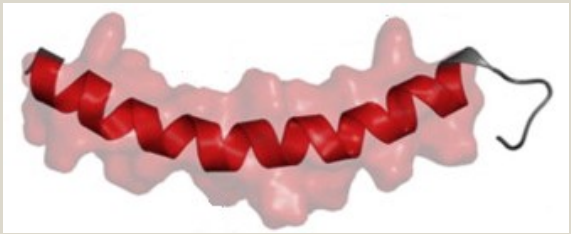
Stiftung Tierärztliche Hochschule Hannover
University of Veterinary Medicine Hannover, Foundation

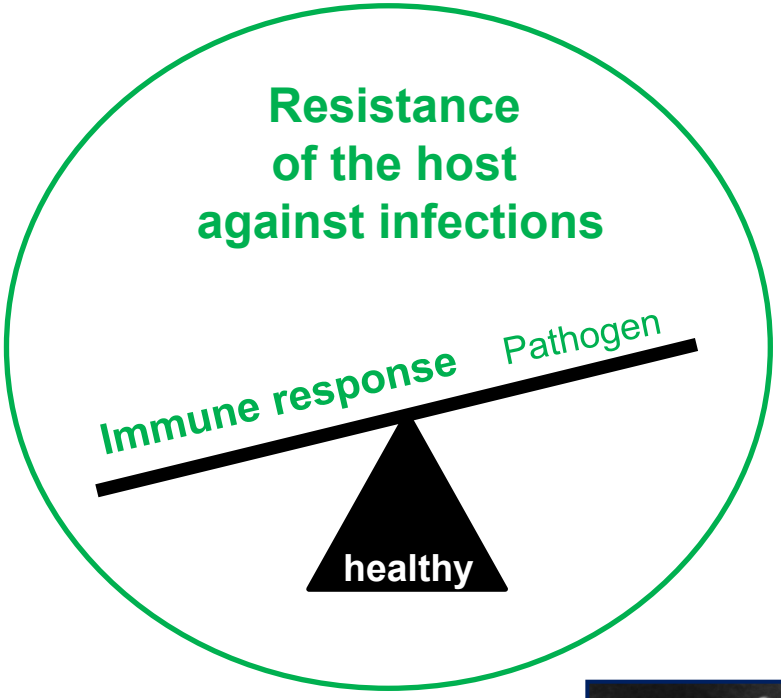
# Wie alte Waffen helfen, neue therapeutische Ansätze gegen Infektionskrankheiten zu finden

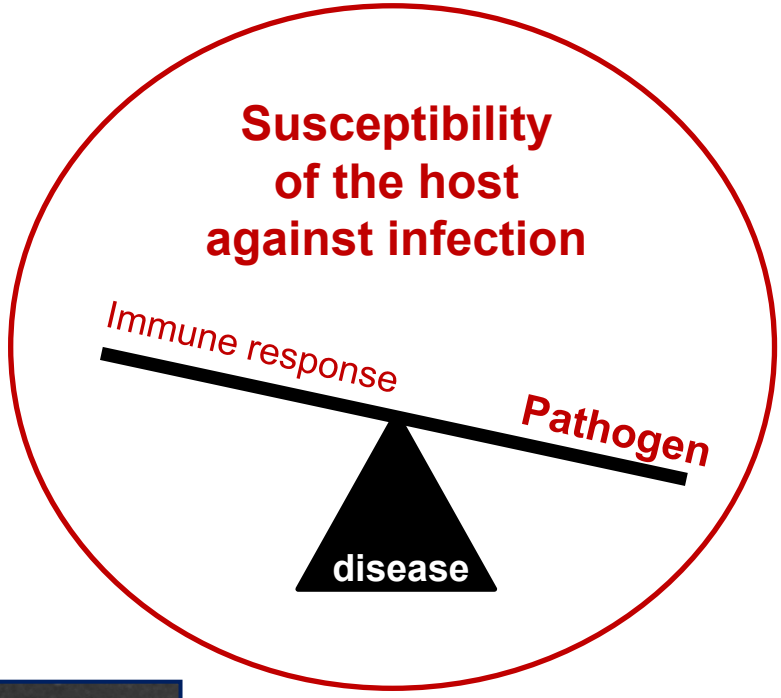
## *„Old weapons against infectious diseases“*

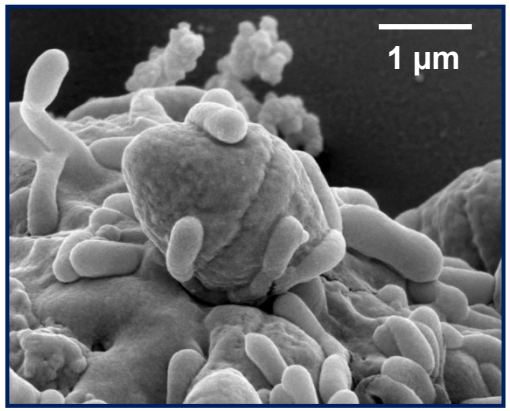


**Prof. Dr. Maren von Köckritz-Blickwede,**
**Infection Biochemistry,**
**Department of Biochemistry,**
**TiHo Hannover, Germany**

# Infections in human and animals




***Streptococcus pyogenes*** **entrapped by immune cell**

***von Köckritz-Blickwede et al., Blood, 2008***

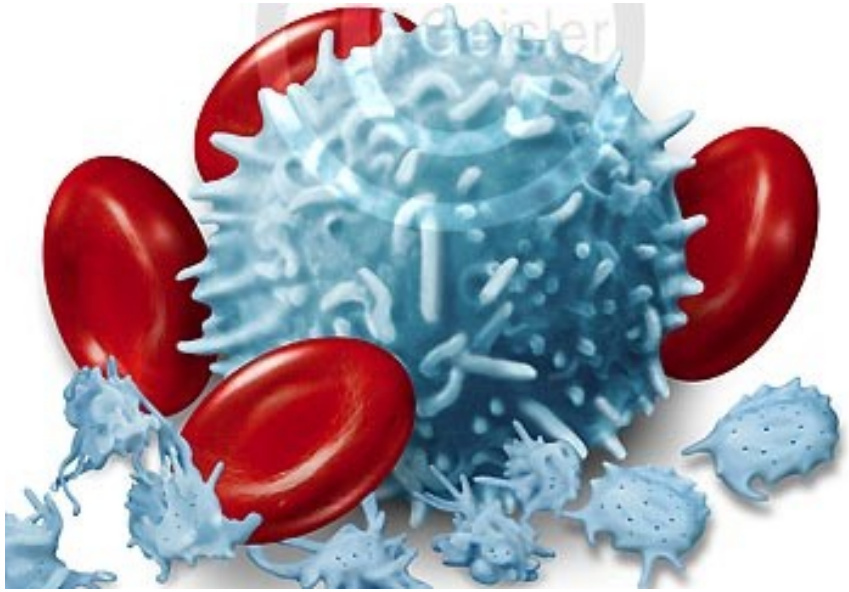
# Alternatives

Development of drugs which

**boost the host immune system**

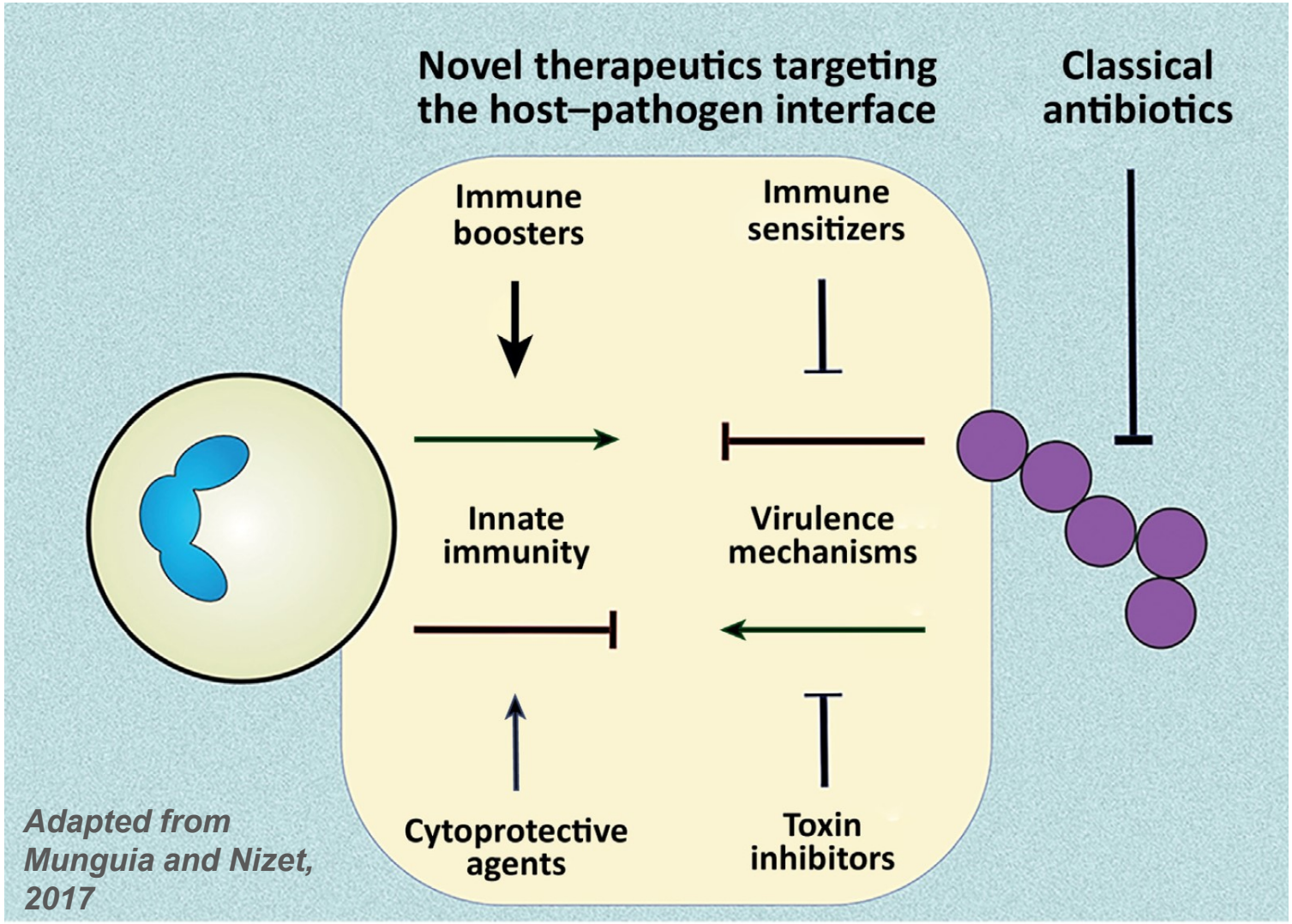
used alongside conventional antibiotic treatment

for successful therapy of difficult infections.



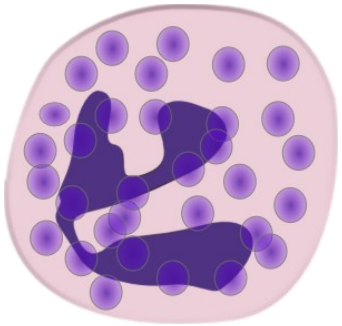
http://www.loadmedical.com/media/landingpages/Immun/Immun-00005.jpg

# Alternatives



# Guardian cell of the immune system

## Neutrophil



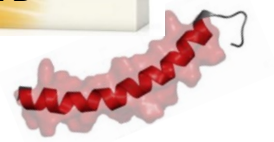
### Phagocytosis

(intracellular uptake and killing)

Guarea kunthiana

### Degranulation

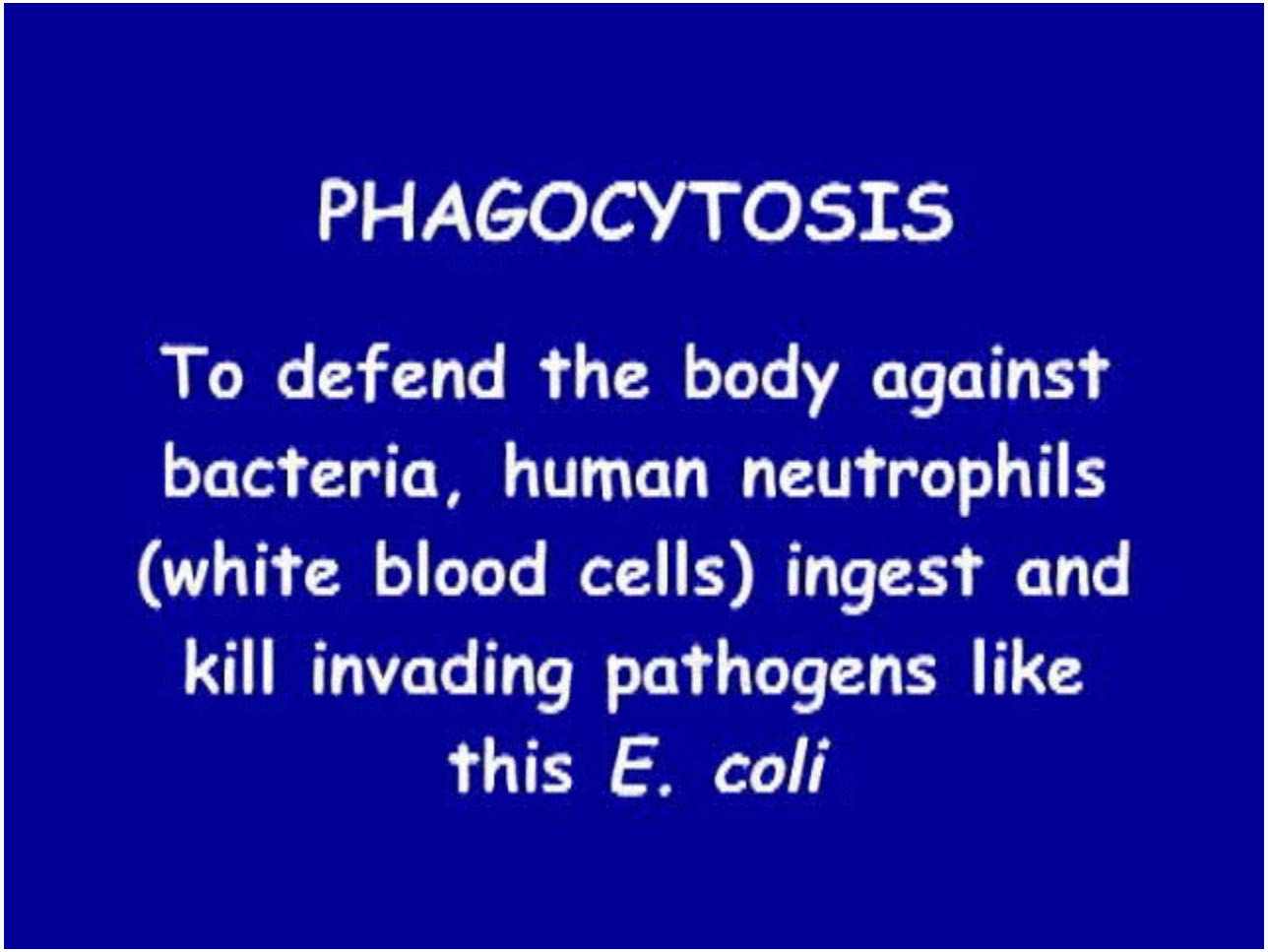
(extracellular release of antimicrobial peptides and others)

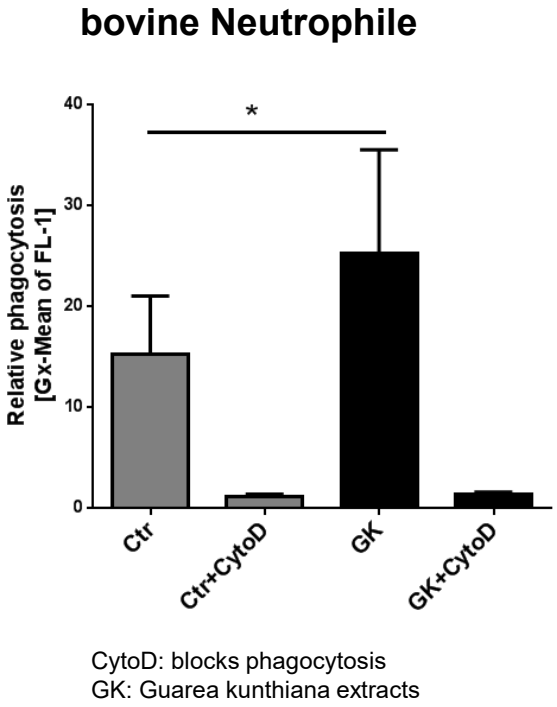
### Neutrophil extracellular traps (NETs)

(extracellular entrapment and killing of bacteria)

# Phagocytosis of bacteria



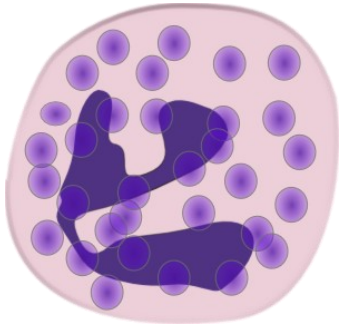
# Natural products that stimulate phagocytosis




CytoD: blocks phagocytosis
GK: Guarea kunthiana extracts

*Jerjomiceva, 2014*

## Neutrophil

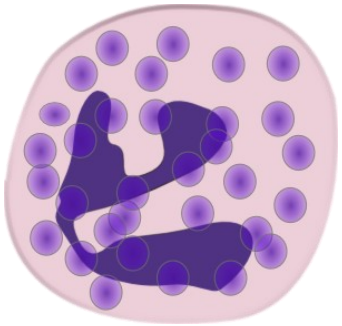




## Defense against pathogens

# Guardian cell of the immune system

## Neutrophil



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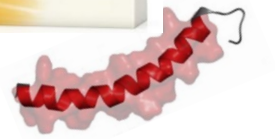
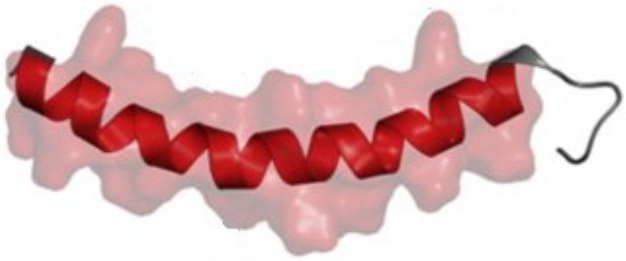


### Neutrophil extracellular traps (NETs)

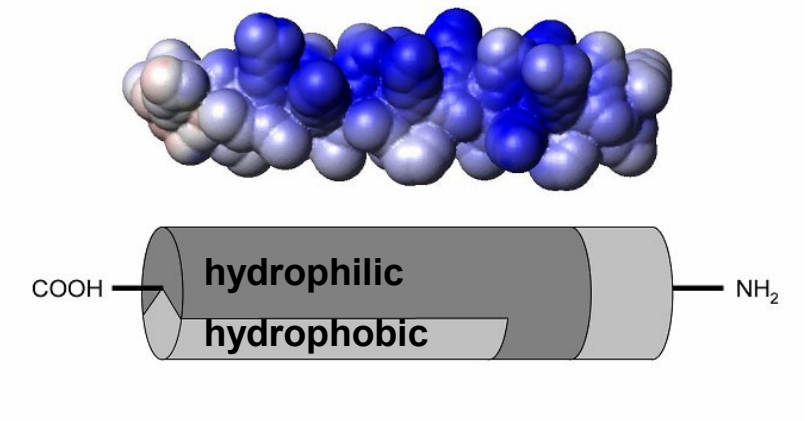
(extracellular entrapment and killing of bacteria)

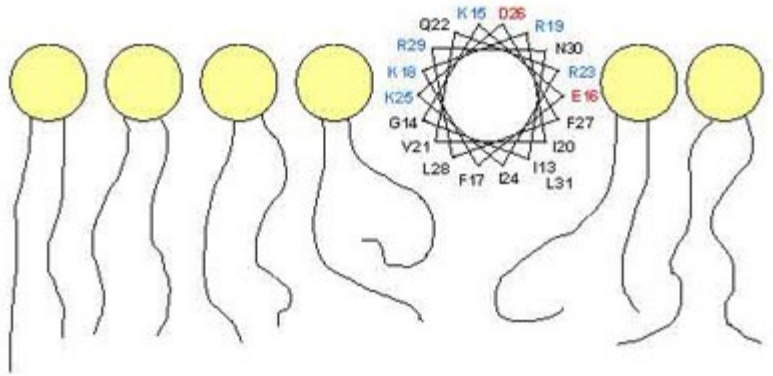
# Host antimicrobial peptides (AMPs)

- Small molecules, positively charged, amphipathic structure
- Released by neutrophils and other cells e.g. keratinocytes
- e.g. cathelicidin LL-37



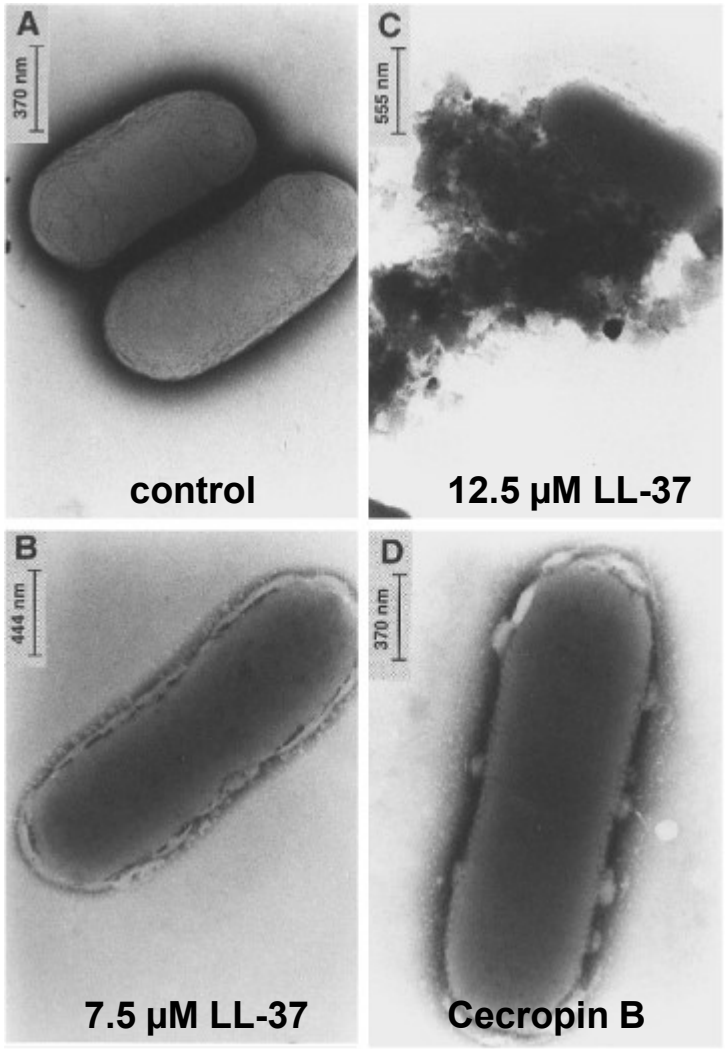
*Vandamme et al., 2012*



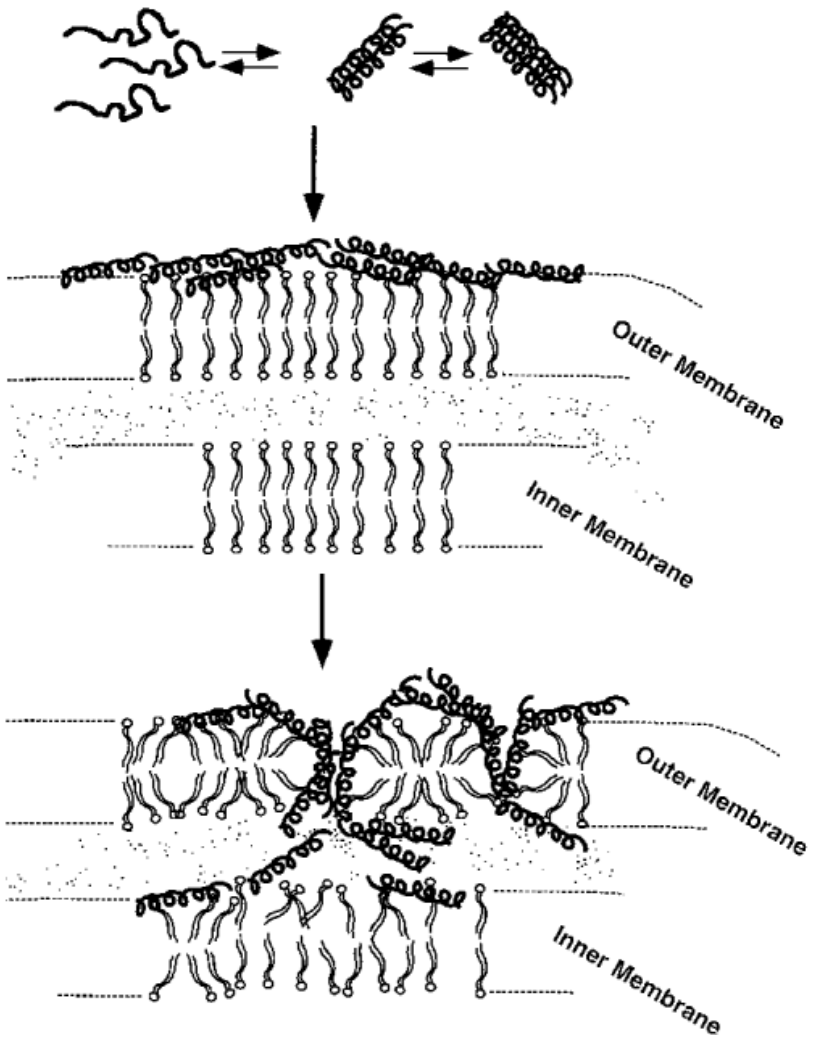




*Henzler Wildman, 2003*

# Host antimicrobial peptides (AMPs)




*Oren,1999*



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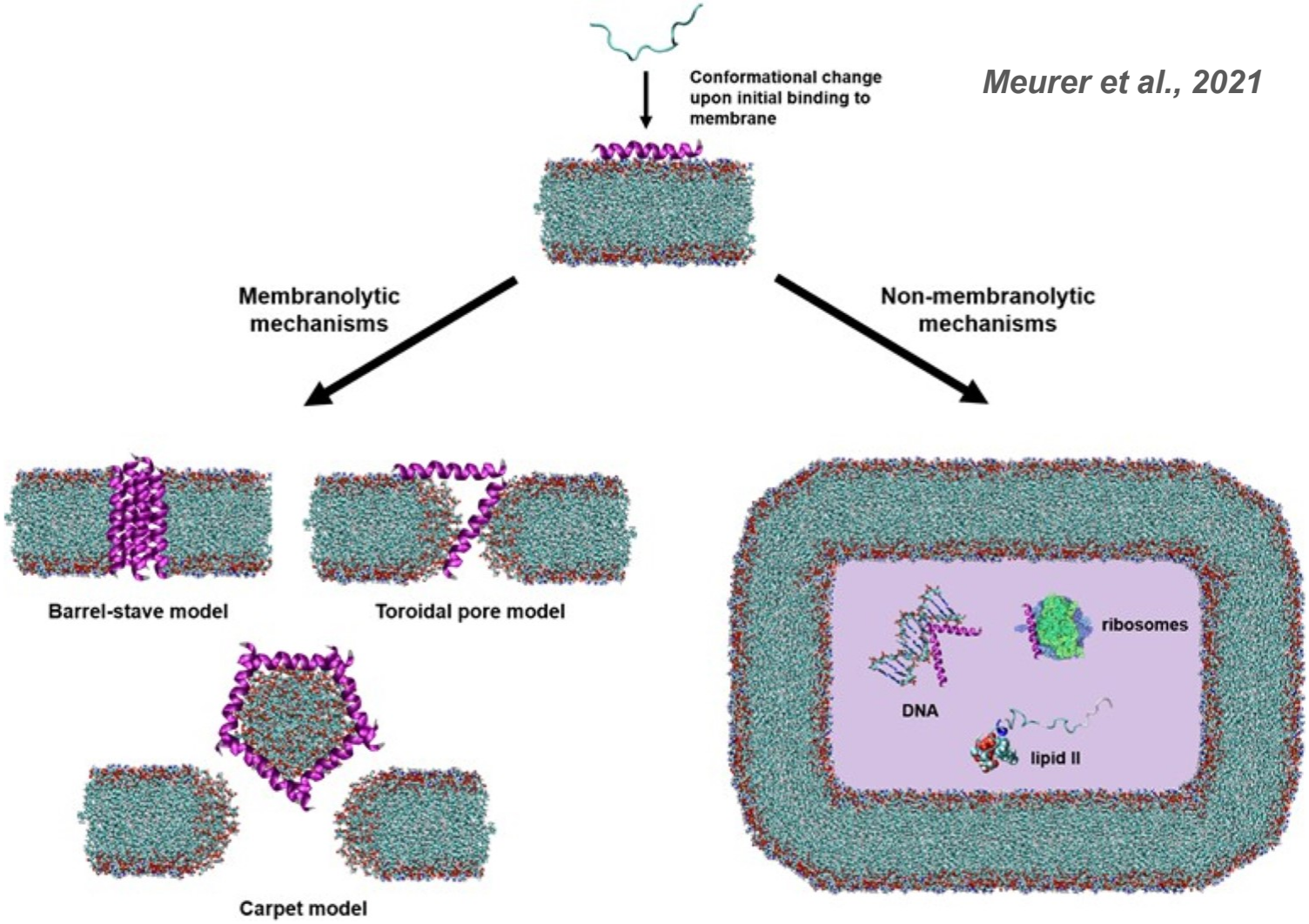
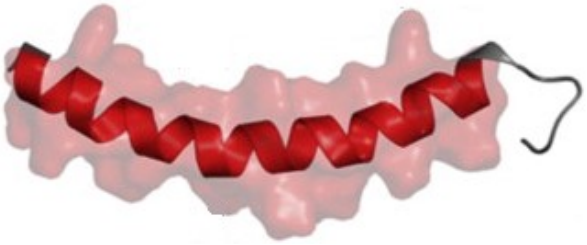
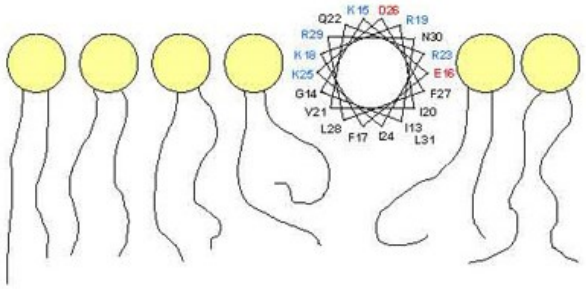



*Meurer et al., 2021*

# Antimicrobial peptides



Vandamme et al., 2012




Henzler Wildman, 2003

Intracellular activity

**Immunomodulation and host defense**

**Disruption of bacterial membranes**

## Advantage of AMPs:

## Low propensity to select resistance

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# The effect of antimicrobial peptides (AMPs) on the innate immune response against zoonotic *S. aureus*

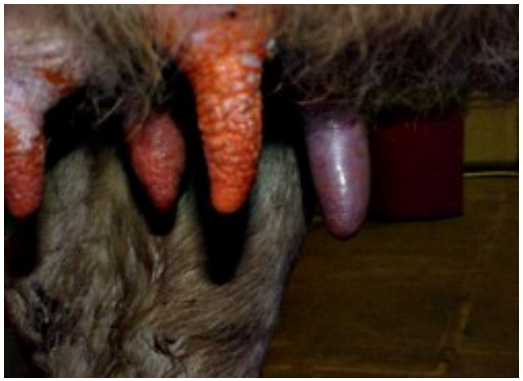
Stefanie Blodkamp

FRIEDRICH-LOEFFLER-INSTITUT
FLI
Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health

# *Staphylococcus aureus*

- Round-shaped, facultative anaerobic
- Typical commensal
- Skin & soft tissue infections
- Necrotizing pneumonia
- Mastitis

https://en.wikipedia.org/wiki/Staphylococcus_aureus



http://cal.vet.upenn.edu/projects/fieldservice/Dairy/Mastitis/staphaur.htm

http://www.20min.ch/diashow/95650/95650wg7Hlrp_Q2zu7SJS4NegBw.jpg

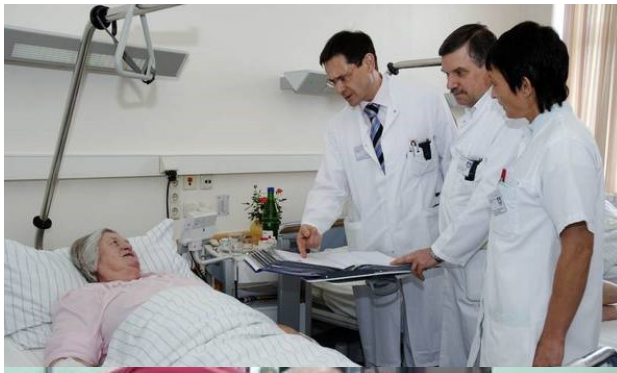
# Methicillin-resistant *Staphylococcus aureus* (MRSA)

HA-MRSA = healthcare-associated MRSA

CA-MRSA = community-associated MRSA

LA-MRSA = livestock-associated MRSA

# Methicillin-resistant *Staphylococcus aureus* (MRSA)



**Goal:**

1. AMP susceptibility testing of LA-MRSA field isolates
2. Factors that alter AMP MICs of *S. aureus*

LA-MRSA = livestock-associated MRSA

# Cathelicidins

| Host | AMP | Sequence |
|---|---|---|
|  | LL-37 | LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES |
|  | mCRAMP | GLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPE |
|  | CAP18 | GLRKRLRKFRNKIKEKLKKIGQKIQGFVPKLAPRTDY |
|  | BMAP-27 | GRFKRFRKKFKKLFKKLSPVIPLLHLG |
| | BMAP-28 | GGLRSLGRKILRAWKKYGPIIVPIIRIG |

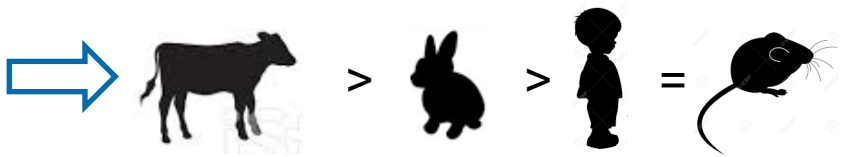
# AMP MICs of field isolates

| Source | No. of isolates | Mode MIC [mg/L] | | | | |
|---|---|---|---|---|---|---|
| | | LL-37  | mCRAMP  | CAP18  | BMAP-27  | BMAP-28  |
| Cattle d. | 16 | ≥128 | ≥128 | 32 | 8 | 2 |
| Chicken h. | 19 | ≥128 | ≥128 | 64 | 8 | 2 |
| Chicken d. | 5 | ≥128 | ≥128 | ≥128 | 16 | 8 |
| Turkey d. | 17 | ≥128 | ≥128 | 64 | 8 | 4, 8 |
| Swine d. | 32 | ≥128 | ≥128 | 64 | 8 | 2 |
| Farm e. | 31 | ≥128 | ≥128 | 64 | 8 | 2 |
| Chicken meat | 10 | ≥128 | ≥128 | 64 | 16 | 4 |
| Turkey meat | 21 | ≥128 | ≥128 | 64 | 8 | 4 |

d. = diseased; e. = environment and healthy employees; h. = healthy

# Antimicrobial peptides

- Antimicrobial activity of cathelicidins derived from different hosts



| Host | AMP | Sequence | Hydrophobic residues % |
|---|---|---|---|
|  | LL-37 | LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES | 35 |
|  | mCRAMP | GLLRKGGEKIGEKLKKIGQKIKNFFQKLVPQPE | 29 |
|  | CAP18 | GLRKRLRKFRNKIKEKLKKIGQKIQGFVPKLAPRTDY | 29 |
|  | BMAP-27 | GRFKRFRKKFKKLFKKLSPVIPLLHLG | 40 |
| | BMAP-28 | GGLRSLGRKILRAWKKYGPIIVPIIRIG | 42 |

*Blodkamp et al., Vet. Micro., 2015*

# Bovine cathelicidins

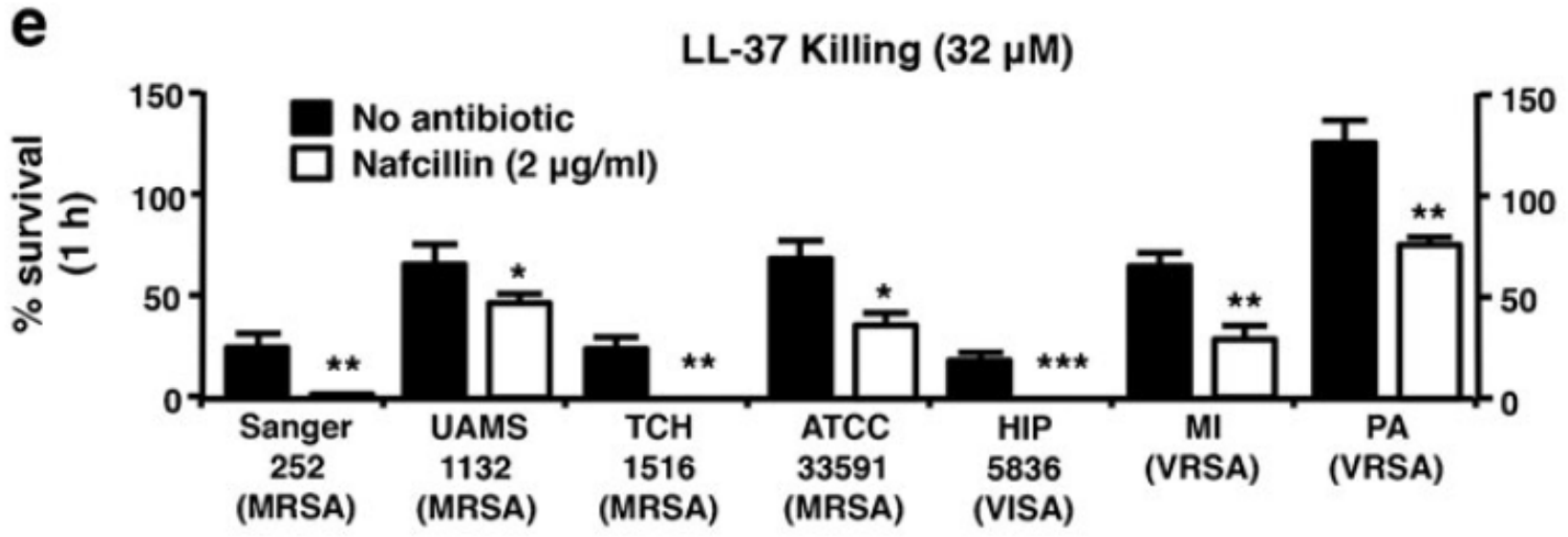
BMAP-27

*Baumann et al., 2017*


Thivierge et al., 2013

# Antimicrobial peptides

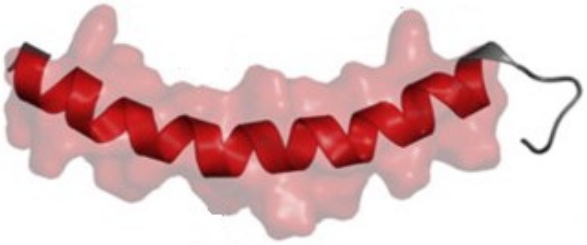
## Synergisms of AMPs with antibiotics



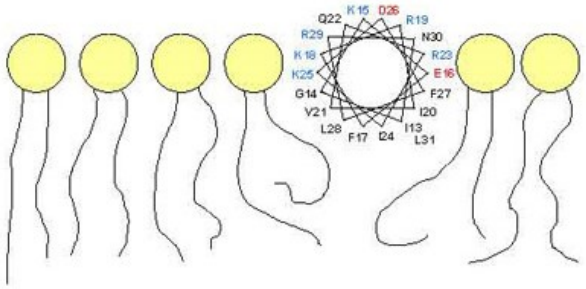

➔ **Nafcillin enhances killing of methicillin-resistant *Staphylococcus aureus* by cathelicidins**

*Sakoulas et al., 2014*

# Antimicrobial peptides



Vandamme et al., 2012




Henzler Wildman, 2003

Intracellular activity

**Immunomodulation and host defense**

**Disruption of bacterial membranes**

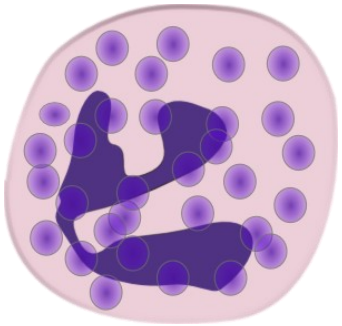
## Advantage of AMPs:

## Synergistic effects with antibiotics

## and other immune defence mechanisms

# Guardian cell of the immune system

## Neutrophil



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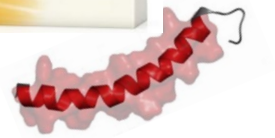
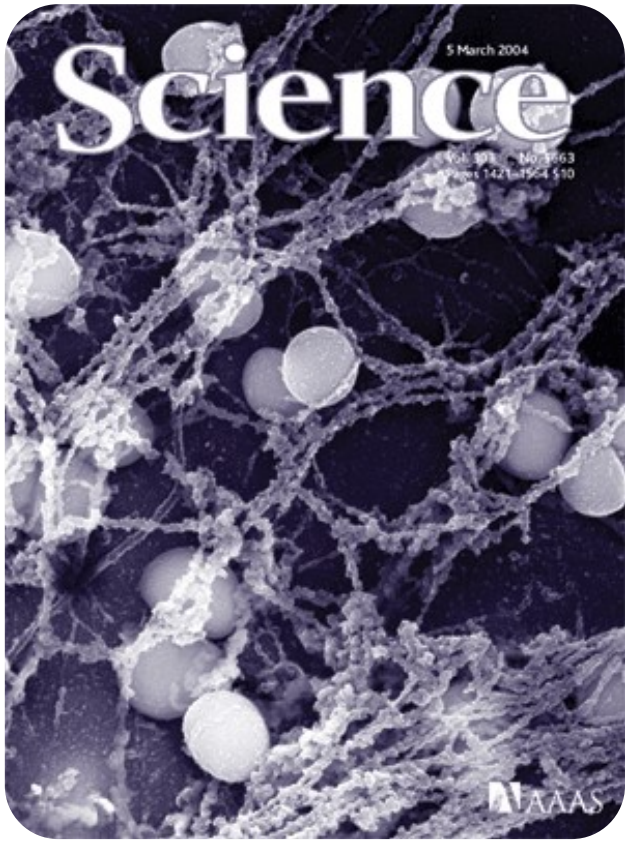


### Neutrophil extracellular traps (NETs)

(extracellular entrapment and killing of bacteria)

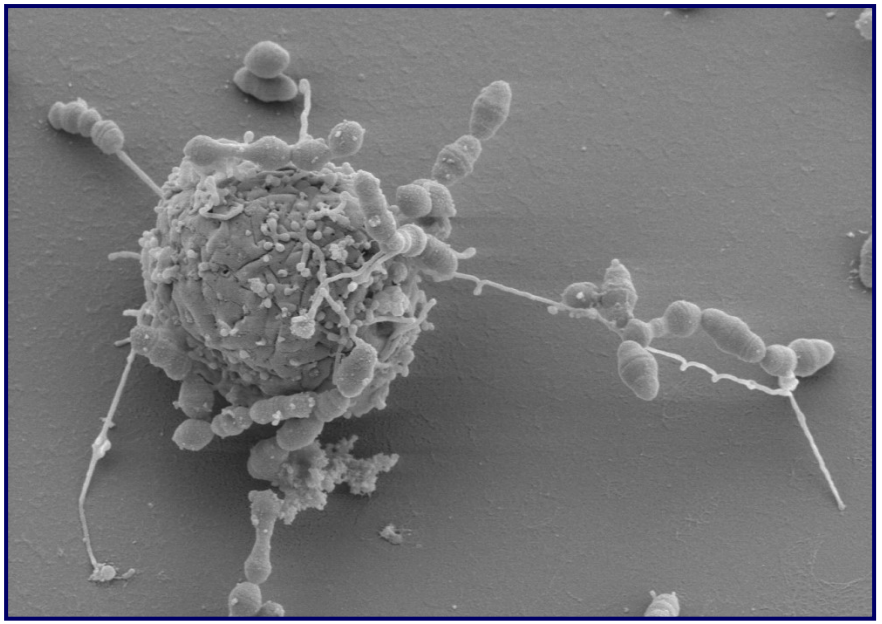
# Neutrophil extracellular traps (NETs)

## Neutrophils



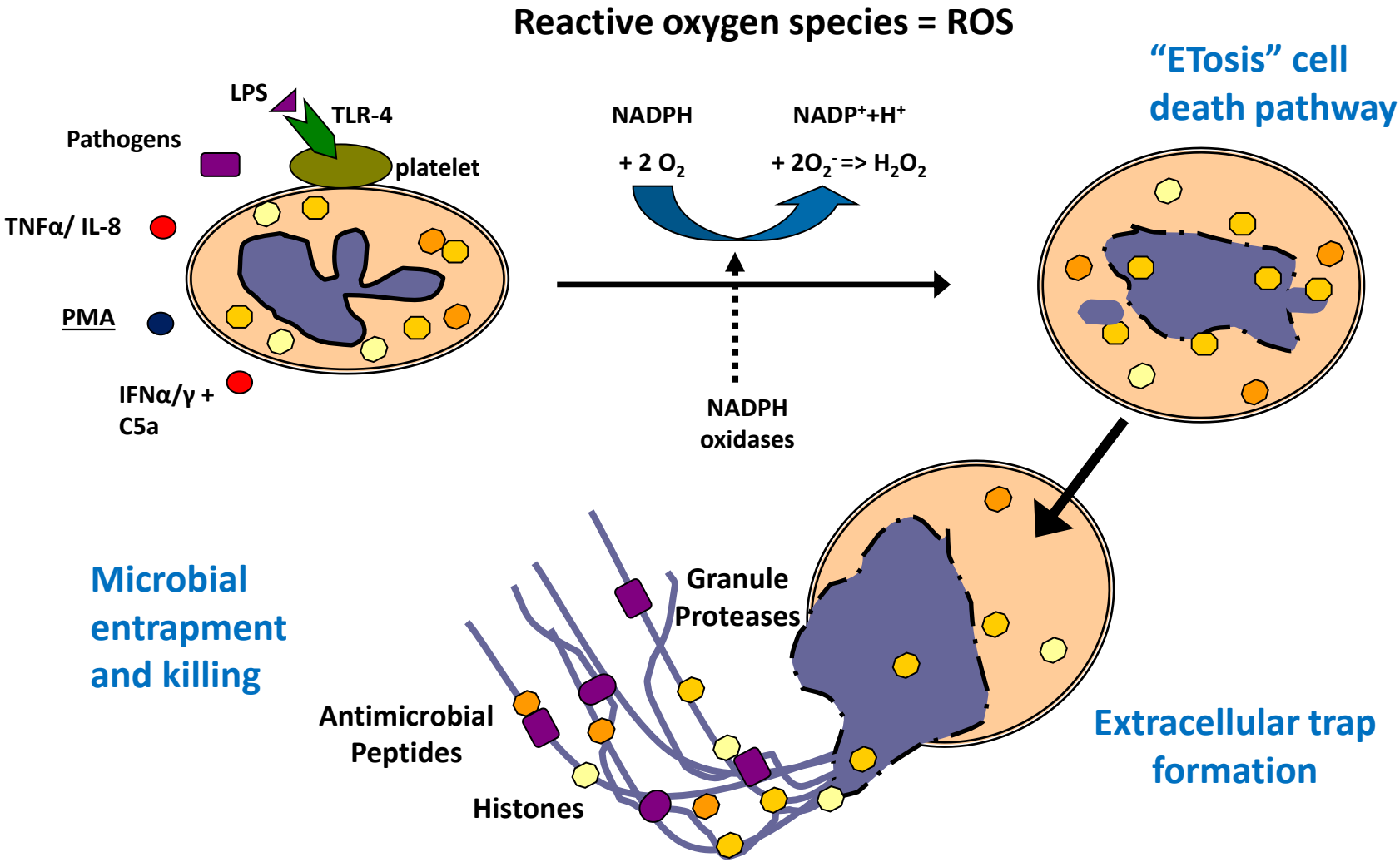

*Brinkmann et al., 2004*

## Mast cells



*von Köckritz-Blickwede et al., Blood, 2008*

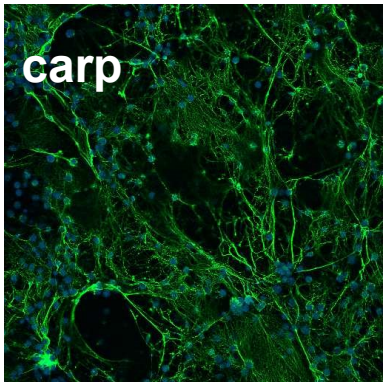
# Extracellular DNA traps (NETs)



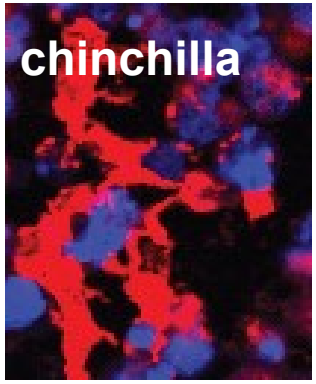

PMA = phorbol myristate acetate

*von Köckritz-Blickwede and Nizet , 2009*

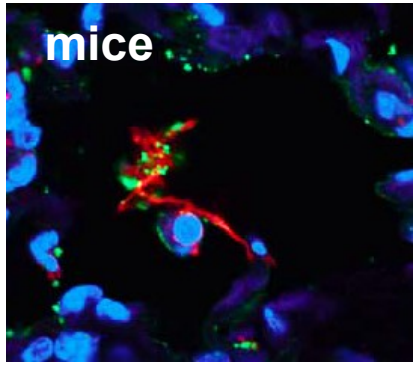
# Extracellular traps in human and animals



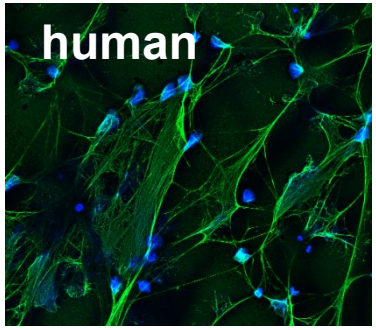

*Brogden et al., Dev. Comp. Immunol., 2014; Brogden et al., Fish and Shellfish Immunol. 2012*



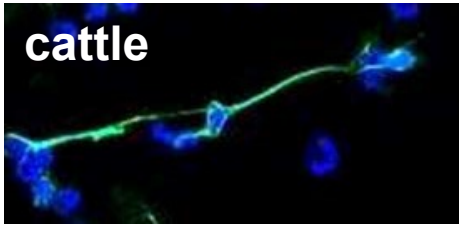

*Short et al., Infect. Immun., 2013*




*Berends et al., J. Innate Immun., 2010*

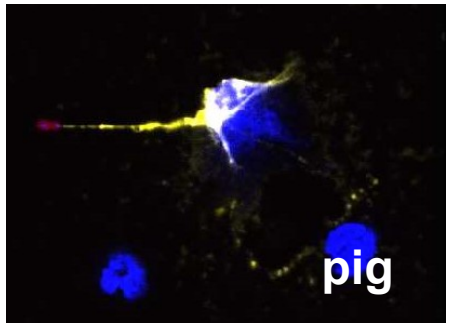



*Neumann et al., J. Innate Immun., 2014; Branitzki-Heinemann et al., Biochem. J. 2012*



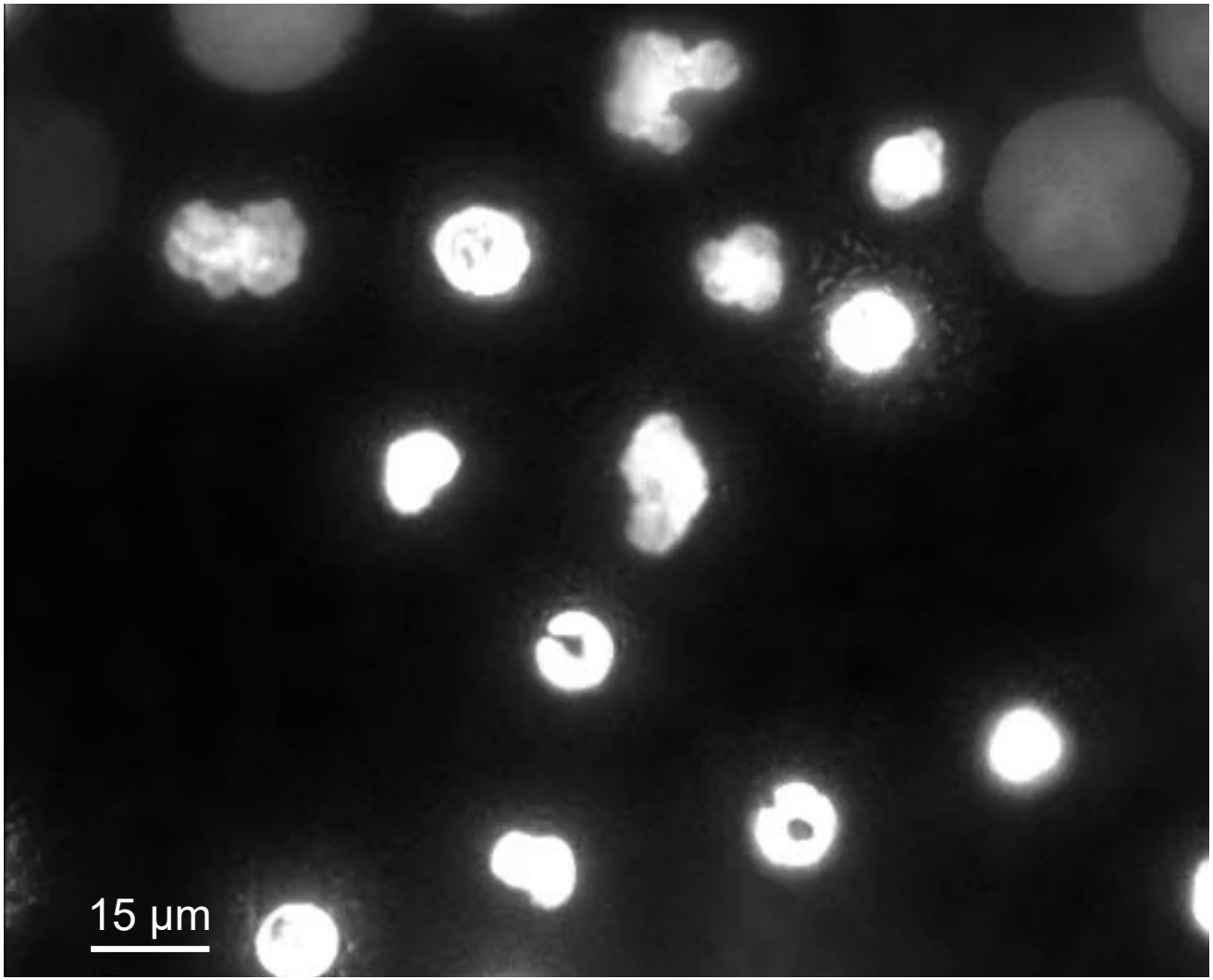

*Jerjomiceva et al., J. Innate Immun., 2014*


*Fingerhut et al., 2021*



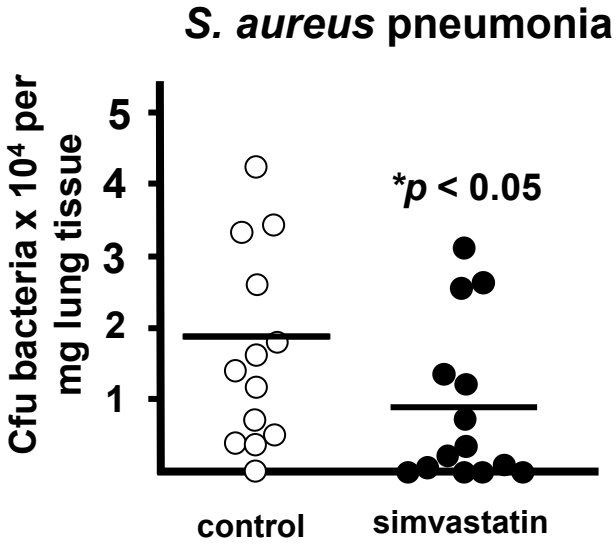

*Meurer et al., Microbiology, 2020*

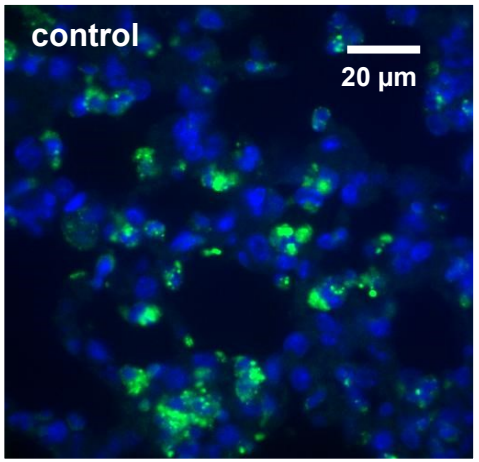
# Statins induce NETs

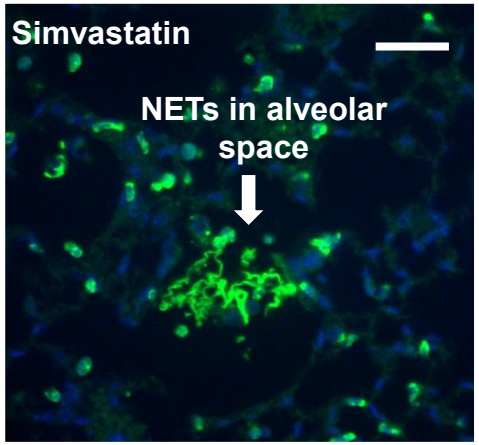


# Protective effect of NETs *in vivo*?

**Murine *in vivo* model**

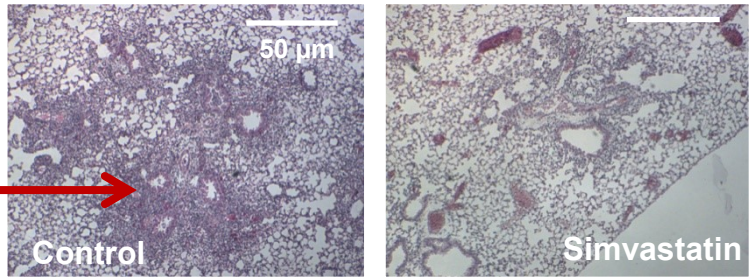



Blue: nucleus
Green: antimicrobial peptide mCRAMP

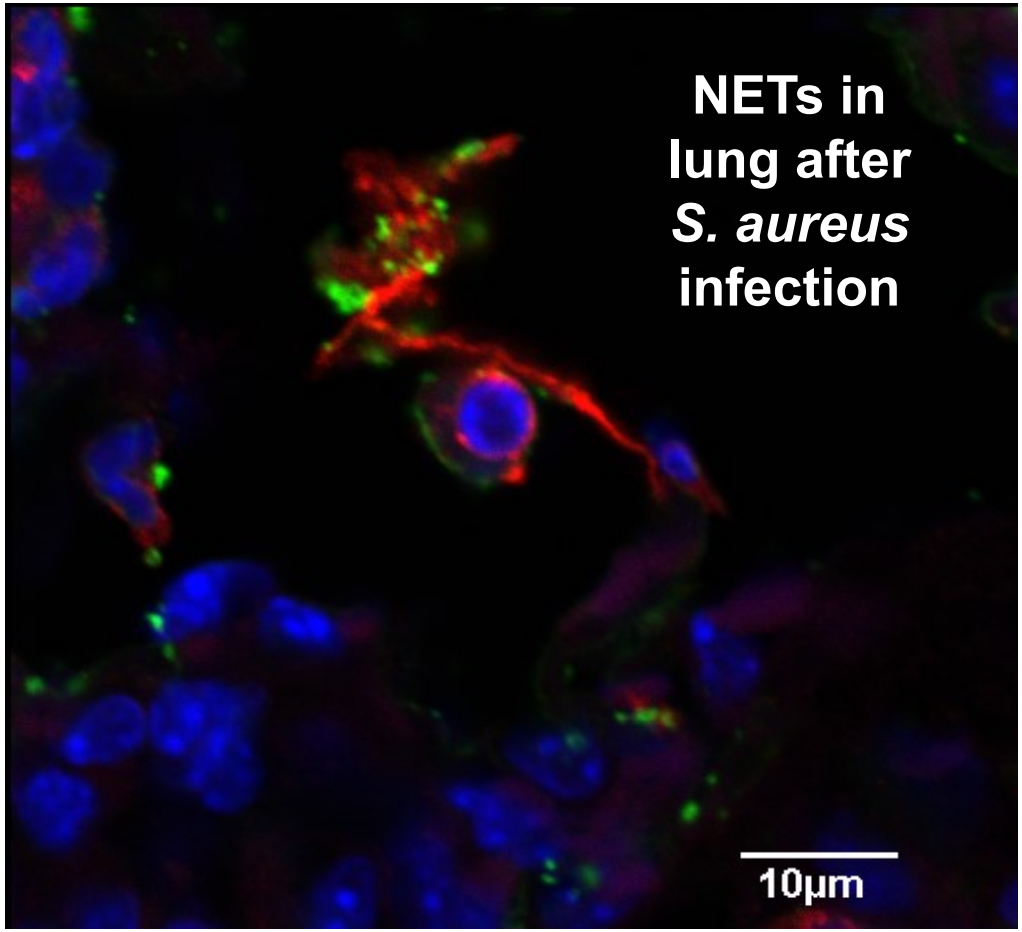
Inflammation of lung tissue




**Mice were prefed for 5 days with 500 mg/kg/day statins and then infected with a sublethal dose of 2 x $10^8$ cfu *S. aureus* Newman intranasally for 48 h.**

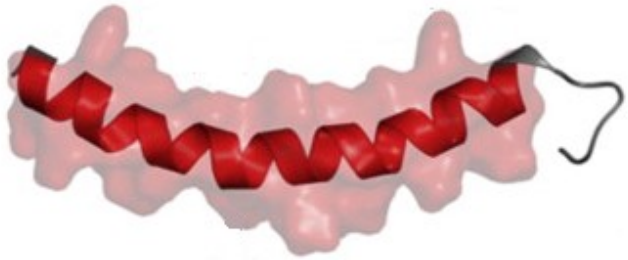
*Chow et al., 2010*

# In vivo NET-formation during infection




**Blue: nucleus**
**Green: histone-DNA-complex**
**Red: antimicrobial peptide mCRAMP**

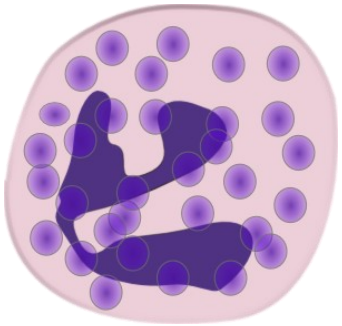
**Host antimicrobial peptides (AMPs)**



*Vandamme et al., 2012*

# Guardian cell of the immune system

## Neutrophil



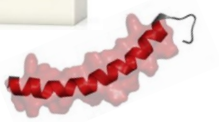
### Phagocytosis

(intracellular uptake and killing)

Guarea kunthiana

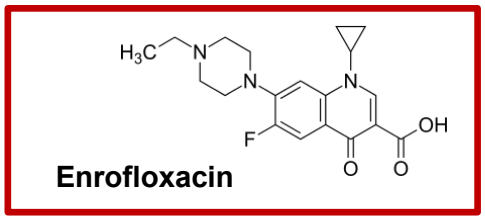
### Degranulation

(extracellular release of antimicrobial peptides and others)

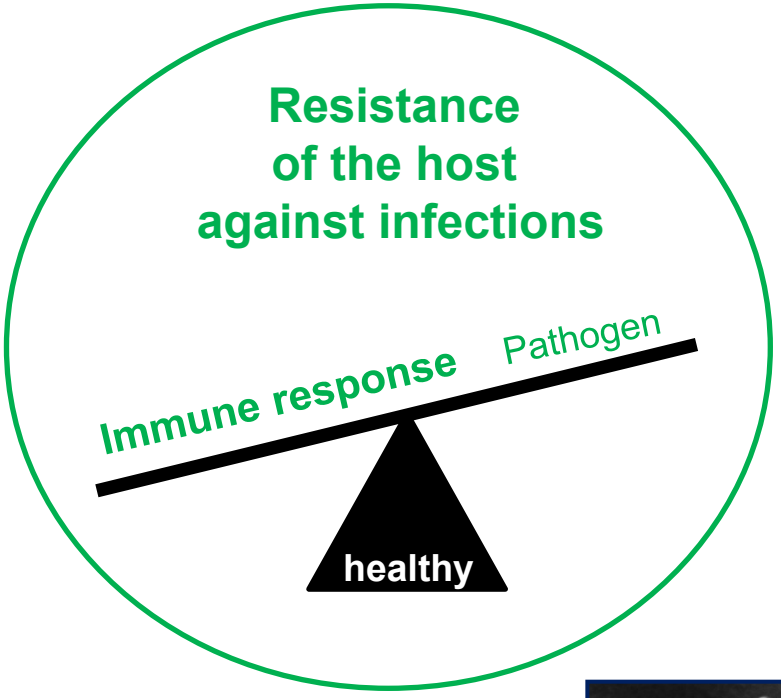
### Neutrophil extracellular traps (NETs)

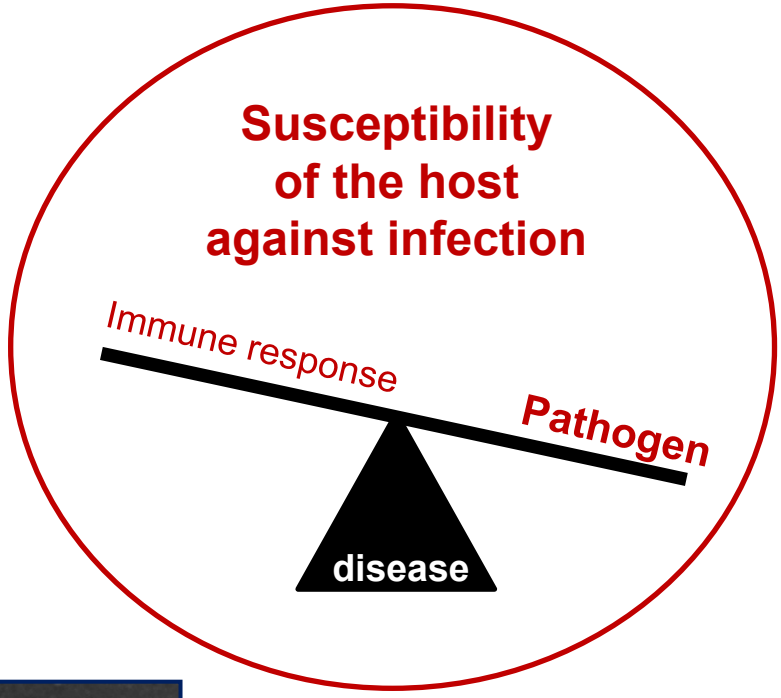
(extracellular entrapment and killing of bacteria)

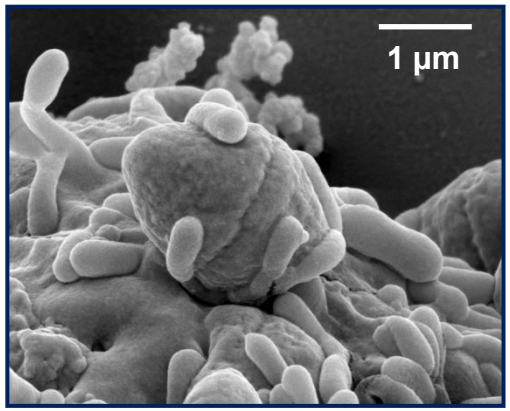




Enrofloxacin

# Infections in human and animals



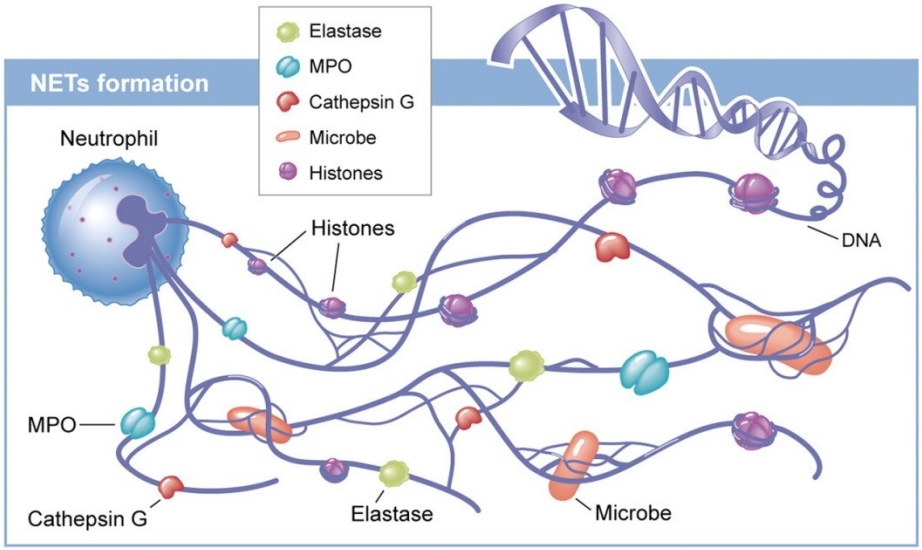

***Streptococcus pyogenes*** **entrapped by immune cell**

***von Köckritz-Blickwede et al., Blood, 2008***

# Balance of NETs and AMPs

**Protective defence of NETs against infections:**

*e.g.* *Staphylococcus aureus* (Berends et al., 2010), *Streptococcus pyogenes* (Lauth et al., 2009), *Streptococcus suis* (de Buhr et al., 2014), *Yersinia enterocolitica and Escherichia coli* (Möllerherm and Neumann et al., 2015)



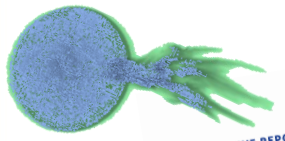

**Detrimental consequences of NETs for the host during infections with:**

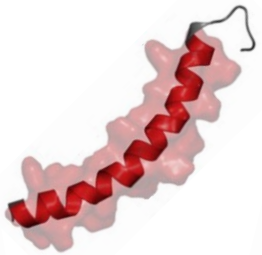
*e.g. Strep. pneumoniae* + influenza (Short et al., 2013)

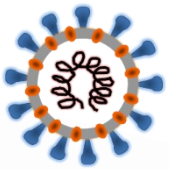
- Entrapment of pathogens
- Growth inhibition of pathogens
- Limit acute inflammation

- Cytotoxicity
- Thrombosis
- Autoimmune reactions
- Chronic inflammation

# NETs & AMPs interact with SARS-CoV-2







JEM

BRIEF DEFINITIVE REPORT

SARS-CoV-2–triggered neutrophil extracellular traps mediate COVID-19 pathology

Interdisciplinary Sciences: Computational Life Sciences (2021) 13:766–777
https://doi.org/10.1007/s12539-021-00462-3

ORIGINAL RESEARCH ARTICLE

HD5 and LL-37 Inhibit SARS-CoV and SARS-CoV-2 Binding to Human ACE2 by Molecular Simulation

Daixi Li[1] · Peiqin Chen[1] · Ting Shi[2] · Aamir Mehmood[2] · Jingfei Qiu[3]

Blood. 2020 Sep 3;136(10):1169-1179. doi: 10.1182/blood.2020007008.

Neutrophil extracellular traps contribute to immunothrombosis in COVID-19 acute respiratory distress syndrome

Elizabeth A Middleton [1 2], Xue-Yan He [3], Frederik Denorme [1], Robert A Campbell [1 2], David Ng [3], Steven P Salvatore [4 5], Amelia Baxter-Stoltzfus [4], Alain C Borczuk [4 5], Massimo Loda [4 5], Matthew Manne [1], Irina Portier [1], Estelle S Harris [2], Aaron C Petrey [1], Anthony Iovino [6 8], Lisa M Abegglen [6 8], Andrew S Weyrich [1], Egeblad [3], Joshua D Schiffman [1 6 8], Christian Con Yost [1 6]

DOI: 10.1182/blood.2020007008

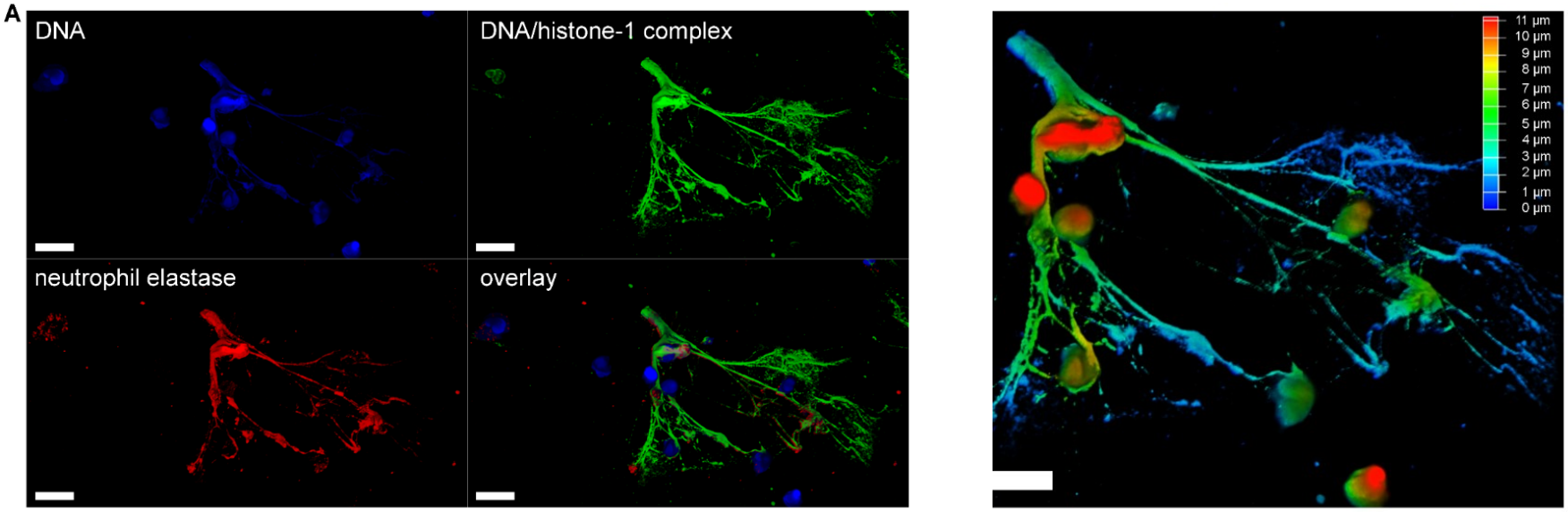
**Are NETs or AMPs counteracting SARS-CoV-2 and beneficial?**

**Are NETs & AMPs detrimental in the COVID-19 pathogenesis?**

**Is it beneficial for COVID-19 patients to modulate NETs & AMPs?**

# NETs & AMPs interact with SARS-CoV-2

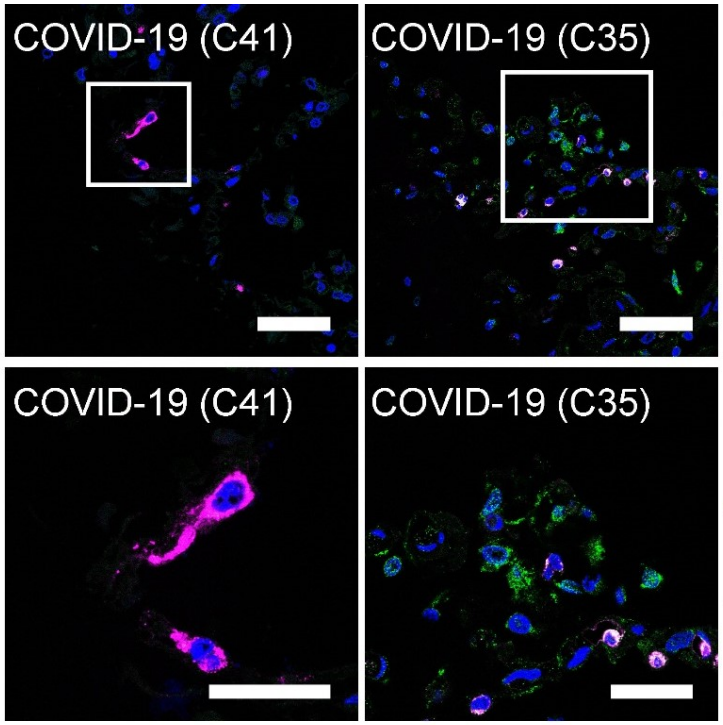
## NETs & SARS-CoV-2 *in vitro, Timo Henneck*



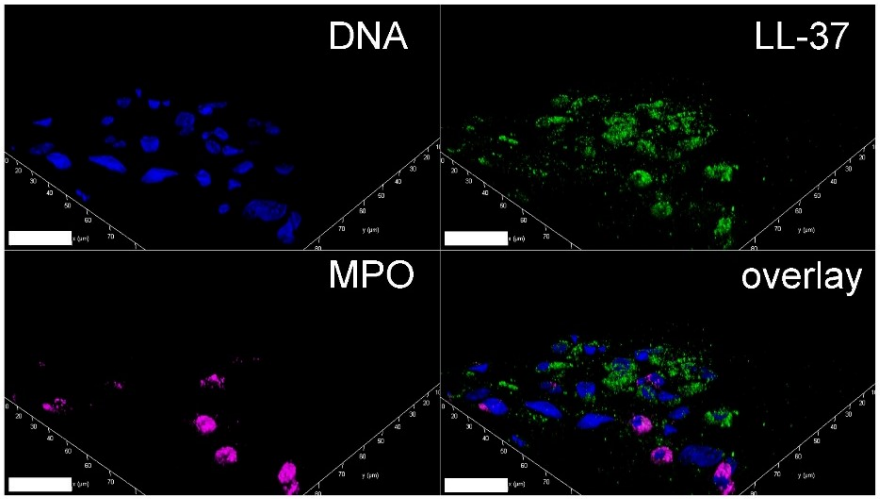

*In vitro*: Primary human blood-derived neutrophils release NETs in response to SARS-CoV-2.

# NETs & AMPs interact with SARS-CoV-2

## NETs & SARS-CoV-2 in human biopsies from COVID-19 patients



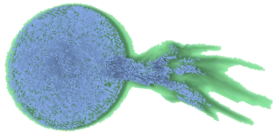

COVID-19 (C35) zoom 3D-image



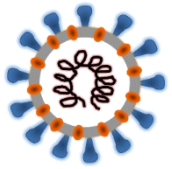

blue = DNA
green = LL-37
magenta = MPO/myeloperoxidase

**Cationic antimicrobial peptide LL-37 stabilizes NETs**
**(Neumann et al., 2014; de Buhr et al., 2017, Meurer et al., 2020)**

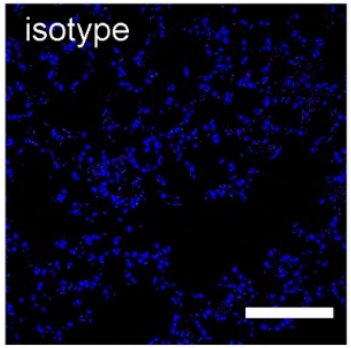
# NETs & AMPs interact with SARS-CoV-2

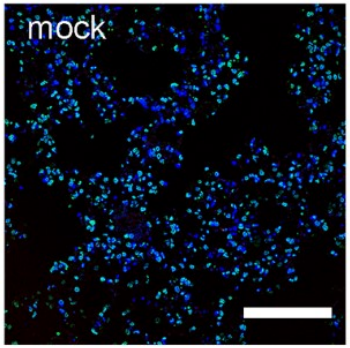


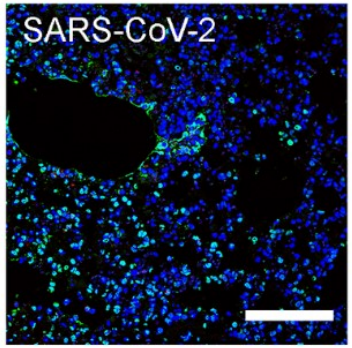
**intranasally infected with $10^5$ plaque forming units (p.f.u.) SARS-CoV-2 or mock**

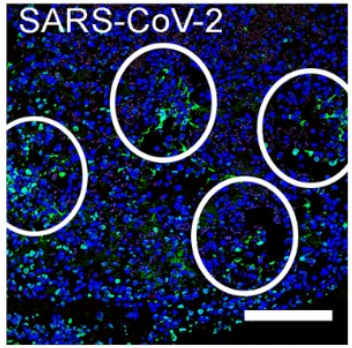


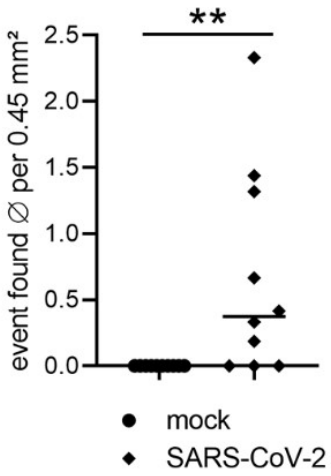
**1, 3, 6 and 14 p.i. ten animals per group were euthanized and lungs were fixed in 4 % paraformaldehyde for microscopic examination.**



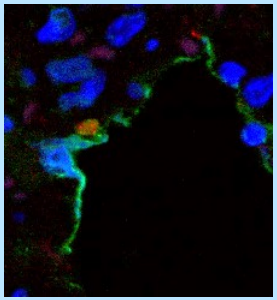



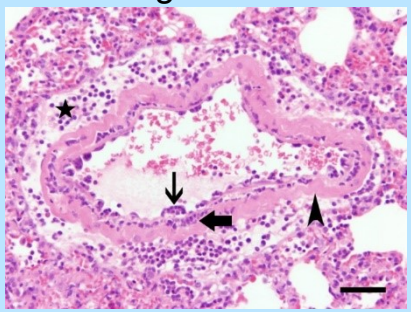

- Semiquantitative analysis of NET formation in SARS-CoV-2-infected animals was significantly increased compared to mock animals.

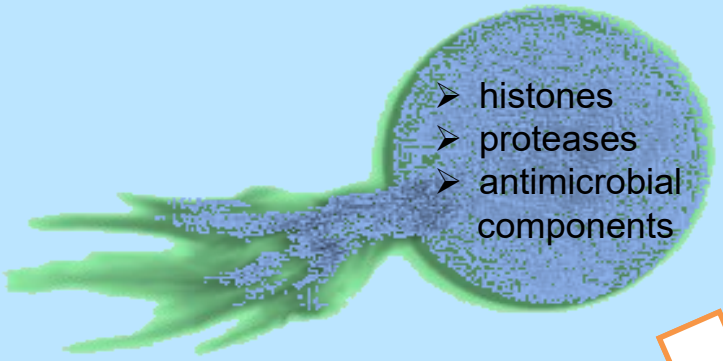
# NETs & AMPs interact with SARS-CoV-2

NETs are formed by infiltrating neutrophils into the lung as response to SARS-CoV-2-infections



Vasculitis during SARS-CoV-2 infection

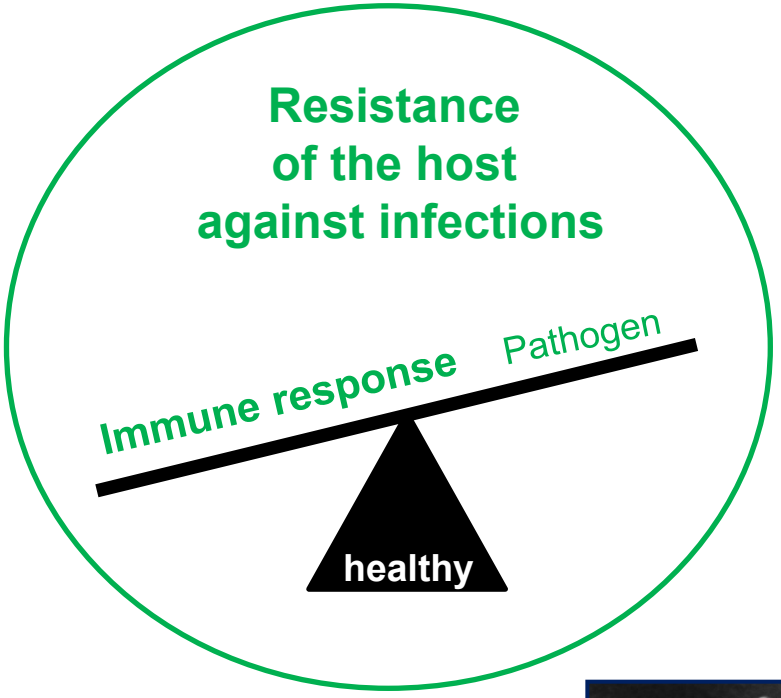


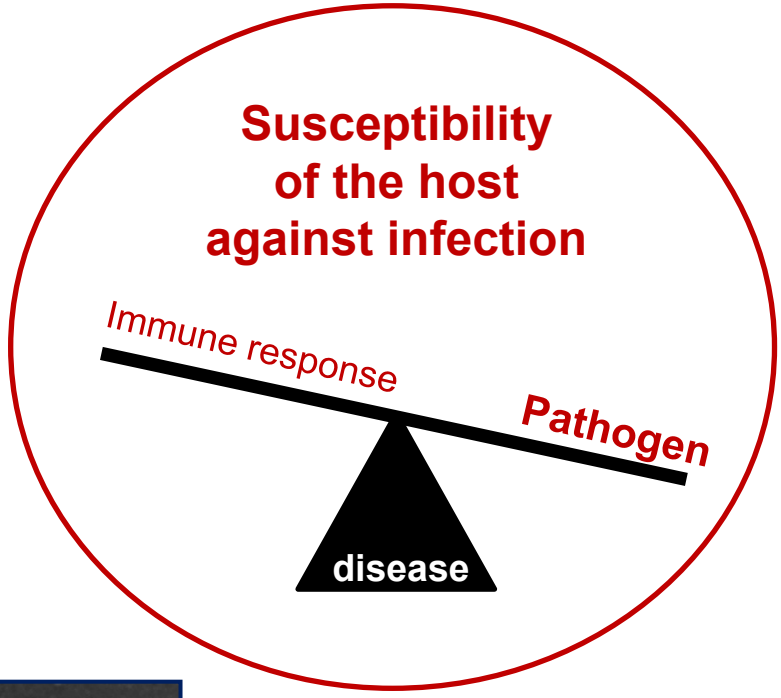


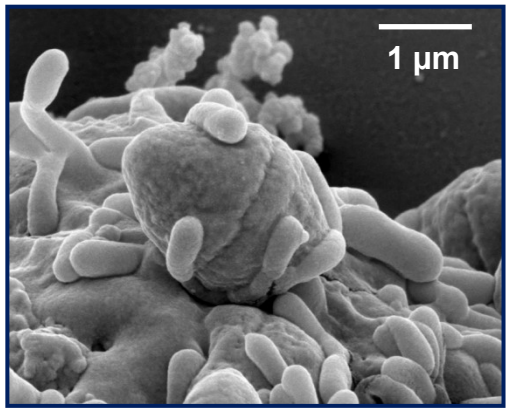

- pore-formation
- cytotoxicity (cationic charge and binding to phospholipid-membranes)

- Increased vascular permeability
- loss of barrier integrity, cytotoxicity
- microvascular injury)

# Infections in human and animals




***Streptococcus pyogenes*** **entrapped by immune cell**

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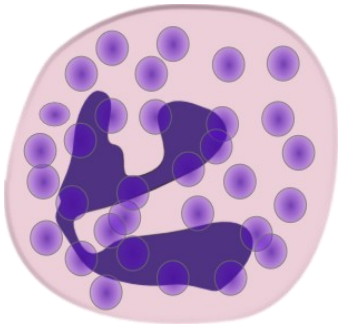
# Guardian cell of the immune system

## Neutrophil



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Guarea kunthiana

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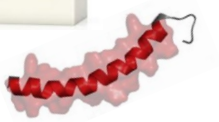

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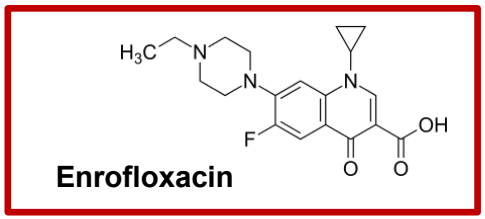
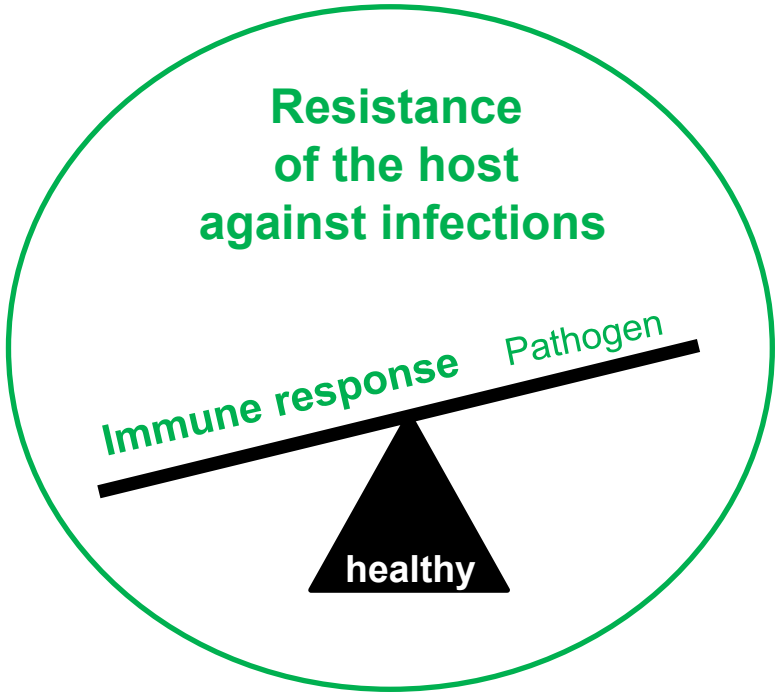
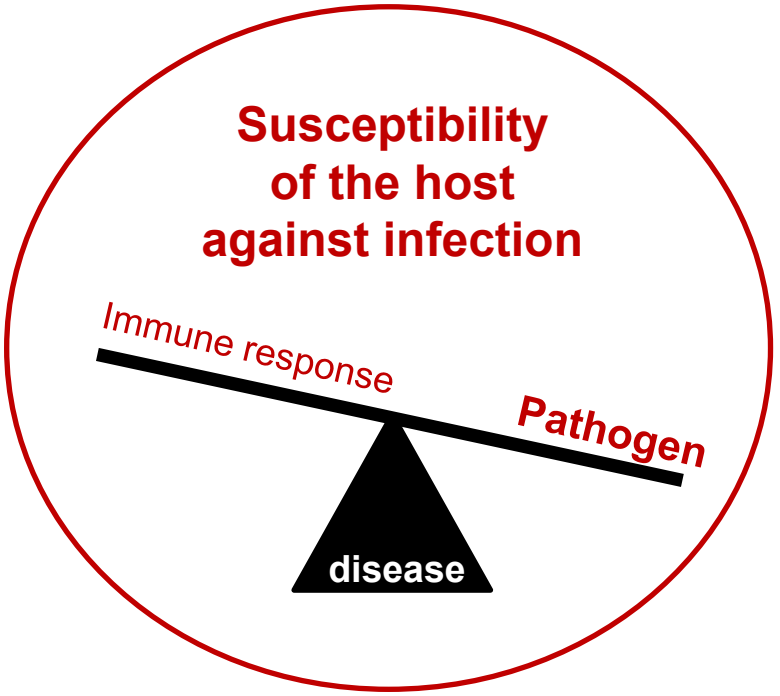



Enrofloxacin

# Infections in human and animals




Future task: Focus on the usage of antibiotics that modulate the innate immune response in a protective way?

# Infection Biochemistry

## Research Group Infection Biochemistry
## Department of Biochemistry
## University of Veterinary Medicine Hannover, Germany

# Support

H. Wilhelm Schaumann Stiftung

Bundesministerium für Bildung und Forschung

DFG

Einzelantrag KO355214

Deutsche Akademie der Naturforscher Leopoldina

J&G Bruns-Stiftung für innovative Veterinärmedizin

Friedrich Ebert Stiftung

VolkswagenStiftung

DAAD
Deutscher Akademischer Austausch Dienst
Servicio Alemán de Intercambio Académico

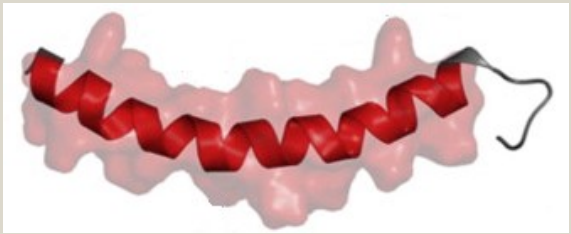
AfT
Akademie für Tiergesundheit

Lifelong Learning Programme

Stiftung Tierärztliche Hochschule Hannover
University of Veterinary Medicine Hannover, Foundation

# Wie alte Waffen helfen, neue therapeutische Ansätze gegen Infektionskrankheiten zu finden

## *„Old weapons against infectious diseases“*



**Prof. Dr. Maren von Köckritz-Blickwede,**
**Infection Biochemistry,**
**Department of Biochemistry & Research Center for Emerging Infections and Zoonoses, TiHo Hannover, Germany**

# Stärkung des Immunsystems

## Unser Ziel:
## Suche nach neuen therapeutischen Ansätzen gegen Infektionskrankheiten: Naturprodukte, die das Immunsystem stärken!

**Testung von Medizinalpflanzen aus dem tropischen Regenwald Costa Ricas:**

➔ Verstärkung der Phagozytose von Neutrophilen

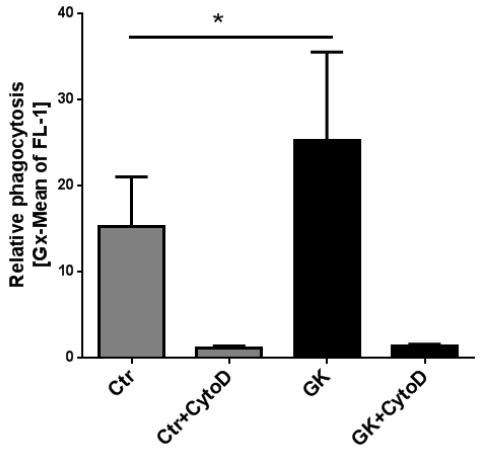
➔Verstärkung der NETs

# GUKUBA stimuliert Immunzellen

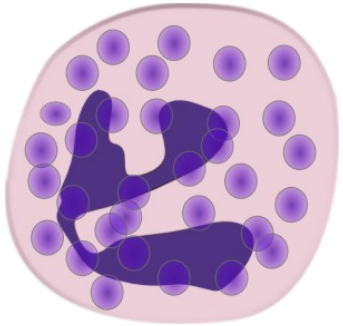
- Rindenextrakt von **Guarea kunthiana** (wächst im tropischen Bergwald von Süd- und Zentralamerika)
- Verwendung als Medizinalpflanze zur Behandlung von Depressionen und Asthma
- Rindensaft wird auch in Ecuador gegen Malaria eingesetzt.
- Mechanismus unklar!!!

# GUKUBA stimuliert Immunzellen

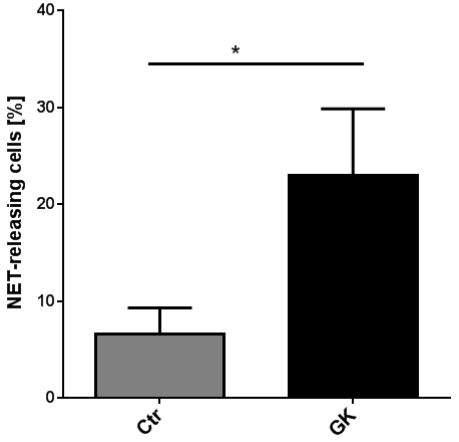
**bovine Neutrophile**




## Phagozyten



**bovine Neutrophile**




### Phagozytose

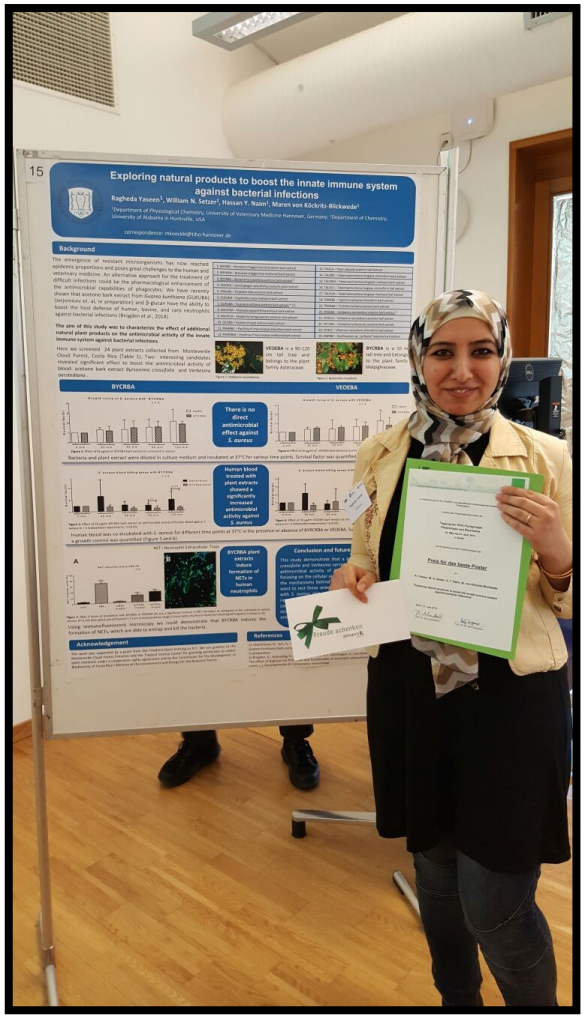
(intrazelluläre Aufnahme und Töten von Mikroorganismen)

### Extrazelluläre DNA-Netze

(Einfangen und Töten von Mikroorganismen)

## Abwehr gegen Infektionserreger

# Posterpreis für Ragheda Yaseen




*“Exploring natural products to boost the innate immune system against bacterial infections”*

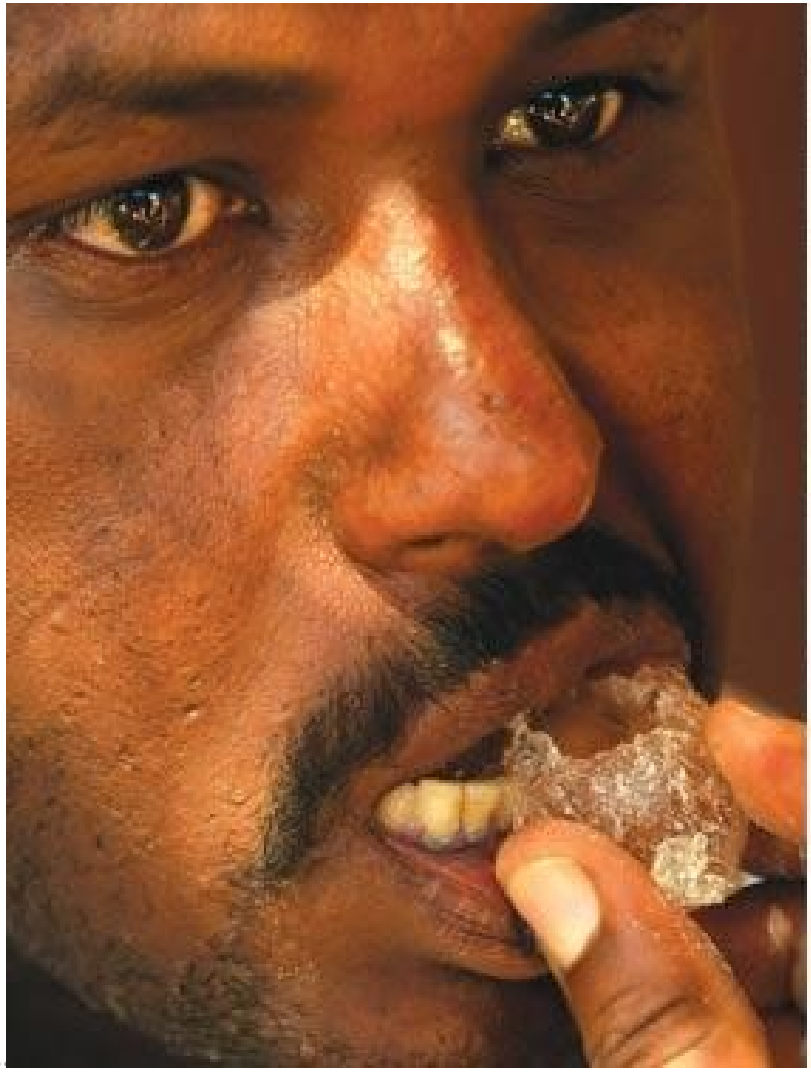
Deutsche Veterinärmedizinische Gesellschaft
Fachgruppe „Physiologie und Biochemie“,
Berlin, April 2016

# Gum Arabic/Gummiarabikum

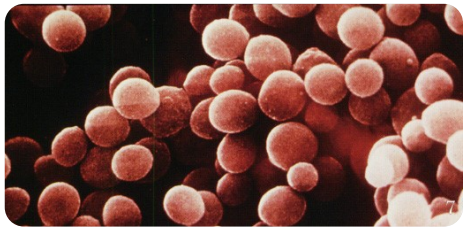
- Gum Arabic (GA), ist ein Exsudat aus dem Pflanzensaft von Verek-Akazien (*Acacia Senegal)* und Seyal-Akazien (*Vachellia seyal*)

- Zusammensetzung
  - Zucker
  - Glucuronsäure
  - Mineralien
  - Proteine


# Medizinalpflanze

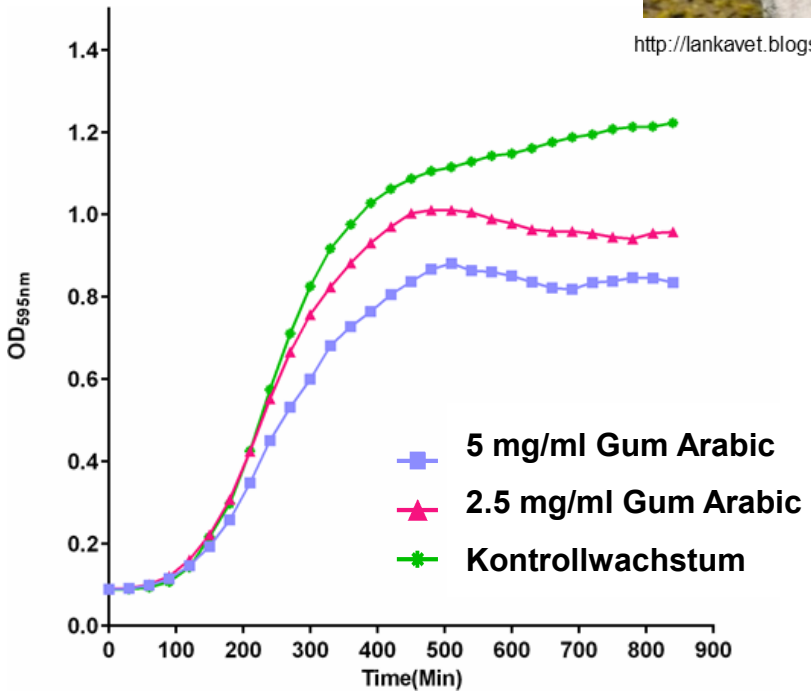


# Gum Arabic hemmt das Bakteriumwachstum



*Staphylococcus aureus*

http://lankavet.blogspot.de/2013/01/mastitis-in-cows.html

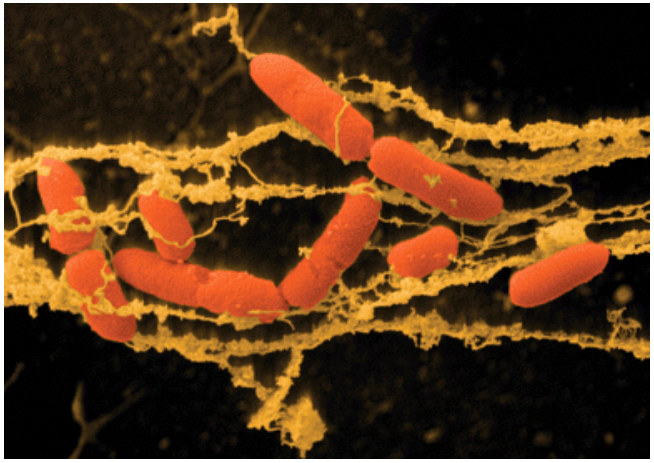


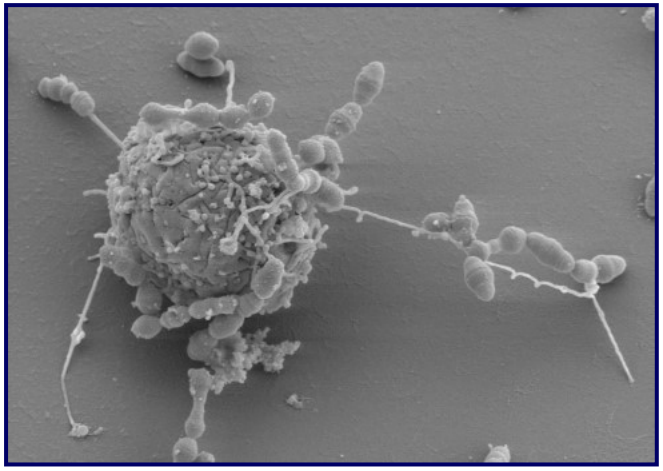


# Institut für Physiologische Chemie

**Unser Ziel:**

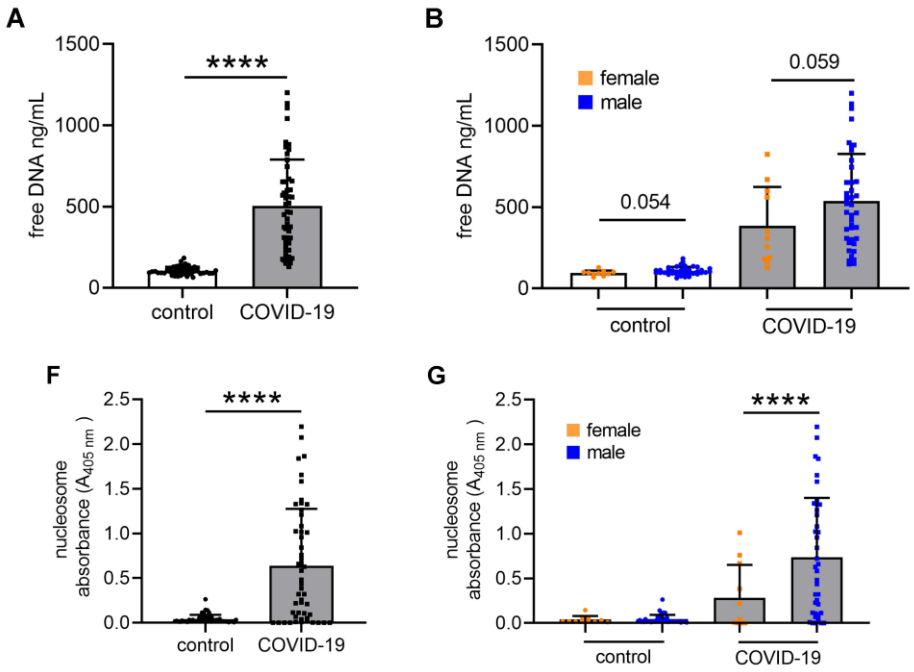
**Suche nach neuen therapeutischen Ansätzen gegen Infektionskrankheiten: Stärkung des Immunsystems!**





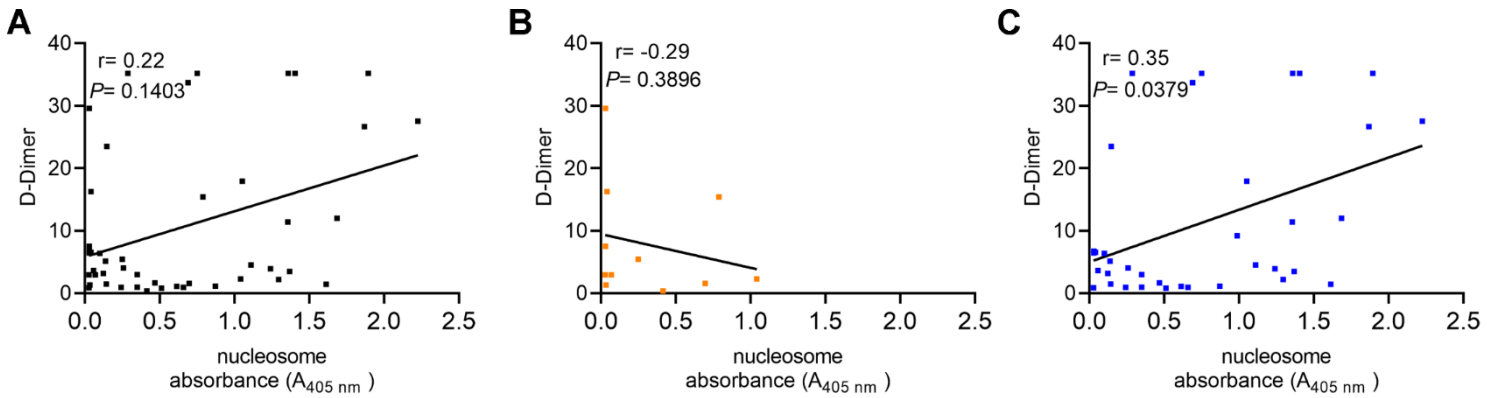
Fotos: Abwehrzellen fangen Bakterien mit NETzen.

# NETs & SARS-CoV-2 in serum of COVID-19 patients



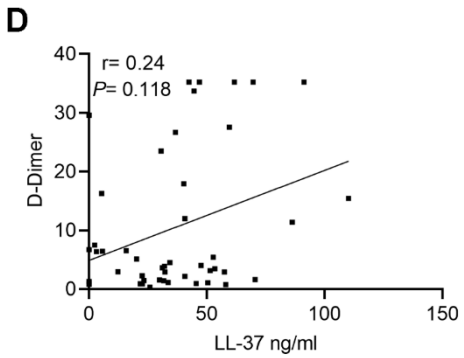

Increased free eDNA and nucleosome level as NET marker were detected in COVID-19 patients compared to healthy controls.

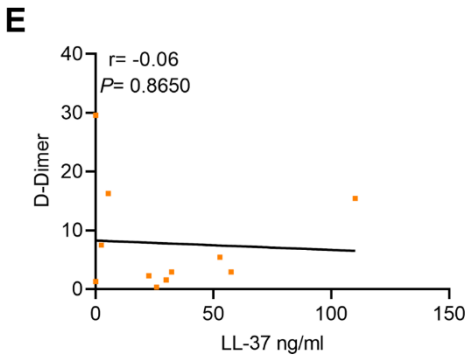
# NETs & SARS-CoV-2 in serum of COVID-19 patients

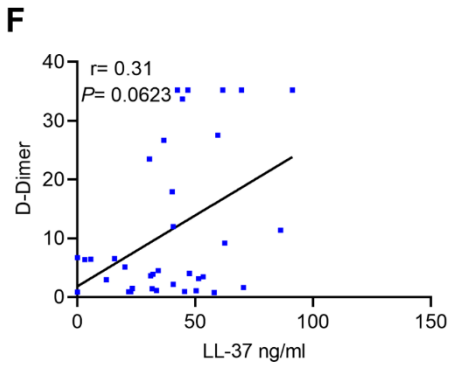



- Severe infection of COVID-19 exhibit more obvious dysregulated coagulation function compared with mild cases. This is shown by a higher level of D-dimer (Snawa et al., 2021)
- Nucleosomes as marker for NETs strongly correlated with D-dimer
- This confirms previous published data that NETs and fibrin from a composite network within thrombin that triggers the risk for thrombosis in COVID-patients.

# NETs & SARS-CoV-2 in serum of COVID-19 patients



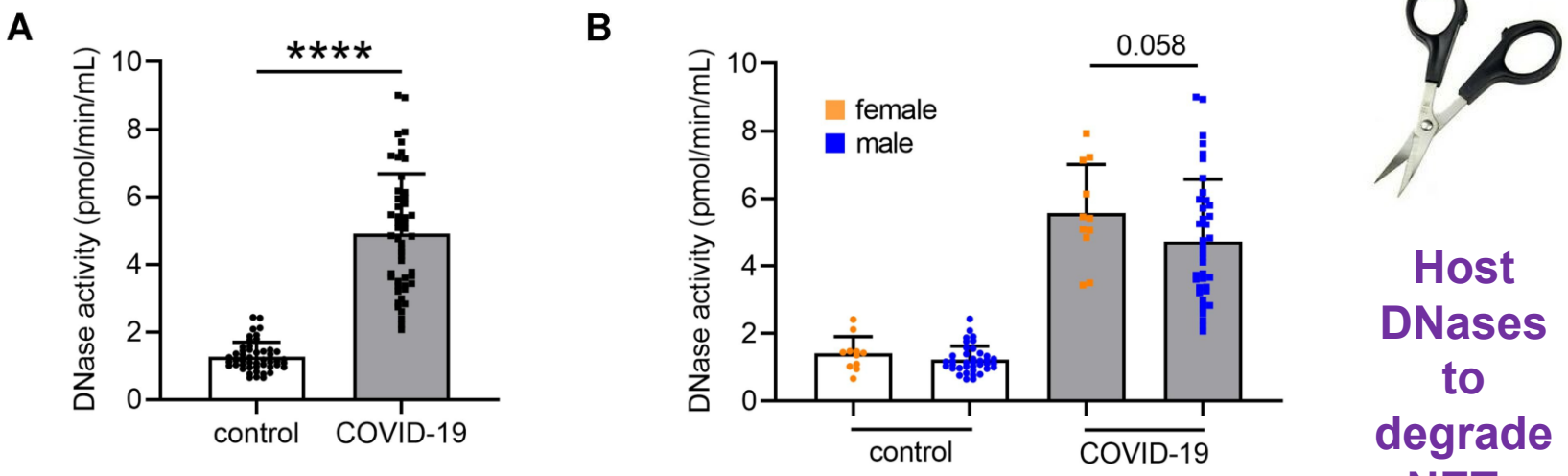




**Cationic antimicrobial peptide LL-37, stabilizes NETs**

- **LL-37 level correlate with D-Dimer level in male COVID-patients**
- **Potential additional role of LL-37 in the risk of NET-associated thrombosis of male COVID-patients by stabilizing NETs?**

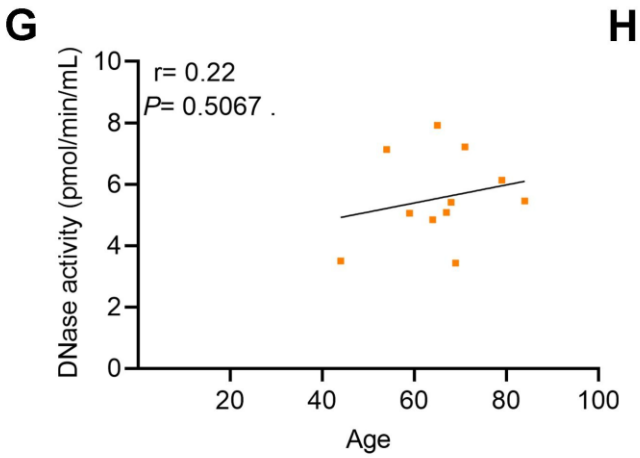
## NETs degradation by nucleases

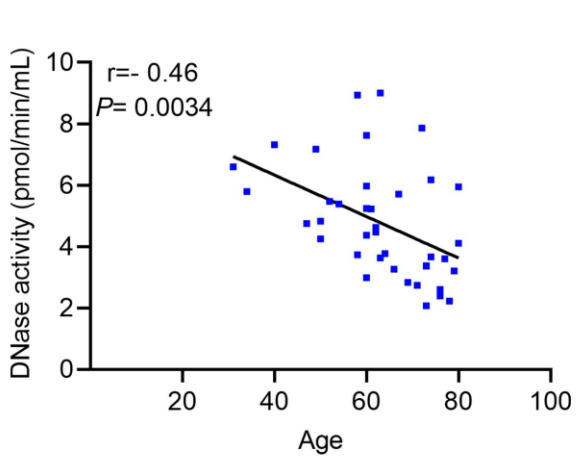



**Host DNases to degrade NETs**

- Increased DNase activity as a response to NET release in COVID patients compared to healthy control.
- No difference in male compared to female.

## NET degradation by nucleases




**Host DNases to degrade NETs**

- Increased DNase activity as a response to NET release in COVID patients compared to healthy control.
- No difference in male compared to female.
- **Lower DNase activity in elderly male patients.**

## Summary: NETs & SARS-CoV-2

1. NETs are highly dicussed in literatur to contribute to COVID-19-severity.
2. Our data confirm increased NET marker in COVID-19 patients compared to healthy controls.
3. NETs are confirmed in biopsies from COVID-19 patients.
4. NET-stabilizing peptide LL-37 is shown to bind NETs that are produced by neutrophils in COVID-patients.
5. LL-37 level correlate with D-Dimer level similar as NET-marker in male COVID-patients, indicating a potential additional role of LL-37 in the risk of NET-associated thrombosis of male COVID-patients.
6. LL-37 in NETs is confirmed in biopsies from COVID-19 patients.
7. Increased DNase activity is confirmed in COVID-patients, showing that patients react to increased NET-formation by producing NET-degrading enzymes e.g. DNase 1.
8. Male elderly patients show decreased DNase 1 activity, hypothesizing a potential risk factor for those patients in ist ability to degrade NETs.

**➔Two risk factors of male elderly patients: Reduced nuclease activity and increased LL-37 level which lead to inefficient NET degradation and**

# NETs & SARS-CoV-2

***Research Article***

## *Impaired degradation of neutrophil extracellular traps: a possible severity-factor of elderly male COVID-19-patients*

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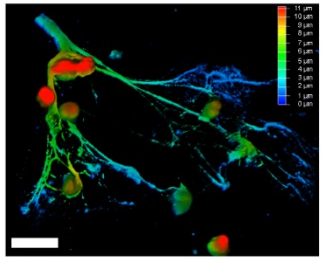

*Major revision, Journal of Innate Immunity*