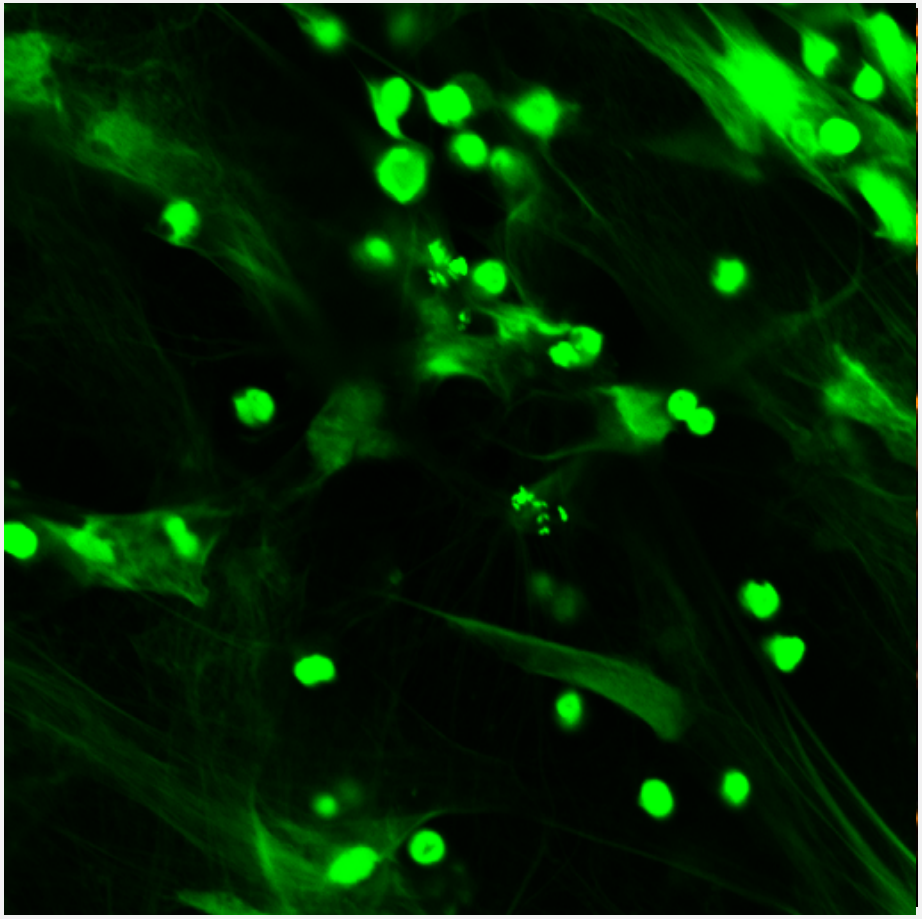
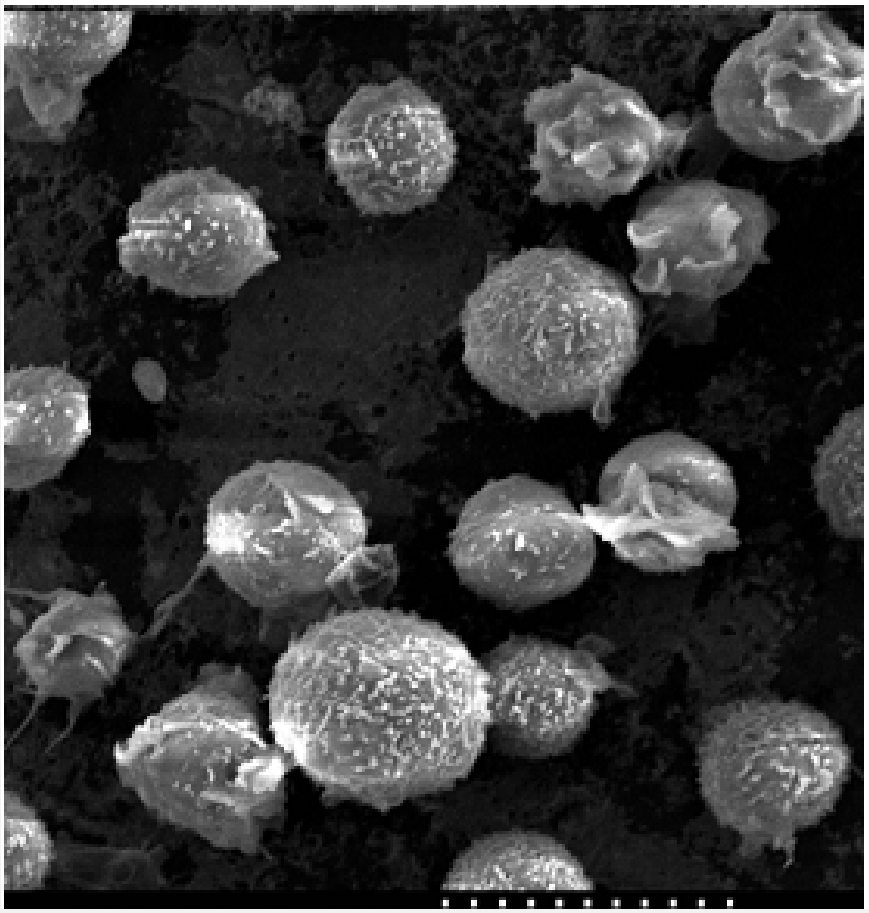


Activated

Activated porcine neutrophils form NETs



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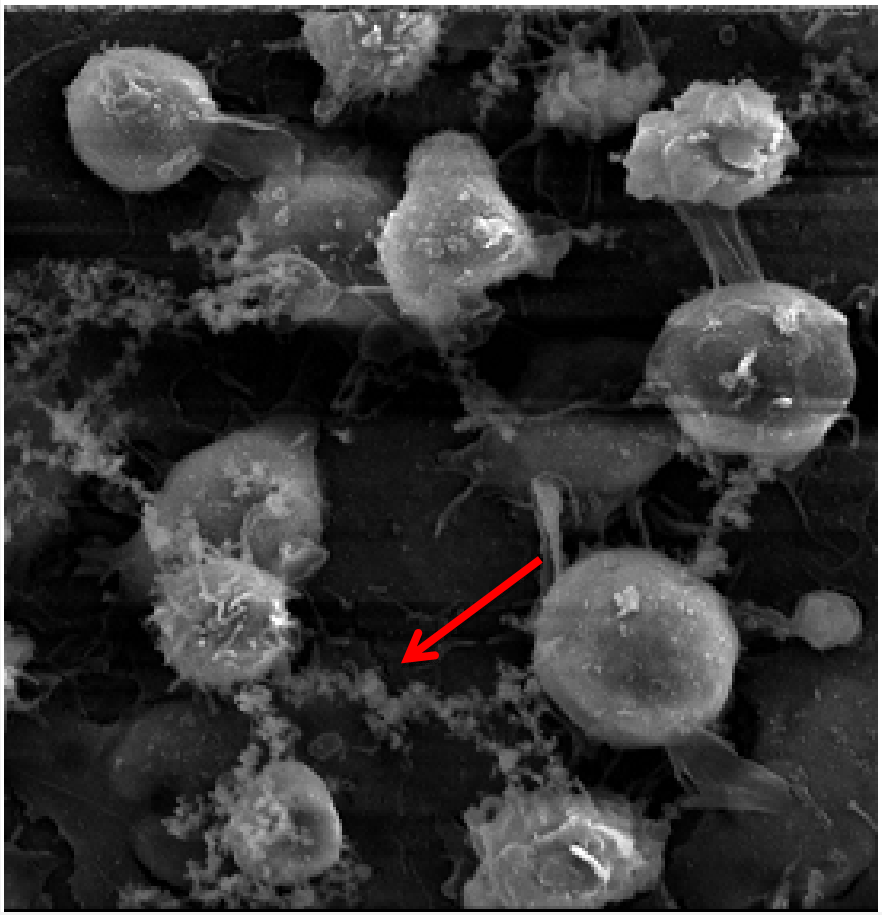
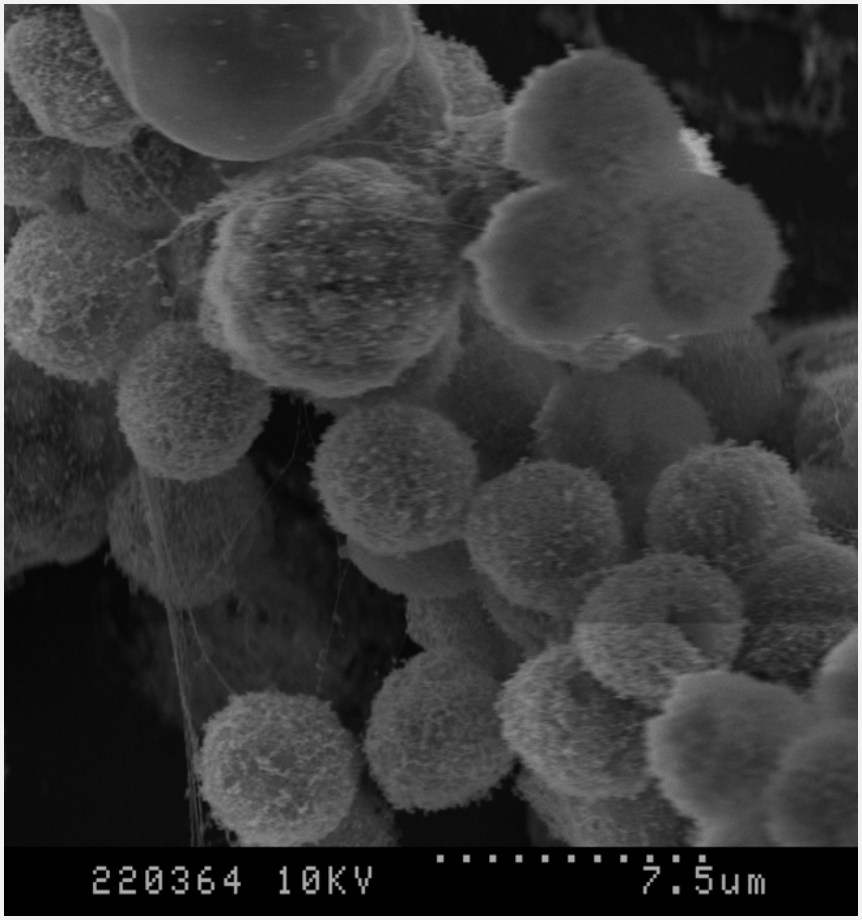


Activated

Sarah Scapinello MSc-Thesis 2009, University of Guelph

Activated equine neutrophils form NET like structures



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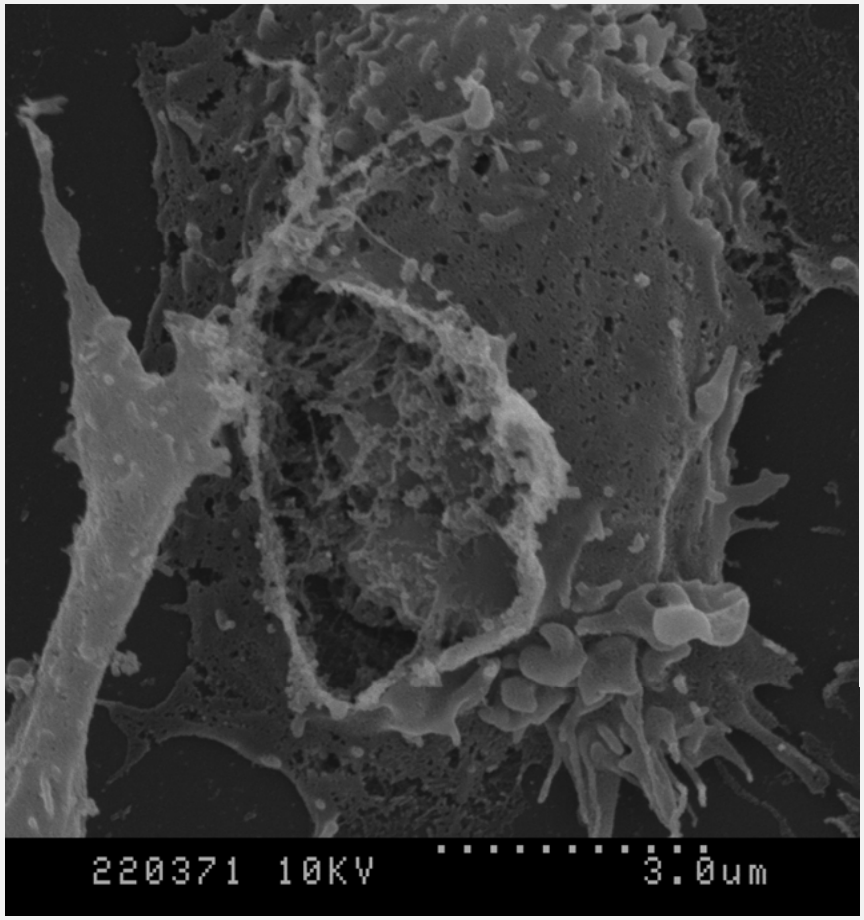
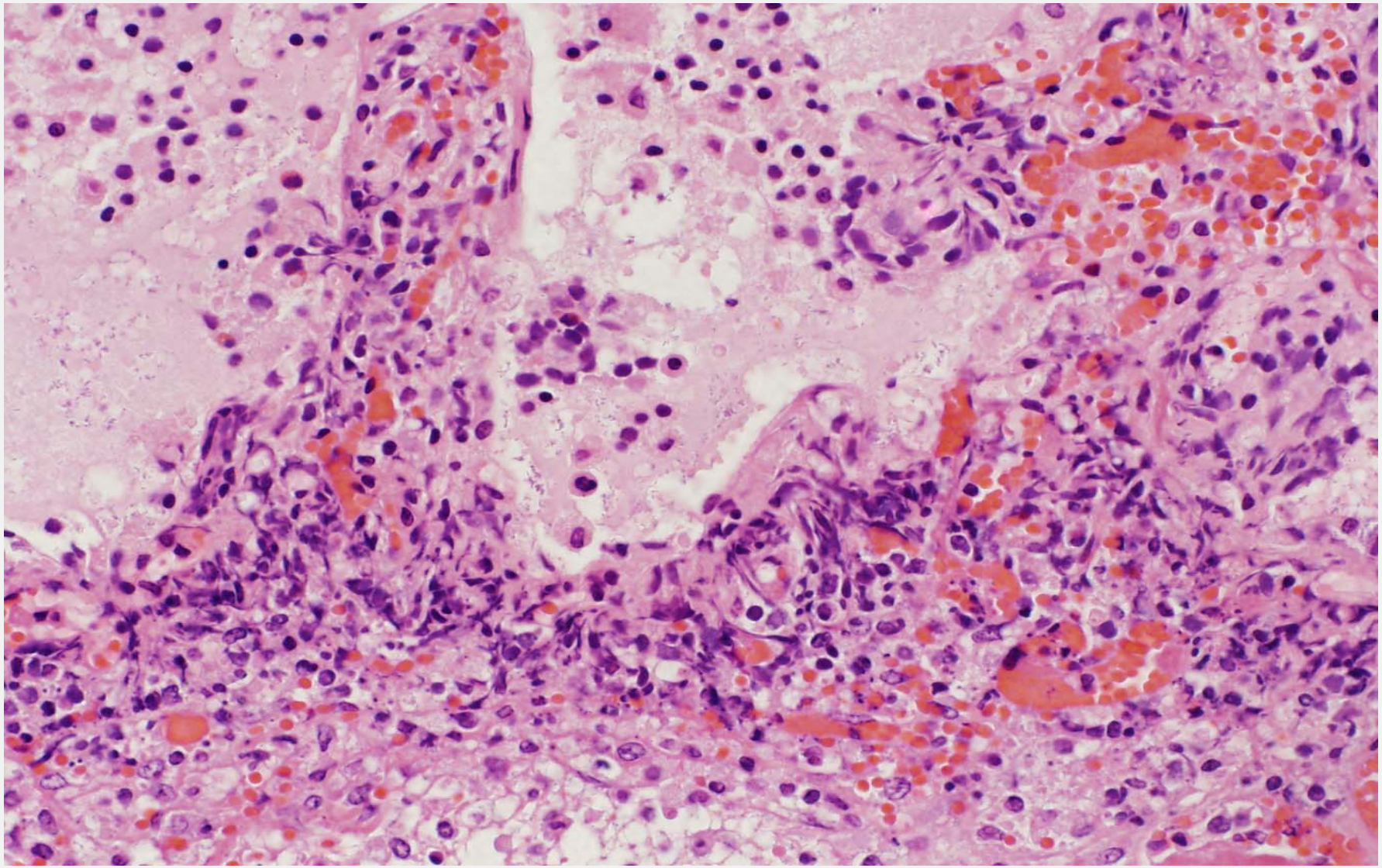
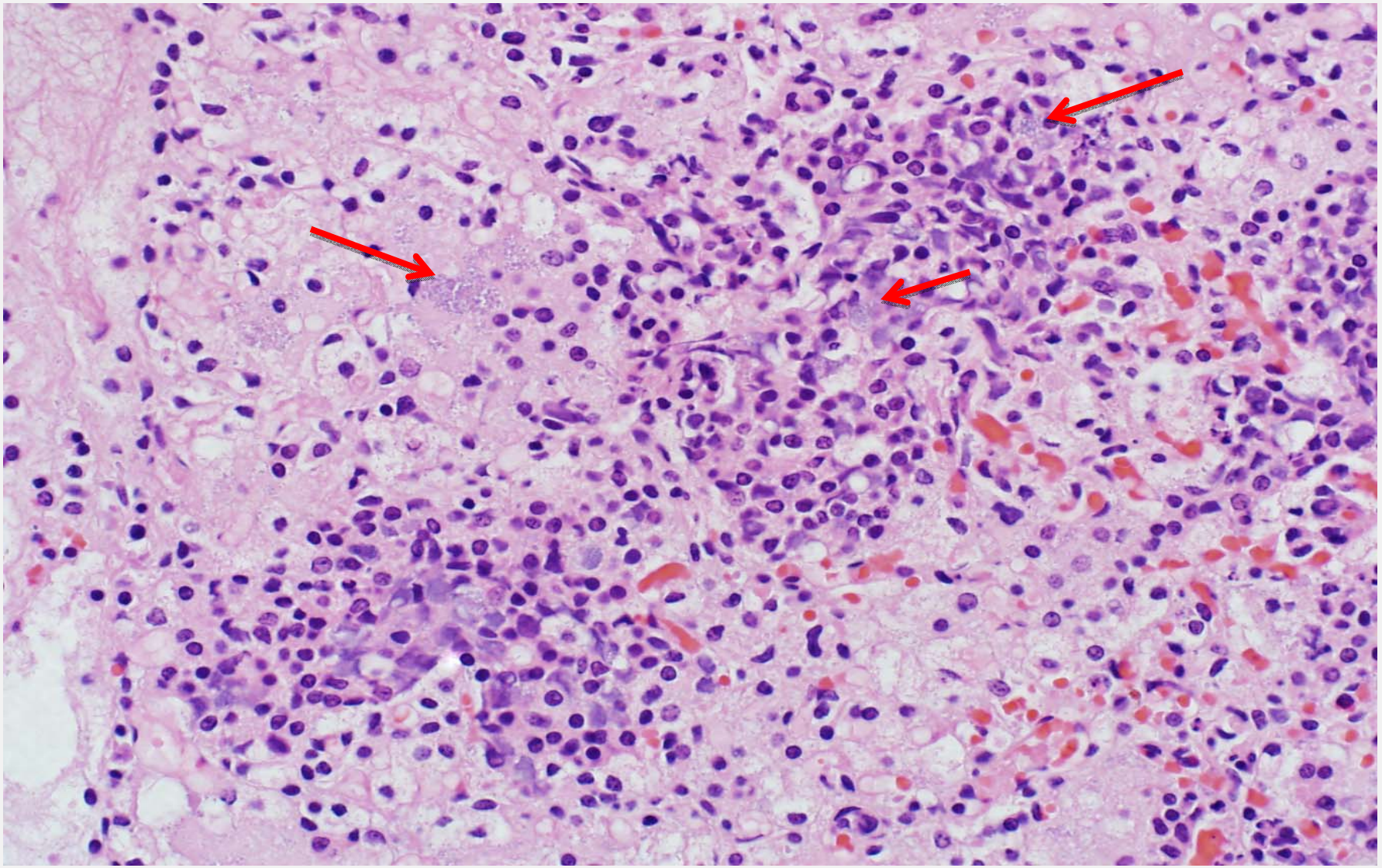


Activated

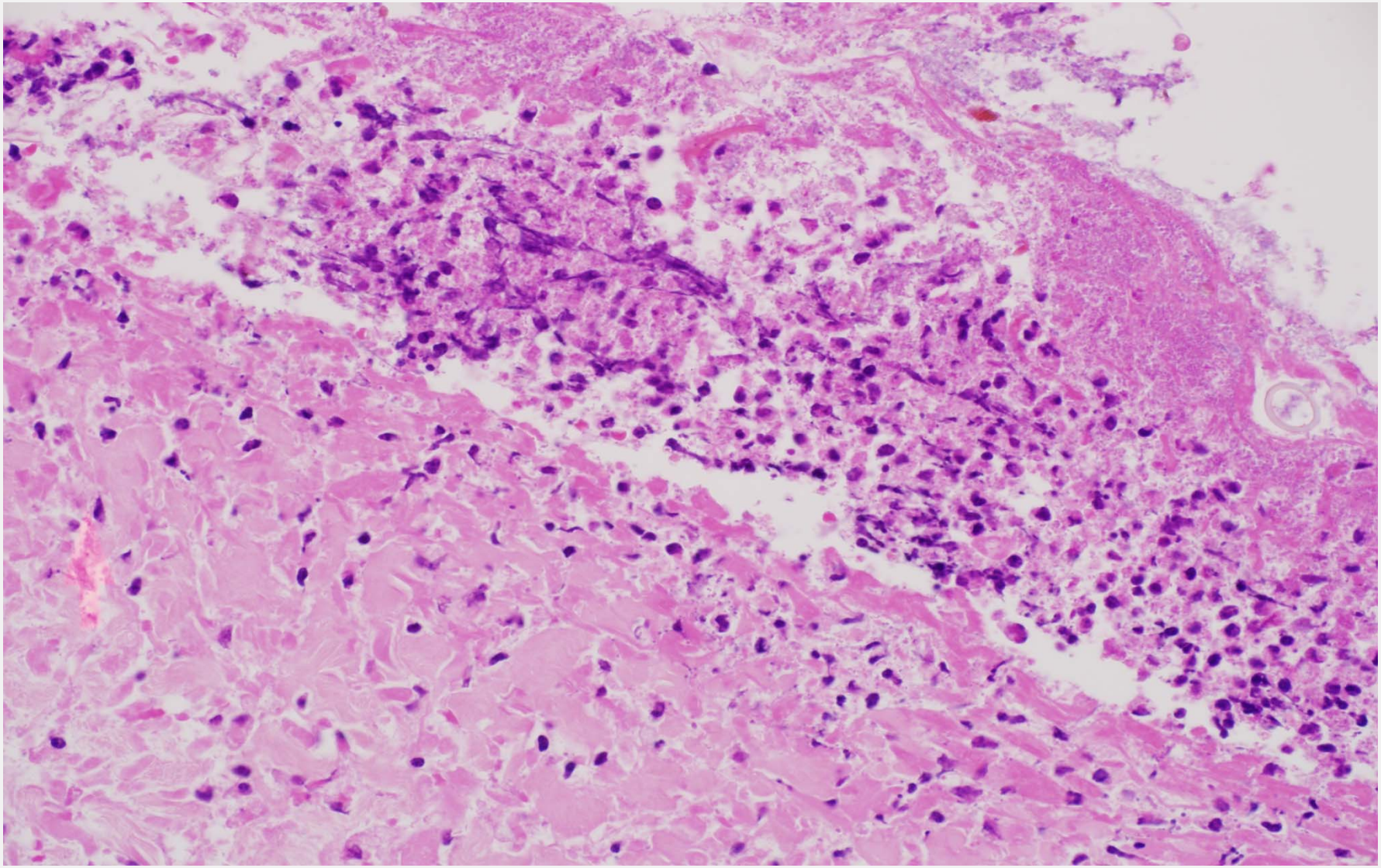
Chromatin deposition in fibrinonecrotizing pneumonia (*A. pleuropneumoniae)*



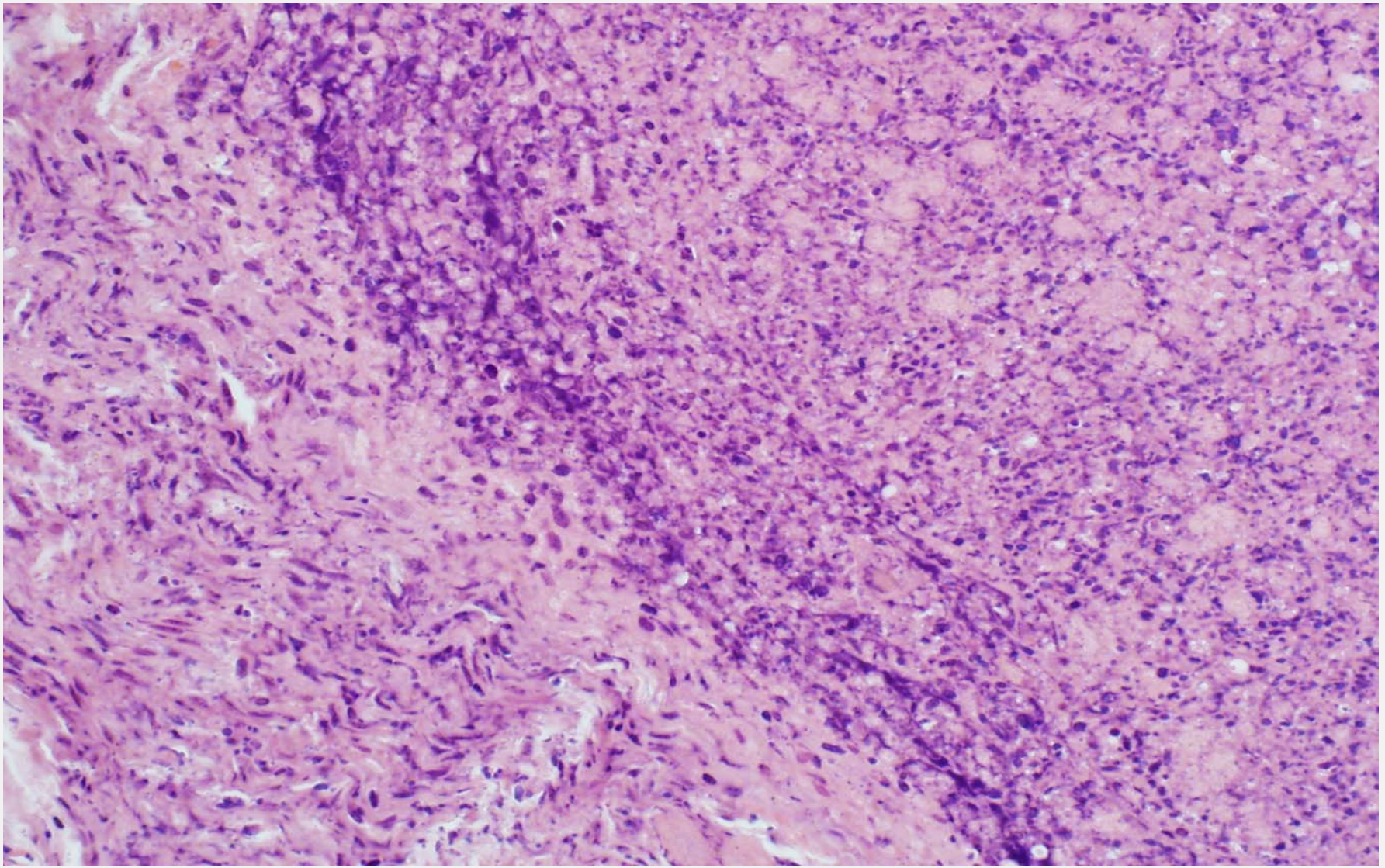
Trapped bacteria in deposited chromatin (APP)



Chromatin deposition in acute fibrinous peritonitis (bovine)



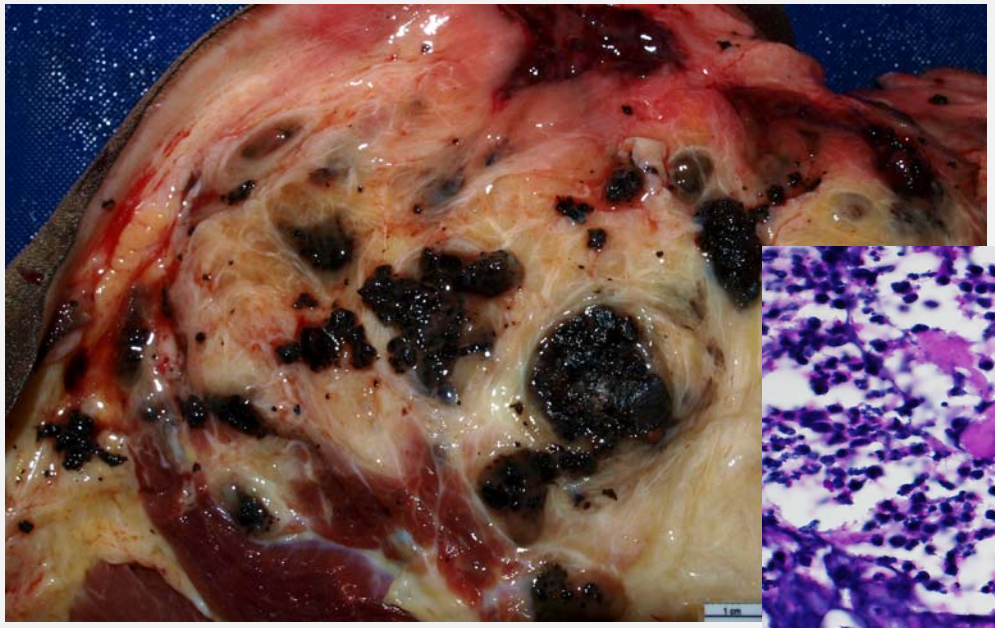
Chromatin deposition in an abscess

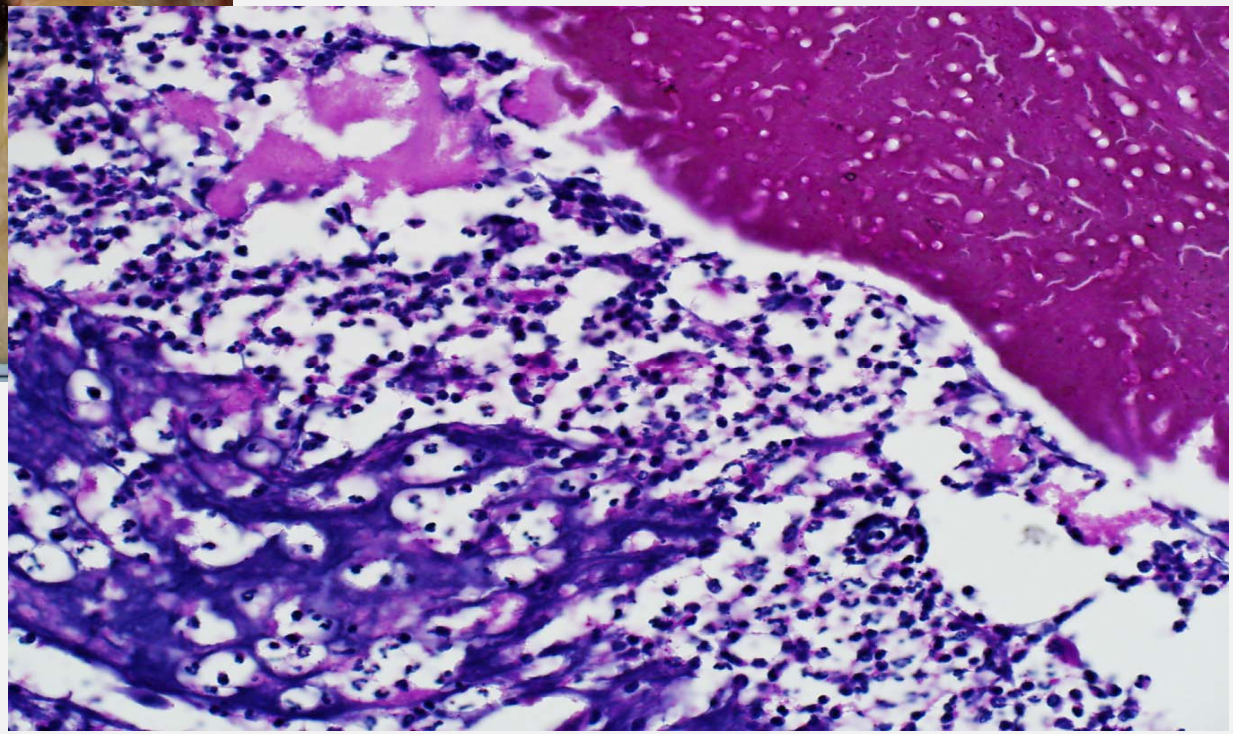


Mycetoma in the hindlimb of a horse

Janet Sunohara-Nielson, et al.

CAHLN-2011 annual meeting poster



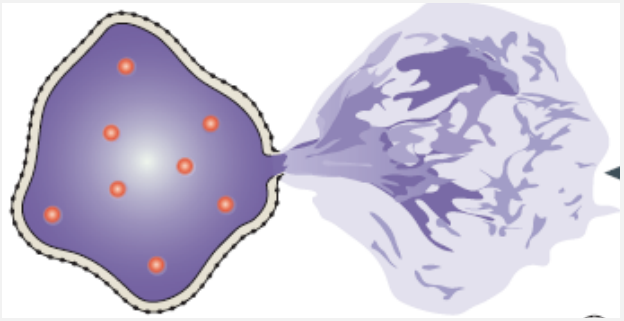


Major equine neutrophil proteins associated with fungal colonies in a mycetoma

Janet Sunohara-Nielson, Bette Anne Quinn et al.
CAHLN-2011 annual meeting poster

Protein	Function	kDa
Lactotransferrin	Iron binding protein	76
Histidine-rich glycoprotein	Antimicrobial protein	43
Lysozyme	Antimicrobial enzyme	17
Myeloperoxidase	Neutrophil lysosomal enzyme	88
Peptidoglycan recognition proteins	Microbial pattern recognition	21
Cathepsin G	Antimicrobial enzyme	48
Bactericidal permeability-increasing protein (BPI)	LPS binding neutrophil protein	53
Lipocalin-1	Molecular recognition protein	33
Calprotectin (S100 A9)	Antifungal neutrophil protein	10
Histone – H4	DNA associated Protein	11
Histone H2A	DNA associated protein	40
Histone H2B	DNA associated protein	14

Significance of chromatin deposition



DAMPs

DNAse I mediated clearance

Inflammatory disease

1. Is neutrophil chromatin more resistant to degradation?
2. Are anti-microbial factors retained in NETs in lesions?
3. Do pathogens (e.g. Staph) have nucleases that degrade NETs?
4. Are NETs adjuvants or DAMPs that mediate immune-mediated diseases?

Conclusions

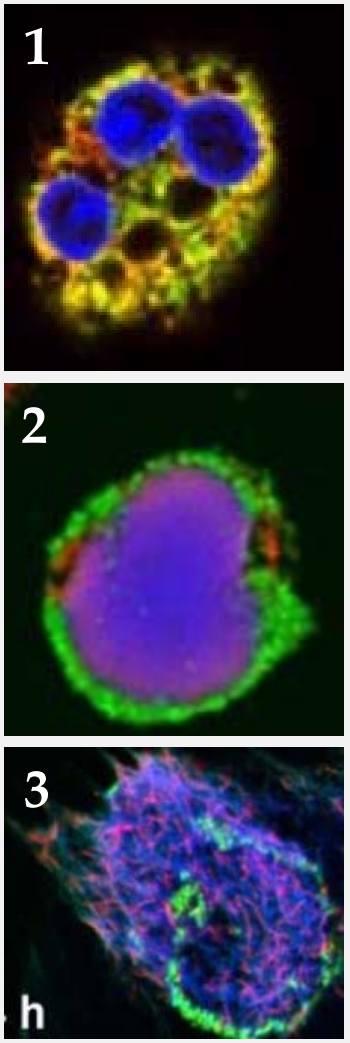
Most reports of NET formation and NETOSIS are based on in vitro findings

Morphological changes in neutrophils around necrotizing and septic lesions resemble NETOSIS

Chromatin deposition is common in septic histological lesions, but we do not know if it is an active or passive process

The location of chromatin deposits in acute septic lesions suggests they may be important in localizing bacteria and fungi

Markers for NET chromatin and associated neutrophil proteins (calprotectin, PGRPs, etc) are needed to study chromatin deposits and possible functions in animal lesions



Acknowledgments

Thesis committee
Dr. Tony Hayes (advisor)
Dr. Geoff Wood
Dr. Jeff Caswell
Dr. Brandon Lillie

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Janet Sunohara-Nielson
Bette Anne Quinn

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Ontario Veterinary College