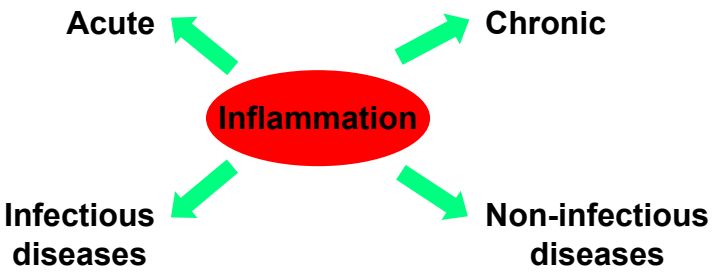
PROTÉASES DU SYSTÈME IMMUNITAIRE: RÔLE PRO- ou ANTI-INFLAMMATOIRE ?

A. Bentaher, Research Director, Inserm
Inflammation et Immunité de l'Epithélium Respiratoire
EA7426

azzak.bentaher@inserm.fr

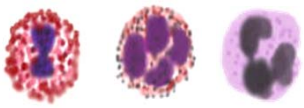
Leukocytes Recruitment : a Characteristic of Inflammation



Wherever inflammation occurs there are certain local mechanisms in common, despite differences in the precipitating factors,…: the recruitment of leukocytes from the circulation to the site of tissue damage.

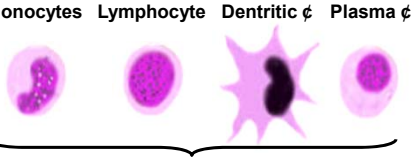
INFLAMMATION: ACUTE OR CHRONIC

Eosinophil Basophil Neutrophil



Granular leukocytes

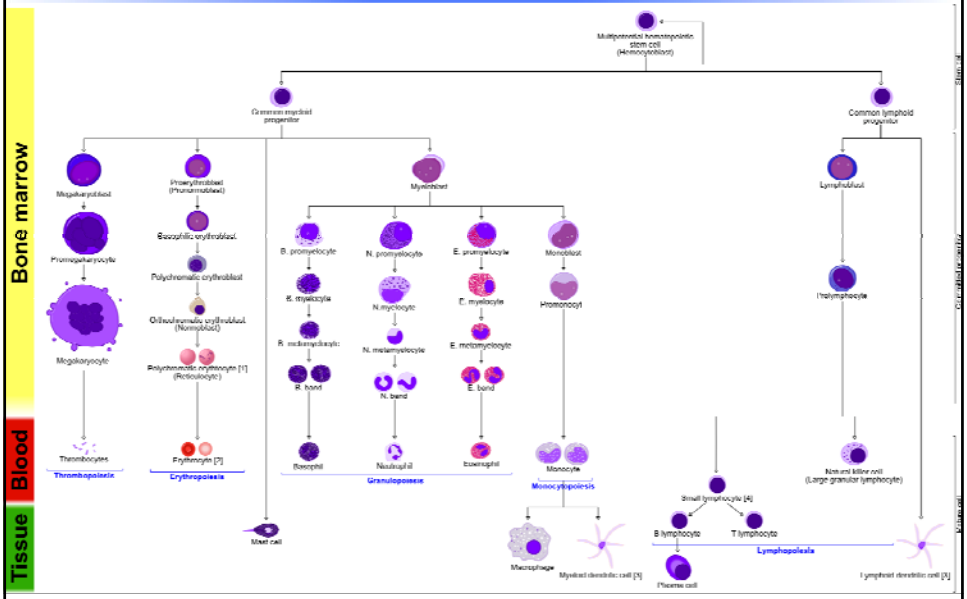
Monocytes Lymphocyte Dentritic ¢ Plasma ¢



Agranular leukocytes

Diseases: Pulmonary, Cardiovascular, Gastrointestinal, Nephrological, Arthritis
Cancer
etc…..

Hematopoiesis in humans



Protéases du Système Immunitaire: rôle pro-inflammatoire ou anti-inflammatoire

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LAB TEAM

Composition de l'équipe « Inflammation et Immunité de l'Epithélium Respiratoire » :

Nom Prénom	Titre	HDR
Bentaher Azzak	DR INSERM/PhD - Responsable	+
Devouassoux Gilles	PU-PH/PhD	+
Menotti Jean	MCU-PharmD/PhD	+
Calender Alain	PU-PH/PhD	+
Pacheco Yves	PU-PH/PhD	+
Pizzoccaro Anne	IE (CDD)	
Rebaud Chloé	AI (CDD)	
Bougherira Nedra	Master 2	
Josse Emilie	Master 1	
Perrichet Julien	Master 1	

- Recrutement d'un(e) étudiant(e) en thèse (IDEX)

- Acceuil de chercheurs: F. Agostini (Technicienne de recherche et de formation)

- Stagiaires à travers des programmes d'échange internationaux

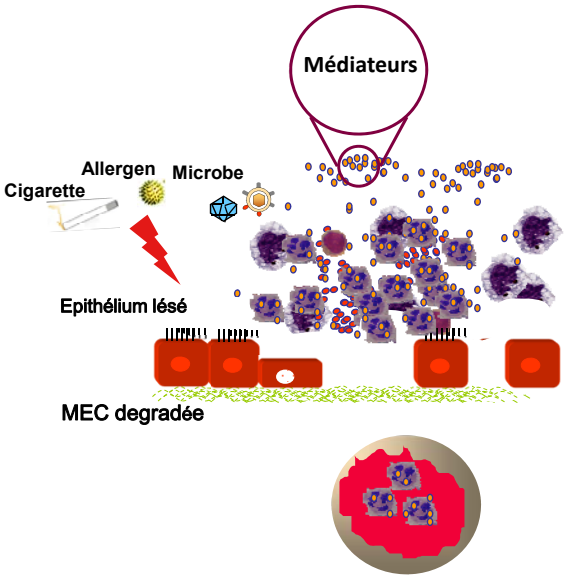
We are here

Locaux: Faculté de Médecine Lyon Sud Charles Mérieu

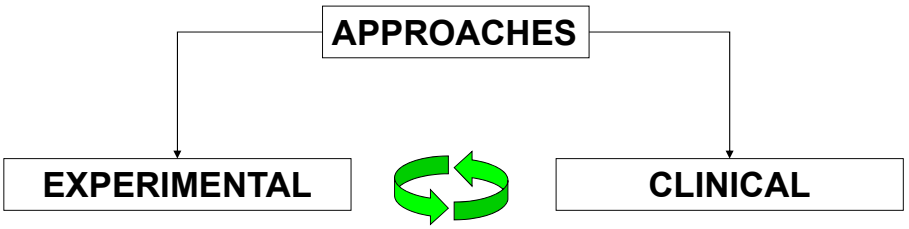
Laboratoire
~200 m² + 6 bureaux

Accès aux plateformes techniques communes dans le bâtiment : Cytométrie et tri cellulaire - Animalerie conventionnelle/sécurisée - Salle sécurisé de microbiologie, Salle d'imagerie, Salle d'histologie….

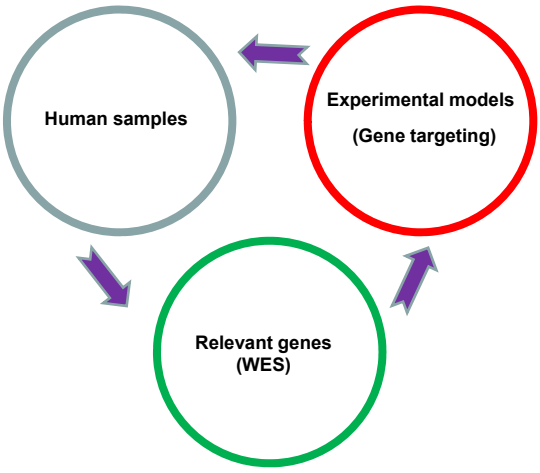
Accès aux plateformes techniques:
SFR Biosciences (https://www.sfr-biosciences.fr) et
SFR Santé Lyon-Est (*https://sfrsantelyonest.univ-lyon1.fr*)



TRANSLATIONAL RESEARCH STRATEGY



TRANSLATIONAL RESEARCH STRATEGY

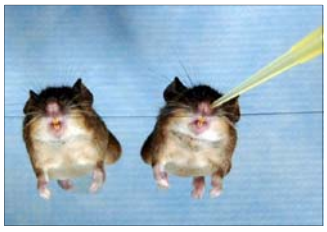


DESIGN: EXPERIMENTAL & TRANSLATIONAL

- *In vivo*: WT & KO mice & lung disease models
- *Ex vivo*: Primary cells & cell lines
- *In vitro*: Purified proteins of interest
- *Human biopsies and fluids*

EXPERIMENTAL MODELS

Intranasal instillation

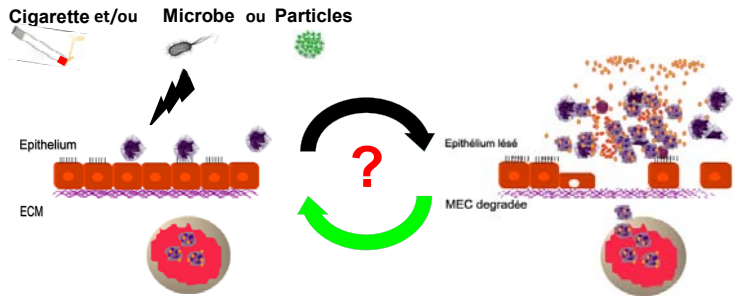


Septicemia

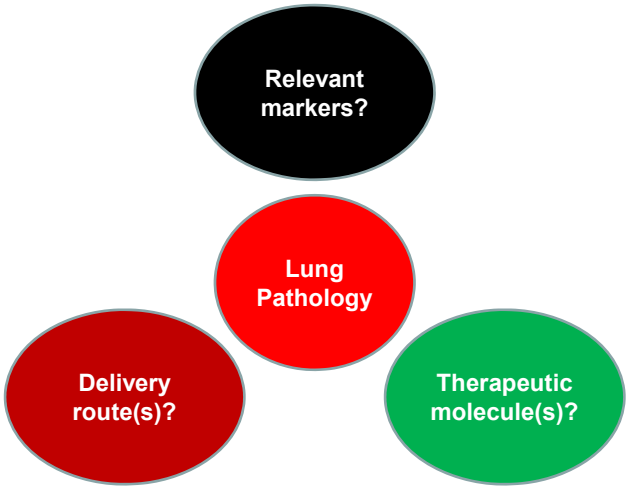
COPD exacerbations

Air pouch

RESEARCH PROGRAM



THERAPEUTIC STRATEGY



PROTEASES and ALLERGIC DISORDERS

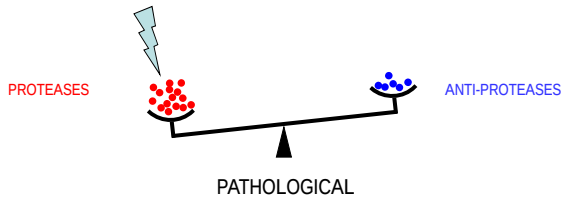
- Proteases: Specific hydrolysis of peptide bonds in proteins

- Irreversible: activation, inactivation
  or degradation of targeted protein

- Physiologic roles:
  Immunity, blood coagulation, apoptosis, inflammation, angiogenesis, tissue remodeling….

- Pathophysioloc roles:
  Pulmonary diseases, arthritis, cancer…..

Proteases-antiproteases imbalance hypothesis



PROTEASES and ALLERGIC DISORDERS

Proteases-antiproteases balance

↓

Maintenance of epithelial barriers

Proteases-antiproteases imbalance

↓

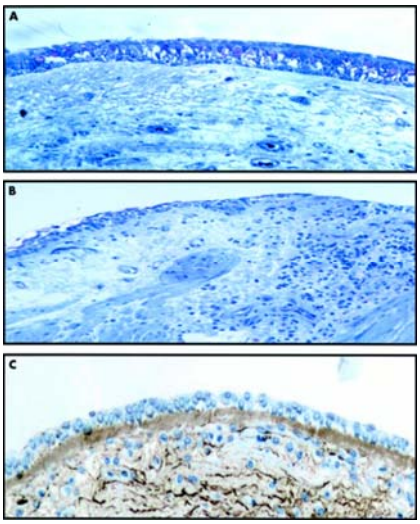
Disruption of epithelial barriers

↙ ↘

Allergic sensitization and inflammation

airway pathophysiology and remodeling in Asthma

Comparison of (A) normal and (B, C) asthmatic airway wall showing epithelial damage, increased smooth muscle, inflammatory cell infiltration, and sub-basement membrane thickening.



P A Beckett, and P H Howarth Thorax 2003;58:163-174

THORAX

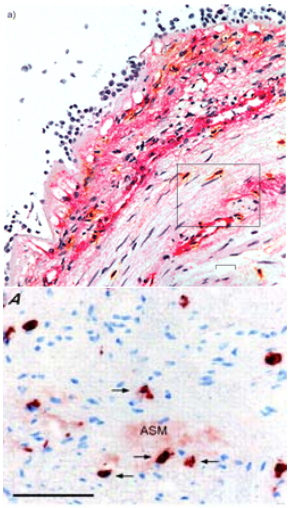
MAST CELLS: KEY PROTEASE SOURCE

- IgE-mediated type I hypersensitivity (e.g., asthma, rhinitis, and dermatitis)

- Within smooth muscle bundles

- Density correlates with bronchial hyperresponsiveness

- Central role in promoting airway remodeling and inflammation



EVIDENCE FOR GENETIC LINKS

Single Nucleotide Polymorphism (SNP)

DPP10 ⇒ Exotaxin and CCL5 (RANTES) cleavage

Chymase promoter ⇒ Increase of IgE levels in AD

α1-antichymotrypsin ⇒ Asthma,

PAI1 ⇒ Asthma

MAST CELL-DERIVED PROTEASES: KEY PLAYER

- Proteases account for around 25% of total MC protein

- MC-specific proteases: tryptase and chymase

- 10-35 pg of tryptase and chymase per one cell

Central role
in promoting airway remodeling and inflammation

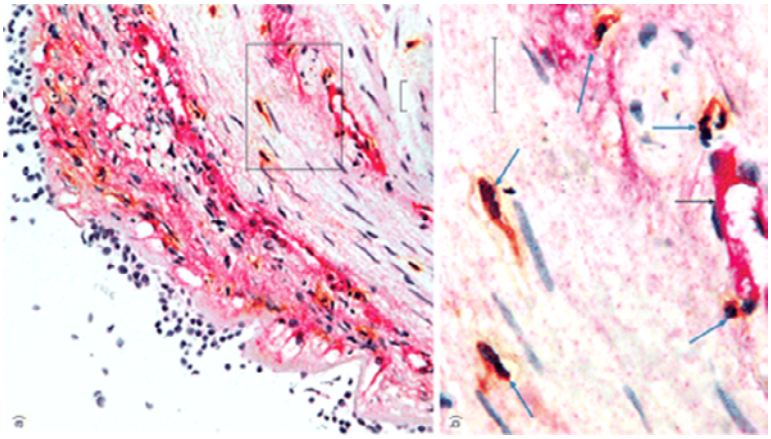
TRYPTASE

- Large quantities following allergen challenge.

- High levels of transcript and immunoreactive protein are found in asthmatic bronchial epithelial biopsies.

- Basal level of tryptase concentration is higher in BALF of atopic asthmatics, further increased in response to allergen challenge

Mast cell-derived tryptase in airway smooth muscle layer



TRYPTASE

- Sérine-protéase tétramérique de masse moléculaire 134 kDa
- Essentiellement sécrétée par les mastocytes
- Médiateur de la réaction d'hypersensibilité immédiate
- Demi-vie plus longue que l'histamine (1,5 à 2,5 heures)
- 20 à 50 % des protéines mastocytaires

TRYPTASE

- Interacts with protease activated receptors (PAR-2) on ASM leading to constriction

- Potentiates the action of known constrictors like histamine

- Cleaves extracellular matrix

- Activates matrix cleaving proteases

TRYPTASE

- Can also act as mitogens

- Causes degranulation of nearby MCs

- Cleaves interleukin IL-33 to generate its potency

- Tryptase inhibition suppresses IL-33-dependent allergic airway inflammation

TRYPTASE

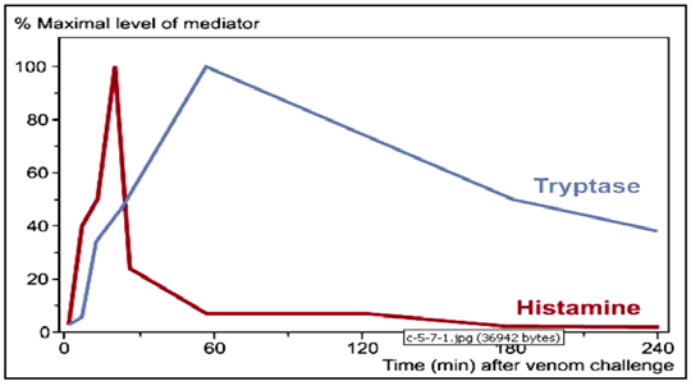
Deux formes moléculaires dans le plasma :

- **Tryptase α :**
  - **Forme physiologiquement sécrétée par les mastocytes**
  - **Considérablement augmentée dans les mastocytoses systémiques**
  - **Responsable du taux basal sérique**

- **Tryptase β :**
  - **Forme stockée dans les granules mastocytaires**
  - **Biologiquement active**
  - **Libérée avec l'histamine au cours des réactions anaphylactiques**

- **Libération de tryptase → activation mastocytaire**

TRYPTASE



TRYPTASE

Table 1. Biological processes in which tryptase has been implicated. MS, Multiple sclerosis; EAE, experimental autoimmune encephalomyelitis; SIDS, sudden infant death syndrome.

	Type of implication			
	Elevated tryptase levels	Tryptase induces process	Tryptase inhibitor reduces response	Reference
Airway hyper-responsiveness/ inflammation	+	+	+	[87,89–92,159–161]
Neutrophil recruitment		+		[26,68,97]
Eosinophil recruitment		+		[97]
Vascular permeability increase		+		[96]
Fibrosis	+			[109]
Sepsis				[121]
Ulcerative colitis			+	[167]
Angiogenesis		+		[122,124]
Arthritis	+			[104,178]
MS/EAE	+			[106,179]
SIDS	+			[103]
Duchenne muscular dystrophy	+			[124]
Psoriasis	+			[107,180]
Joint inflammation		+	+	[150]
Intestinal inflammation			+	[151]
Atopic dermatitis	+			[109]
Tumor cell proliferation		+		[144]
Itching		+		[152]

TRYPTASE

Table 2. Tryptase substrates. VIP, Vasoactive intestinal peptide; PHM, peptide histidine-methionine; CGRP, calcitonin gene-related peptide; HDL, high density lipoprotein; pro-uPA, pro-urokinase plasminogen activator; proMMP, pro-matrix metalloprotease; PAR, protease activated receptor.

	Cleavage identified in/when:			
	Mixture of purified components	Tryptase added to cell culture or tissue	*In vivo*	Reference
Kininogen		+		[126]
Prekallikrein		+		[126]
Fibrinogen	+	+		[30,125]
Gelatin	+			[135,136]
VIP	+			[128]
PHM	+			[129]
CGRP	+			[129]
Pro-uPA	+			[137]
Fibronectin	+	+		[54,83–85]
HDL	+	+		[127]
proMMP-3	+	+		[132,133]
PAR-2		+	+	[120,140,141,151]
Type VI collagen	+	+		[181]
Pre-elafin	+			[182]

CHYMASE

- Degrades matrix proteins

- Activates matrix metalloproteases

- Cleaves tight junction proteins. Thus,
  increasing epithelial permeability,
  sensitization by increasing access to foreign antigens

- Cleaves and activates:
  proIL-1 β, proIL-18, CCL-6, CCL-9, and CCL-15

NON MAST CELLS-SPECIFIC PROTEASES

Cathepsin G

- Cleave both tryptic and chymotryptic substrates.
- Functions as chymase
- Activates matrix metalloproteases

Cathepsin C

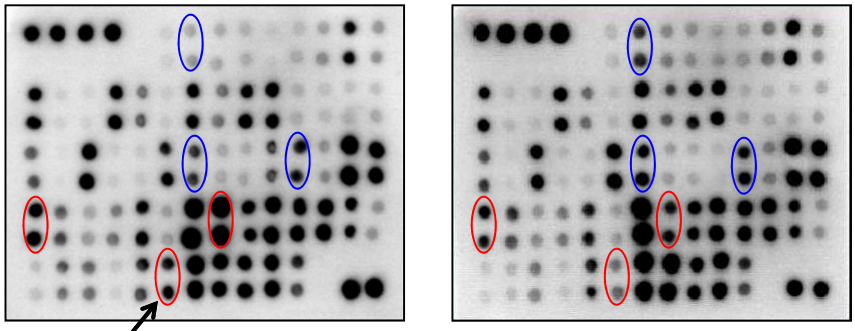
- Has endoproteolytic activity
- Activates of chymases, cathepsin G, and tryptases

Matrix metalloprotease 9

- activated by chymases,
- degradation of extracellular matrix

Altered cytokine levels in infected cell-free BALs in the absence of NE

Cytokine antibody microarray



ALLERGEN-DERIVED PROTEASES

- No unique structure or function responsible for allergenicity

- Enzymatic activity (particularly protease activity) of some proteins contributes to allergenicity.

- Various clinically relevant sources:
  - house dust mite (HDM),
  - cockroach,
  - pollen,
  - and fungi

ALLERGEN-DERIVED PROTEASES

Mite allergens		
Blo t 1	*B. tropicalis* (mite)	Cysteine protease
Blo t 3	*B. tropicalis* (mite)	Trypsin
Blo t 6	*B. tropicalis* (mite)	Chymotrypsin
Der m 1	*D. microceras*	Cysteine protease
Der P 1	*D. pteronyssinus*	Cysteine protease
Der P 9	*D. pteronyssinus*	Collagenolytic serine protease
Eur m 1	*E. maynei*	Cysteine protease
Cockroach allergens		
Bla g 2	*B. germanica*	Aspartic protease
Per a 2	*P. americana*	Aspartic protease-like
Per a 10	*P. americana*	Serine protease
Food allergens		
Act d 1	*A. deliciosa* (kiwi fruit)	Cysteine protease
Cuc m 1	*C. melo* (muskmelon)	Alkaline serine protease

A. mellifera - Apis mellifera, B. pensylvanicus - Bombus pensylvanicus, A. aegypti - Aedes aegypti, A. artemisiifolia - Ambrosia artemisiifolia, A. alternate - Alternaria alternate, A. flavus Aspergillus flavus, A. fumigatus - Aspergillus fumigatus, A. niger - Aspergillus niger, A. oryzae - Aspergillus oryzae, A. versicolor - Aspergillus versicolor, C. cladosporioides - Cladosporium cladosporioides, C. herbarum - Cladosporium herbarum, C. lunata - Curvularia lunata, E. purpurascens - Epicoccum purpurascens, F. proliferatum - Fusarium proliferatum, P. brevicompactum - Penicillium brevicompactum, P. chrysogenum · Penicillium chrysogenum, P. citrinum · Penicillium citrinum, P. oxalicum · Penicillium oxalicum, T. rubrum · Trichophyton rubrum, T. tonsurans · Trichophyton tonsurans, R. mucilaginosa - Rhodotorula mucilaginosa, B. tropicalis - Blomia tropicalis, D. microceras - Dermatophagoides microceras, D. pteronyssinus - Dermatophagoides pteronyssinus, E. maynei - Euroglyphus maynei, B. germanica - Blattella germanica, P. americana - Periplaneta Americana, A. deliciosa - Actinidia deliciosa, C. melo - Cucumis melo, P. dominula - Polistes dominula, CUB - Complement C1r/C1s, Uegf Bmp1 domain, IUIS - International union of immunological societies

PROTEASES-ANTIPROTEASE IMBALANCE

- Correlation between severity of nasal allergen challenge and the amount of endogenous protease inhibitor

- α1-antitrypsin, secretory leukoprotease inhibitor (SLPI), and elafin

- Secreted in the lung lining fluids and protect the respiratory tract from proteolysis by proteases.

PROTEASES-ANTIPROTEASE IMBALANCE

- SLPI blocks and inactivates mast cells and leukocyte serine proteases that are implicated in allergic diseases

- An imbalance between proteases and antiproteases: reported in the nasal mucosa of allergic rhinitis patients

- Der p 1 is known to cleave and inactivate α1-antitrypsin.

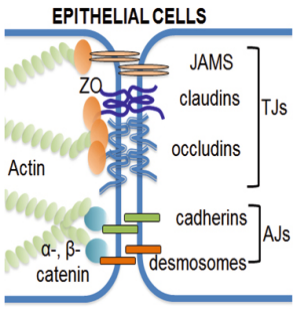
Inflammatory responses at the epithelial surfaces?

DISRUPTION OF EPITHELIAL BARRIER

- The airway epithelium: first line of defense against inhaled insults (pollutants, irritants, pathogens, and aeroallergens)

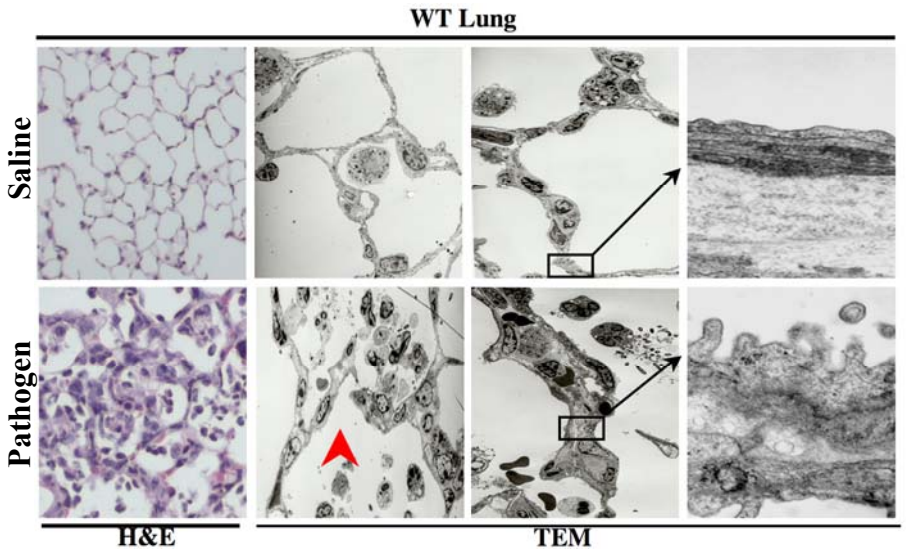
- Intercellular epithelial junctions comprise of tight junctions, adherens junctions, and desmosomes

- Maintain the epithelial barrier and protect the underlying tissue from the inhaled substances.

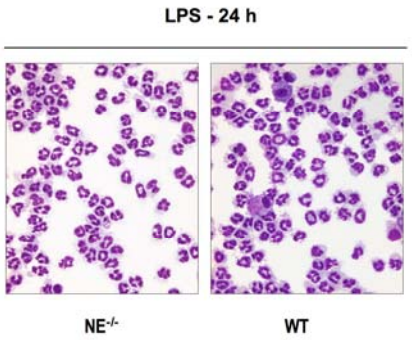


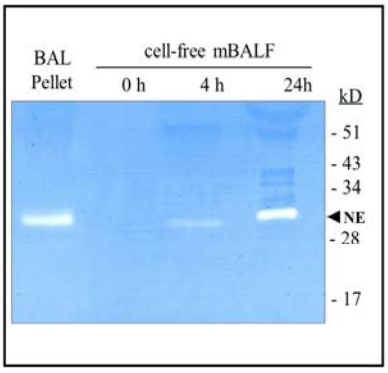
- Defective and disrupted epithelial barrier in allergic diseases such as asthma and dermatitis

Pathogen-induced acute lung injury



LPS-induced acute lung inflammation and injury



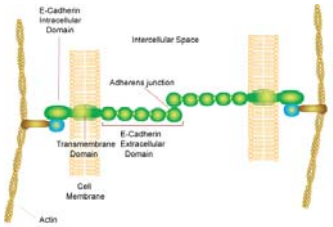


E-cadherin (E-cad)

- Member of the cadherin superfamily

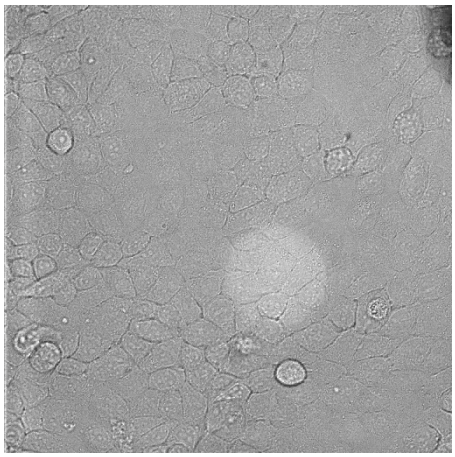
- Expressed in various epithelia (e.g., Lung)

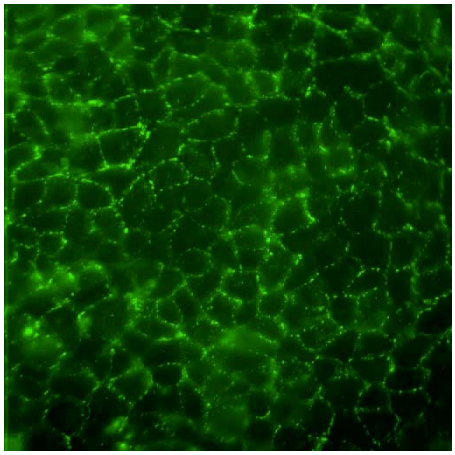
- Physiologic functions include:



- ✓ Cell-cell adhesion
- ✓ Cytoskeletal and tissue organisation
- ✓ Morphogenesis (cell recognition and sorting)
- ✓ Maintenance of cell structure
- ✓ Tissue polarity
- ✓ Cell migration, proliferation and survival

Loss of membrane integrity in the presence of NE





DISRUPTION OF EPITHELIAL BARRIER

- Allergens with protease activity shown to disrupt airway epithelial barrier by cleaving tight junction proteins.

- Der p 1 :
  - cellular detachment of epithelial cells
  - epithelial injury
  - increasing permeability to serum albumin.

- HDM fecal pellets (HDMFPs):
  - increased epithelial permeability
  - and disrupted tight junctions

DISRUPTION OF EPITHELIAL BARRIER

- Der p 1 in HDMFP:
  - disruption of epithelial barrier
  - cleavage sites are present on occludin and claudin 1.

- Similar studies with pollen proteases with similar findings:
  - Cleavage of tight junction proteins
  - Disruption of epithelial barrier integrity

Allergic sensitization
(delivery of aeroallergens across disrupted epithelium
and allergic inflammatory reactions)

OTHER PROTEASE EFFECTS

- Activation of Airway and Bronchial Epithelial Cells
  secretion proinflammatory cytokines

- Modulation of Functions of Immune Cells
  Mediator expression and cell polarisation

- Cleavage of Cell Surface Receptors
  cleavage of CD23 increasing IgE synthesis

POTENTIAL THERAPEUTIC STRATEGIES

- ✓ *Control of excessive immune cell recruitment*
- ✓ *Modulation of cell activation/degranulation (e.g., Protease release)*
- ✓ *Protease inhibition, but with caution*

PROTEASE INHIBITORS AS POSSIBLE THERAPEUTICS

- A balance between endogenous proteases and their inhibitors is necessary for normal homeostasis, e.g. maintenance of epithelial barrier.

- A disruption in this balance leads to the disruption of epithelial barrier resulting in allergic sensitization and inflammation.

- Proteases: targets for developing therapeutics against allergic diseases.

PROTEASE INHIBITORS

- bis-amidines, when used with peptidic inhibitors:
  ↙ airway inflammation

- MOL6131, a nonpeptide inhibitor of lung MC tryptase:
  ↙ allergic features

- Tryptase inhibitor:
  ↙ bronchoconstriction in mild atopic asthmatics.

- APC366, a tryptase inhibitor:
  ↙ antigen-induced late asthmatic response

PROTEASE INHIBITORS

- Gabexate mesylate (FOY) and nafamostat mesilate (FUT), synthetic serine protease inhibitors attenuated

↓ airway eosinophilia
↓ IgE production,
↓ IL-4, and tumor necrosis factor-α levels

↑ IL-12 and IL-10 levels

↓ Der p 1-induced
↓ airway hyperresponsiveness
↓ airway remodeling
↓ Th2 cytokines
↓ Th17 cell function
↓ nuclear factor-κB activation

- AEBSF, a serine protease inhibitor

↓ allergic airway inflammatory parameters

PROTEASE INHIBITORS

SUN C-8257, Y-40613, and SUN C-8077, chymase inhibitors, therapeutic potential in AD in animal models

Human chymase and cathepsin G inhibitors

↓ airway hyperresponsiveness
↓ airway neutrophilia in a mice model exposed to tobacco smoke

NATURAL PROTEASE INHIBITORS

SLPI and urinary trypsin inhibitor (UTI) have been evaluated potential therapeutic agents.

SLPI,

- **allergen-induced pathophysiologic airway responses**
- **bronchoconstriction,**
- **AHR**
- **airway inflammation**

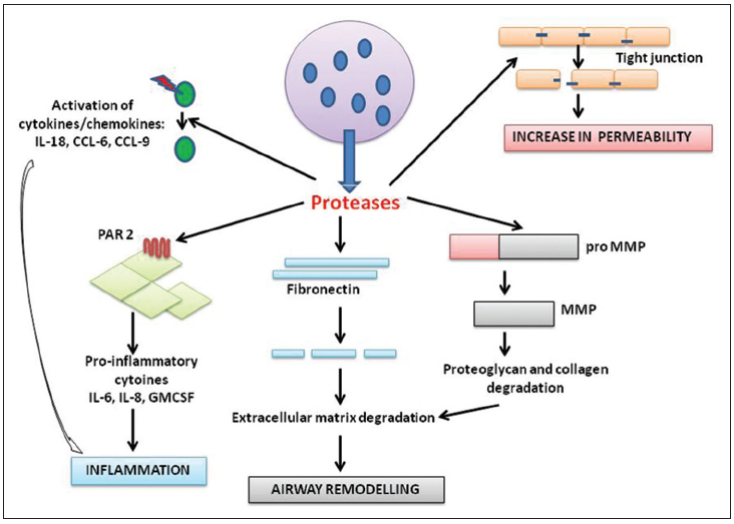
UTI, purified from a human source

- **allergic inflammatory symptoms in house dust mite challenged**

ONGOING PROJECT

Human serum albumin nanoparticles as a nanovector carrier of therapeutic molecules: Application to neutrophil elastase and secretory leukocyte protease

TAKE HOME MESSAGE



TAKE HOME MESSAGE

- Protease-mediated mechanism in allergic responses, still poorly understood???

- Gaining insight ⇒ new targets for therapeutic

- Corticosteroids / allergic symptoms ?!
  side effects???

POTENTIAL THERAPEUTIC STRATEGIES

"Don't tell them we failed. Tell them we decided to temporarily postpone our success"

THANK YOU

TRYPTASE

- Grande variabilité inter-individuelle des valeurs usuelles :
  - valeur de base propre à chaque individu
  - unique et stable au cours du temps.
- Sujets sains: concentrations détectables varient de 1,9 à 13,5 µg

Activation of Airway and Bronchial Epithelial Cells

In vitro studies:
protease allergens activate airway epithelial cells
secretion proinflammatory cytokines.

Mounting evidence:
sensitization occurs at mucosal surfaces
proteolytic activity breaking the normal state of tolerance

Repeated exposure of airway mucosa:
lung eosinophilia and higher IgE/IgG1 production in a protease activity-dependent manner

Airway epithelial cells exposed to mite, timothy grass pollen, or birch pollen extracts showed secretion of IL-6, IL-8, granulocyte macrophage colony-stimulating factor, and monocyte chemotactic protein-1. [58],[59

Use of purified proteases Der p 1and Der p 9 demonstrated that this release of cytokines from the airway epithelial cells was dependent on the protease activity of the allergens. [60]

Der p 1 and Der p 5 activated human derived airway epithelial cells by both protease-dependent and protease-independent mechanisms. [61]
Asokananthan et al. showed that Der p 1-induced proinflammatory cytokine release from the respiratory epithelial cells was in part mediated by PAR-2. [62]

However, other reports have suggested that though Der p 1 is capable of cleaving PAR-2 peptide, it activates airway epithelial cells in a PAR-2-independent manner. [63]

Cockroach serine protease allergen Per a 10 has been shown to activate airway epithelial cells in a PAR-2-dependent manner. [64]

Modulation of Functions of Immune Cells

After crossing the epithelial barrier, protease allergens interact with cells of immune system and can modulate their functioning.

Per a 10 has been shown to potentiate dendritic cells derived T-cell polarization toward type II by upregulating CD86, OX40 L expression and lowered IL-12 secretion. [65],[66]

These lowered IL-12 levels were associated with lower CD40 expression on DCs probably by cleavage of CD40 by Per a 10. [67]

Priming of naive CD4+ T-cells with active Per a 10 pulsed DCs showed high Th2 cytokines IL-4, IL-5, and IL-13 and lowered IL-12 secretion as compared to inactive Per a 10 pulsed DCs. [68]

Der p 1 has also been reported to lower IL-12 expression by monocyte-derived dendritic cells by CD40 cleavage. [68]

A study has also demonstrated that Th2 response development after protease challenge requires a cooperation between DCs and basophils and it occurs through ROS. [69]

Protease allergens can also induce basophils in an IgE-independent manner to produce IL-4 and IL-13. Basophils may act as an early source of IL-4. [70] This early IL-4 is speculated to be involved in the establishment of type 2 immune responses. [71],[72]

Naive T-cells can also act as an early source of IL-4 as they have been shown to express PAR-2 receptors and secrete IL-4 on interaction with papain. [73]

Along with basophils, proteases can also activate MCs leading to the production and secretion of IL-4. [72]

Cleavage of Cell Surface Receptors

Protease allergens promote Th2 responses by hampering Th1 and Treg responses, and this is achieved by cleavage of a myriad of receptors on different cells.

Der p 1 a major cysteine protease from HDM may enhance IgE responses by cleaving CD23 from the surface of activated B-cells. [74]

Membrane-bound CD23 sends a negative feedback signal when bound to IgE that downregulates IgE secretion, cleavage of CD23 switches off this negative feedback signal thereby increasing IgE synthesis. [75]

Subsequently, it has also been demonstrated that Der p 1 can cleave CD25, α-subunit of IL-2 receptor which inhibits IL-2 mediated T-cell proliferation and interferon-γ production thereby shifting the Th1/Th2 balance toward Th2. [76]

Der p 1 has also been shown to cleave DC-SIGN and DC-SIGNR. Cleavage of DC-SIGN reduces binding of DC-SIGN to ICAM-3. [77]

ICAM-3 is an endogenous DC-SIGN receptor expressed by naive T-cells and along with ICAM-1 is involved in DC trafficking, DC-T-cell interaction, and polarization of immune response toward Th1. [78],[79]

DC-SIGN cleavage by Der p 1 can hamper Th1 responses thus favoring Th2 immune responses. [77]
Recently, cysteine protease allergen papain has been shown to cleave CD123 (IL-3α), an IL-3 receptor and suppress IL-3 mediated expansion of basophils. However, the implications of CD123 cleavage in allergic responses need further studies. [80]

PROTEASE ARSENAL

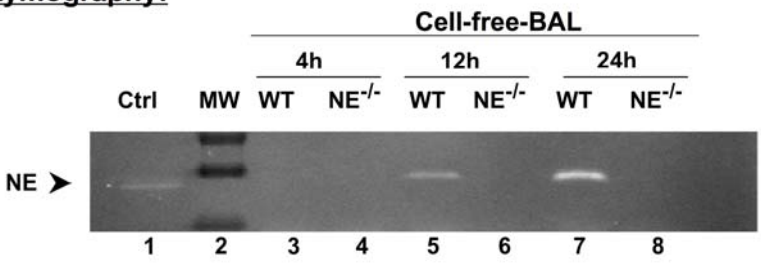
Classes and families

- Murine counterparts

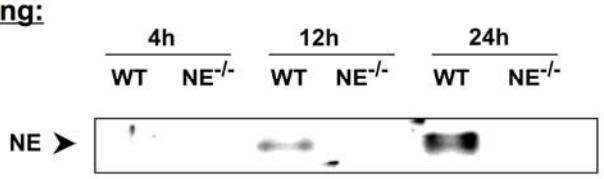
- Gene targeting

Extracellular NE activity increases over time in the setting of *P. aeruginosa* lung infection

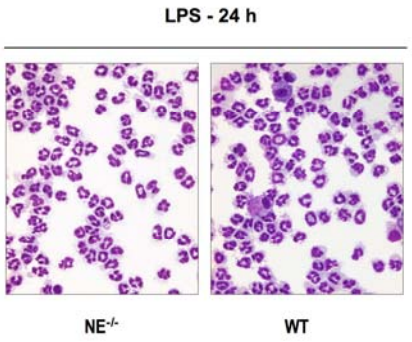
Elastin zymography:

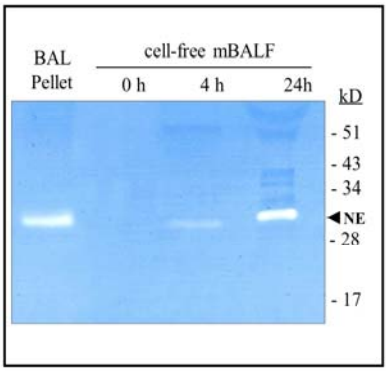


Western blotting:



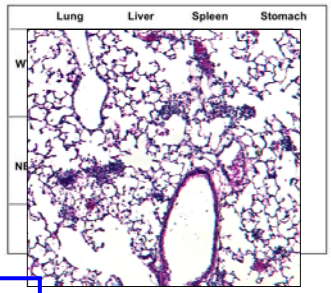
LPS-induced acute lung inflammation and injury





EXPERIMENTAL MODELS

Pneumonia



Collection of blood, BALs and lungs

Characterization of lung inflammation and tissue changes

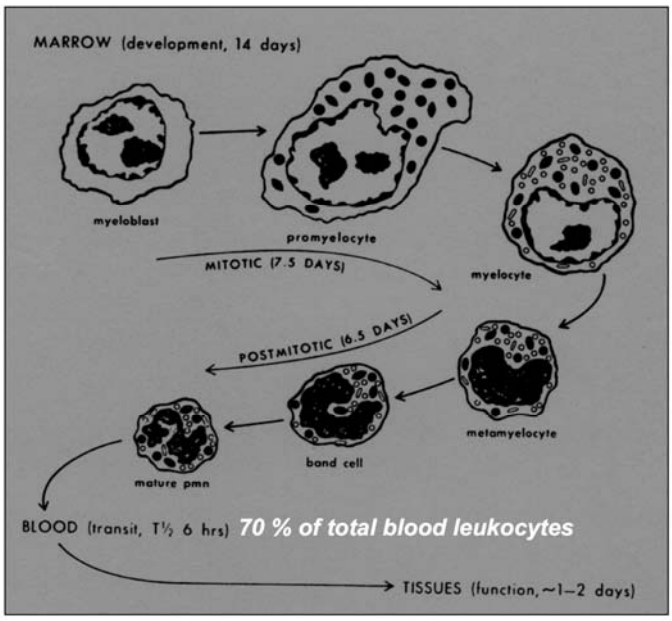
PROTEASE ARSENAL

Classes and families
Focus on one protease family

- Murine counterparts

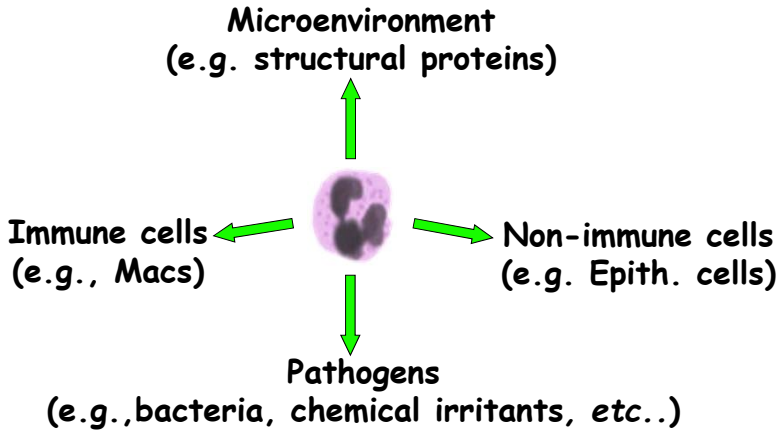
- Gene targeting

NEUTROPHIL DEVELOPMENT



INFLAMMATION: ACUTE OR CHRONIC

Neutrophil interactions:



The Polymorphonuclear Neutrophil
Pathogen killer or Pathogenic?

Abderrazzak Bentaher, Research Director, Inserm
Inflammation et Immunité de l'Epithélium Respiratoire
EA7426

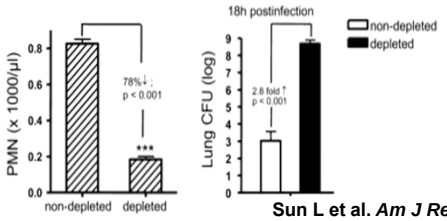
azzak.bentaher@inserm.fr

NEUTROPHIL DISORDERS

- Chronic Granulomatous Disease (CGD)
- Deficiency:
  - Myeloperoxidase
  - Secondary granule
  - CD18 (LAD)
- Neutropenia:
  - CN, SCN

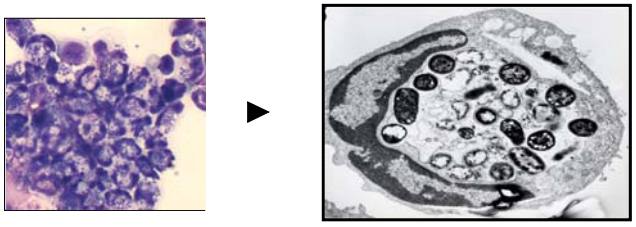
NEUTROPHILS AND BACTERIAL INFECTIONS

Neutrophil depletion increased lung bacterial burden



Sun L et al. *Am J Respir Cell Mol Biol. 2009*

Neutrophil recruitment in the setting of lung infection



Hirche T. et al. *J. Immunol. 2008*

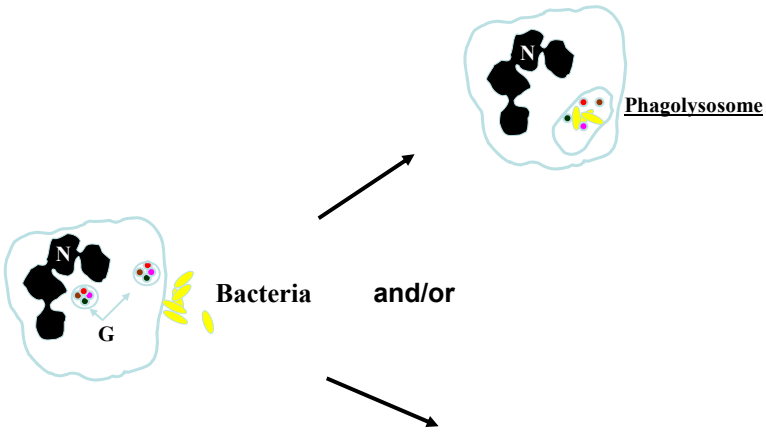
NEUTROPHIL ARSENAL

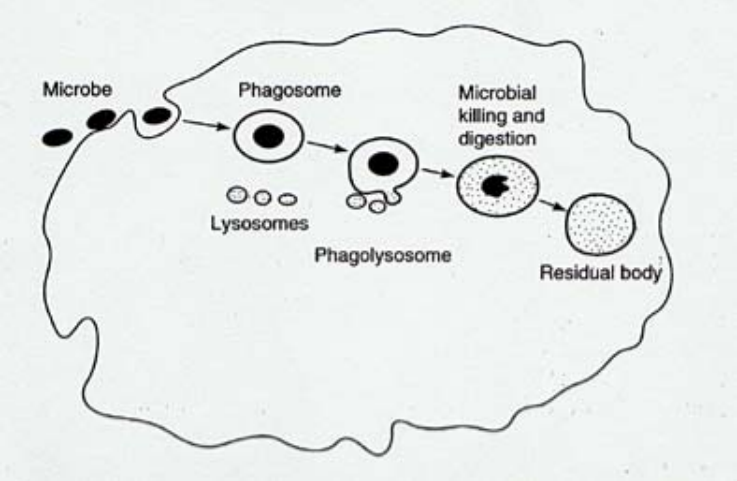
O_2-dependent system:
NADPH Ox^{ase}
MPO (HOCl)
and others…

O_2-independent system:
Elastase, Cathepsin G
Proteinase 3
and others…

- Murine counterparts

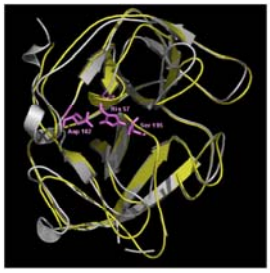
- Gene targeting (e.g., Loss of Function)





NEUTROPHIL ACTIVE SERINE PROTEASES (NSPS)

- Neutrophil elastase (NE)
- Proteinase 3 (PR3)
- Cathepsin G (CG)



Common features:

- Neutrophil specific serine proteinases
- Readily active forms at high concentrations (mM range)
- Large repertoire of targets: e.g.,
  - ✓ cells,
  - ✓ extracellular matrix proteins: elastin,…..
  - ✓ Inflammatory mediators: cytokines,……

NEUTROPHIL ARSENAL

- Murine counterparts

- Gene targeting (e.g., Loss of Function)

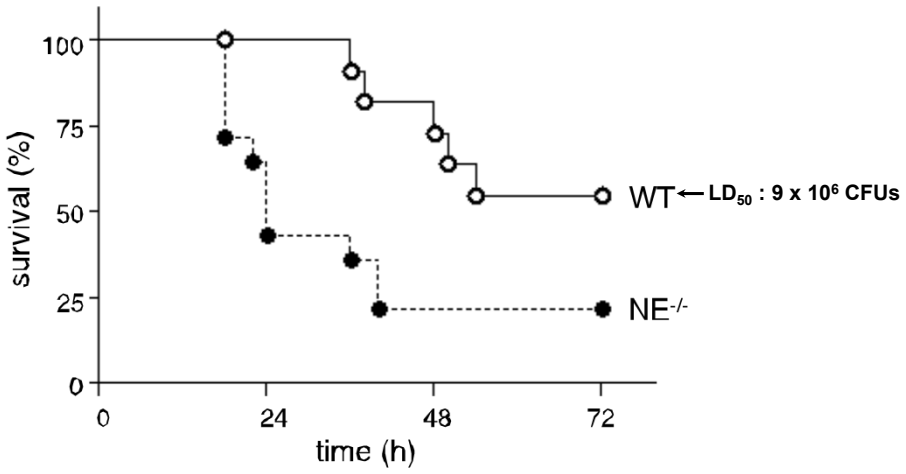
Mice deficient in neutrophil serine proteases:

Single KO:	Double KO:	Triple KO:
NE, CG, PR3±	NE-CG, NE-PR3	NE-CG-PR3

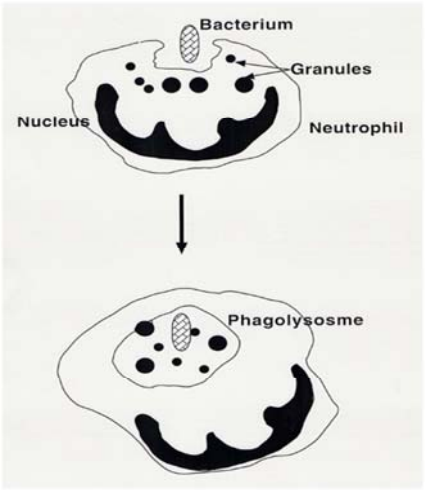
Neutrophil Serine Proteases in Inflammation: a Friend or a Foe ?!

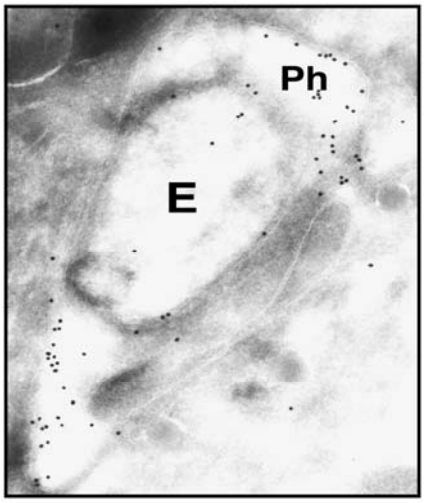
- ***Nat. Med. 1998***
- ***Blood. 1999***
- ***Science. 2000***
- ***J. Immunol. 2004***
- ***J. Immunol. 2008***
- ***J.B.C. 2012***

NE deficiency increases mouse mortality to *P. aeruginosa* in an intranasal infection model

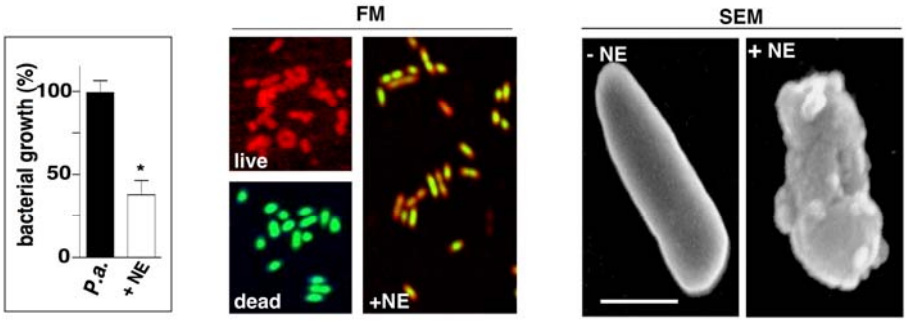


NE mediates maximal killing of pathogens by neutrophils

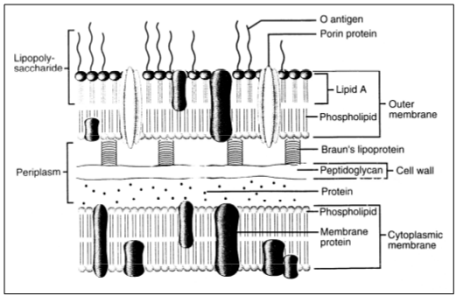




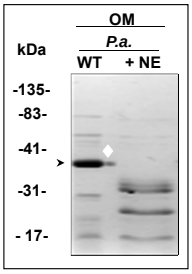
NE mediates *P. aeruginosa* killing by neutrophils

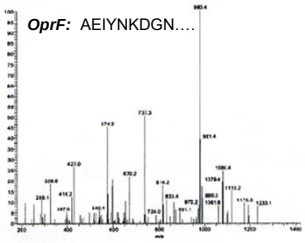


Cell envelope of Gram-negative Bacteria



NE degrades *P. aeruginosa* major outer membrane protein F

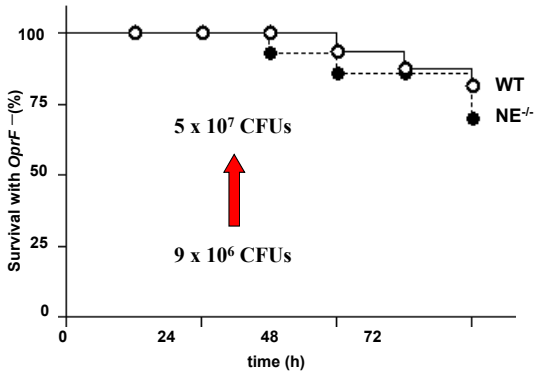




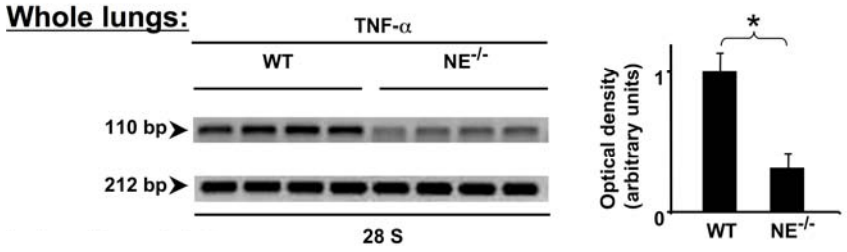
Opr F functions:

- **Maintenance of structural integrity**
- **Porin activity**
- **Sensor of host immune system activation**

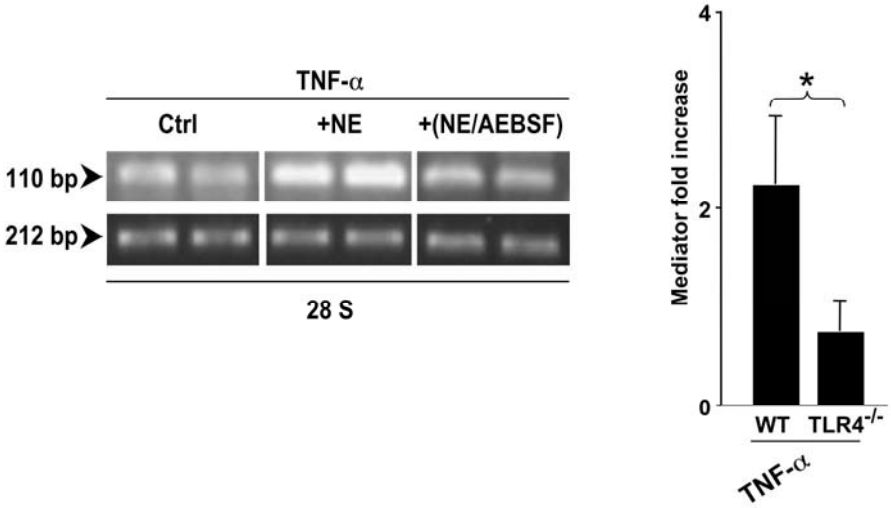
The absence of OprF negates NE role in host defense against *P. aeruginosa*



Decreased levels of TNF-α protein and transcript in infected NE-deficient mice



Purified active NE increases mRNA expression of TNF-α in macrophages involving at least TLR4



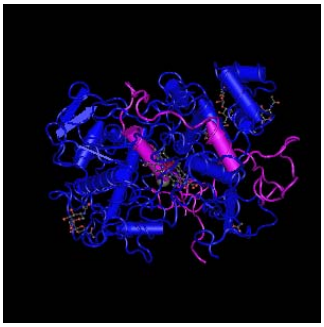
NEUTROPHIL ARSENAL

O_2-dependent system:
NADPH Ox^{ase}
MPO (HOCl)
and others…

- Murine counterparts

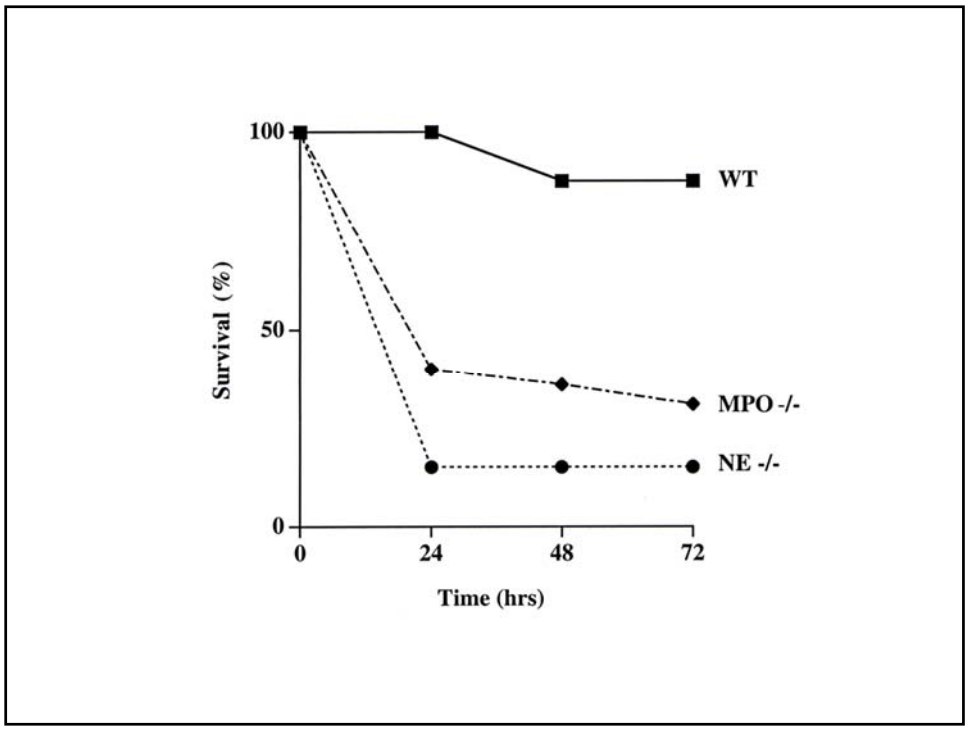
- Gene targeting (e.g., Loss of Function)

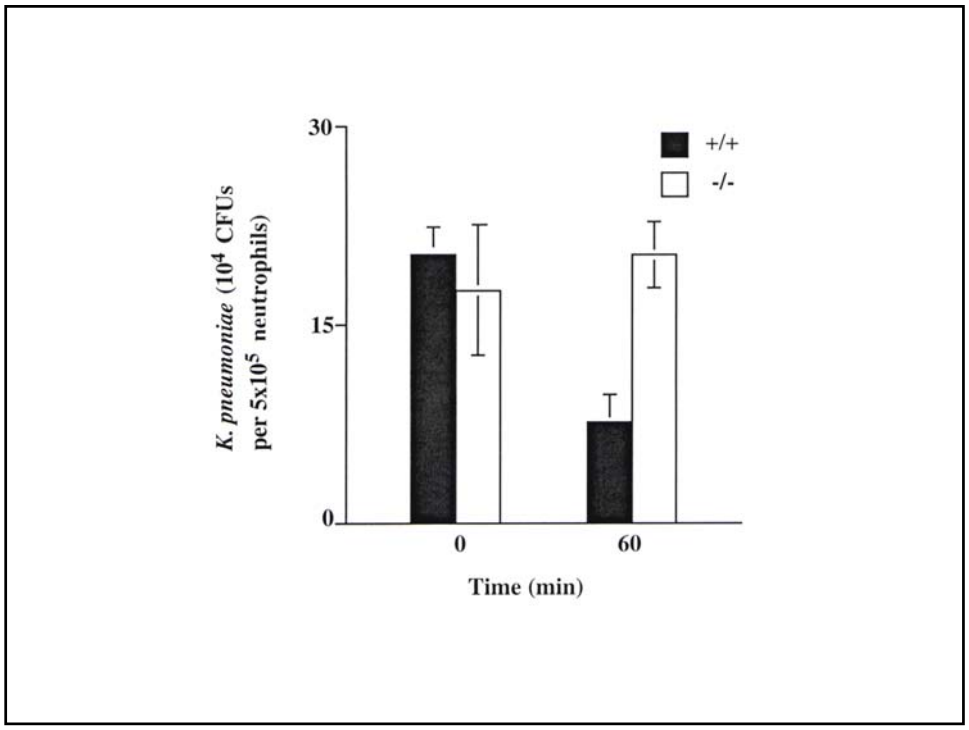
MYELOPEROXIDASE

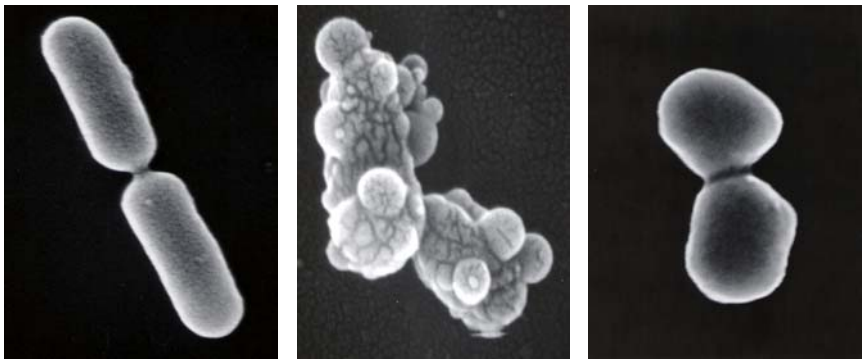


$H_2O_2 + Cl^- \xrightarrow{MPO} HOCl$

MPO:
- composed of two heavy (59 kDa) and light (14 kDa) chains
- only enzyme known to generate HOCl, potent oxidant
- stored in the neutrophil primary granules
- 5% of dry weight of the neutrophil

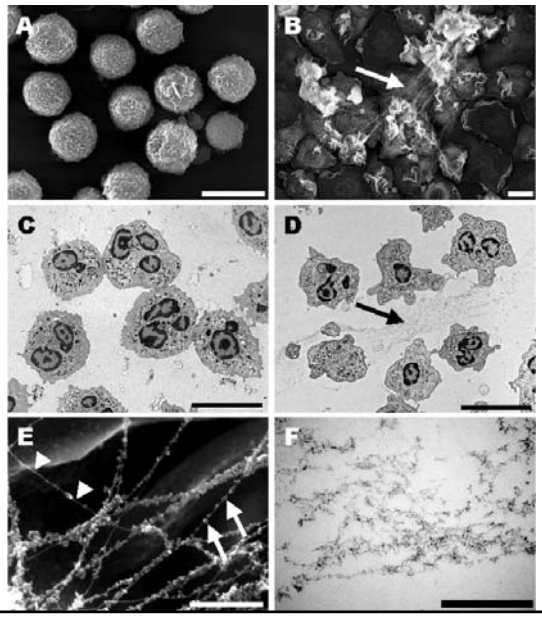

100
50
0
Survival (%)
0
24
48
72
Time (hrs)
WT
MPO -/-
NE -/-


30
15
0
K. pneumoniae (10^4 CFUs per 5x10^5 neutrophils)
0
60
Time (min)
+/+
-/-

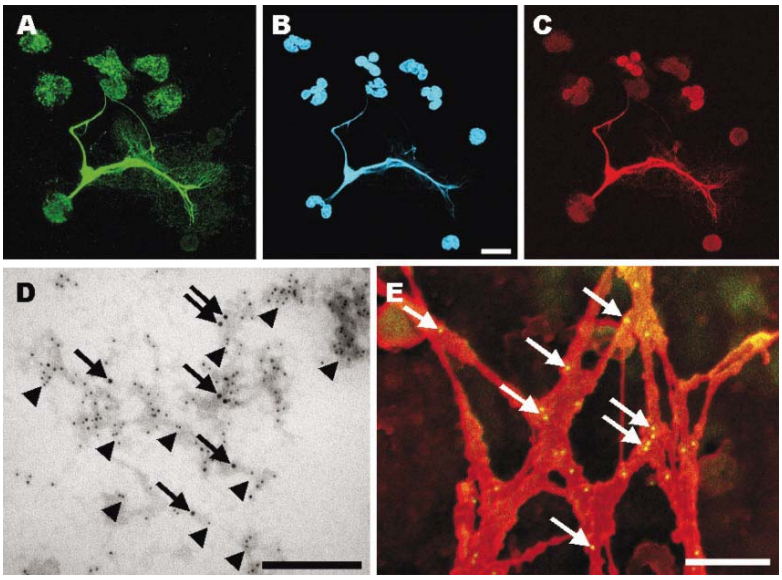


Neutrophil Extracellular Traps Kill Bacteria

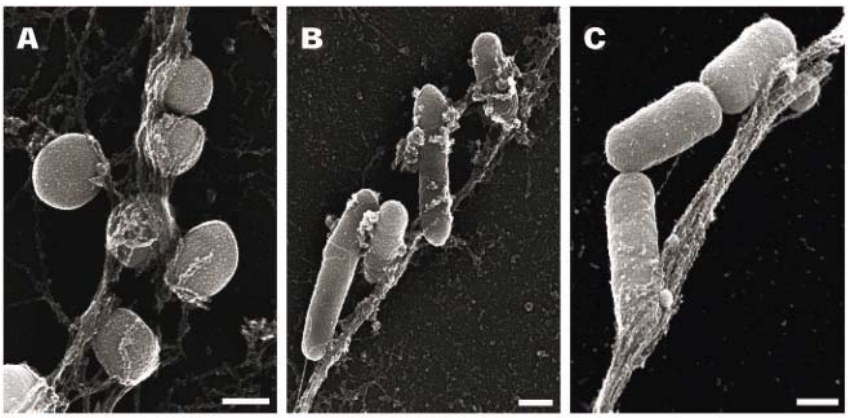
Brinkmann V. et al, Science 2004 303:1532-



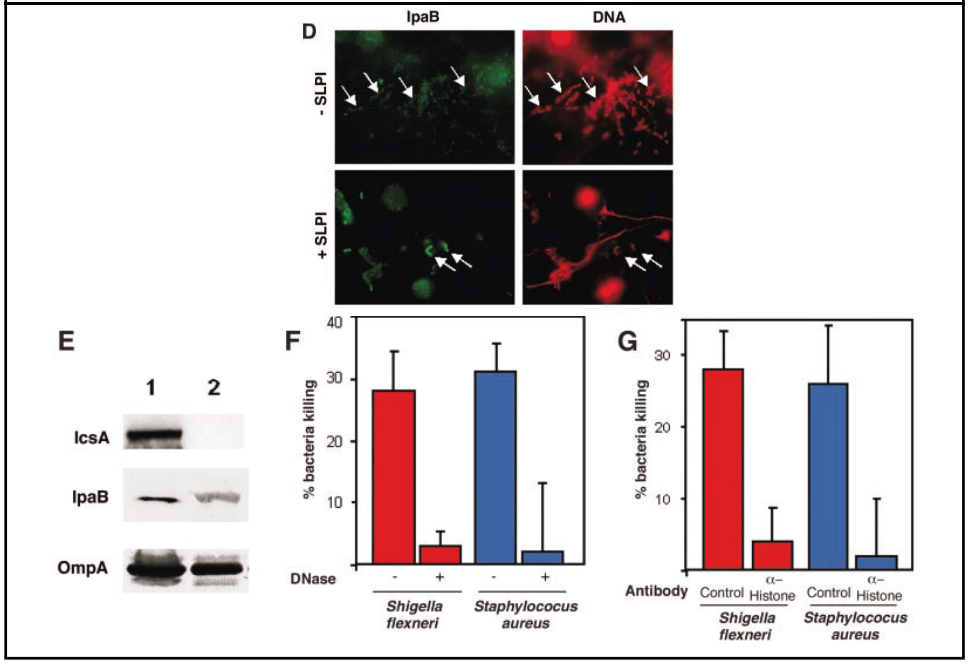
Neutrophil Extracellular Traps Kill Bacteria



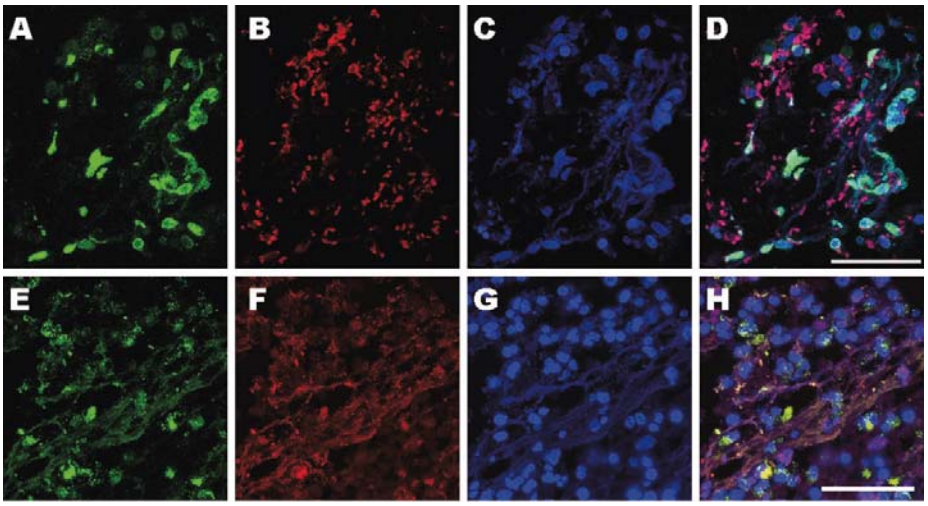
Neutrophil Extracellular Traps Kill Bacteria



Neutrophil Extracellular Traps Kill Bacteria

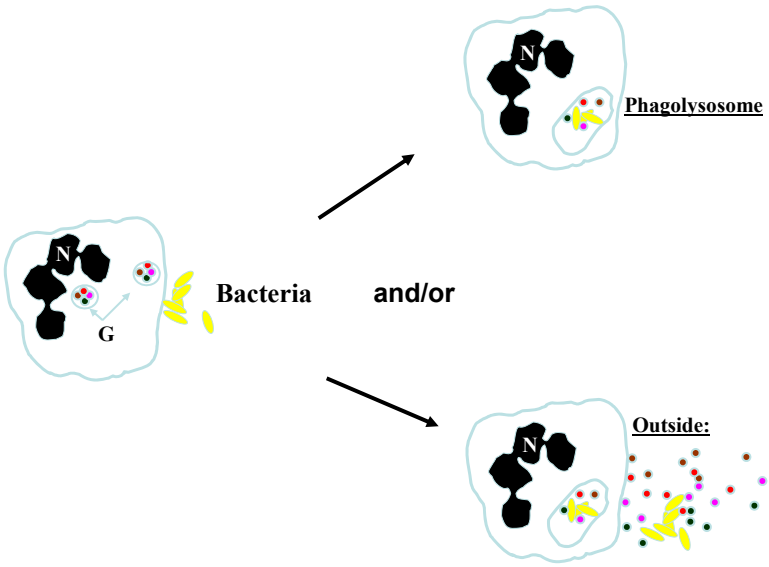


Neutrophil Extracellular Traps Kill Bacteria

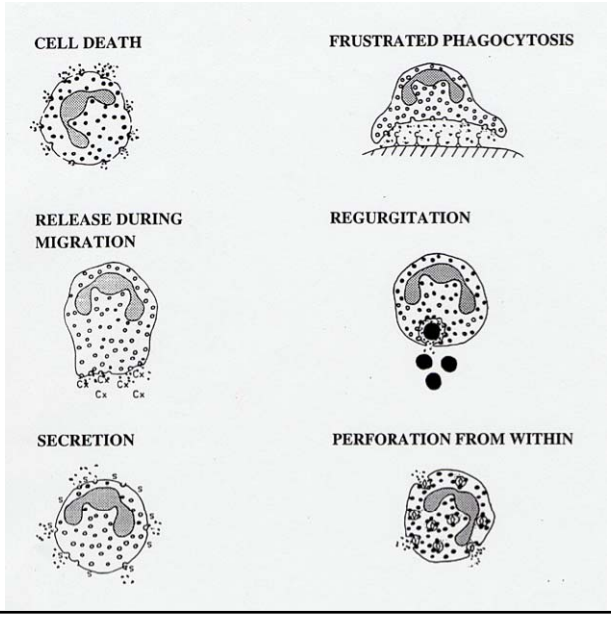


CONCLUSIONS

- Antibacterial role of neutrophil arsenal
  - *Direct killing*
  - *Inactivation of virulence factors*
  - *Induction of early responssive cytokines*



Extracellular discharge of neutrophil content



NEUTROPHIL FUNCTIONS?

1,500 - 7,000 cells/µl

In clinics:

High numbers

- **ALI, COPD**

Tissue damage

NEUTROPHIL FUNCTIONS?

1,500 - 7,000 cells/µl

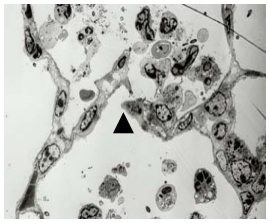
In clinics:

High numbers

- **ALI, COPD**

Tissue damage

In the lab:



Lung injury

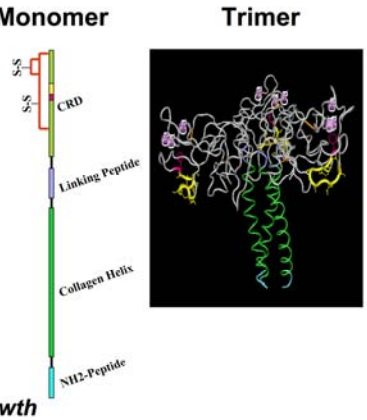
Neutrophil Serine Proteases in Inflammation:
a Friend or a Foe ?!

Role(s) of Neutrophil Serine Proteases in the Setting of Acute Inflammation

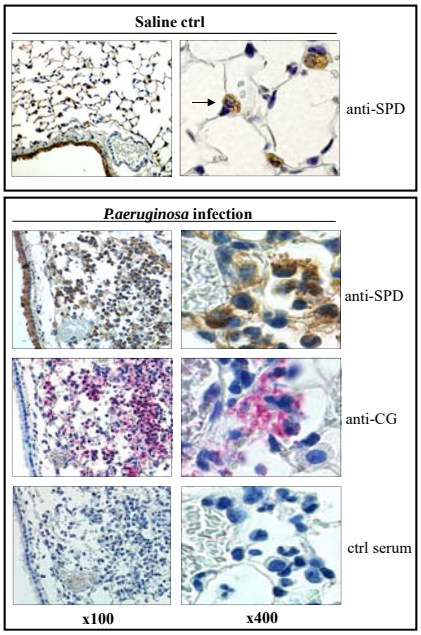
- *Am. J. Respir. Cell. Mol. Biol. 2004*
- *J. Biol. Chem. 2004*
- *J. Immunol. 2005*
- *J. Immunol. 2007*
- *J. Biol. Chem. 2009*
- *Manuscript in revision*

Surfactant protein D (SP-D)

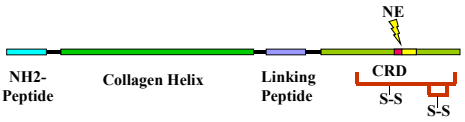
- Member of the collectin family
- Expressed mainly in the lung
- Physiologic functions include:
  - Surfactant homeostasis
  - Innate host defense
    - ✓ *Microbial agglutination*
    - ✓ *Opsonization*
    - ✓ *Modulation of phagocyte function*
    - ✓ *Antigen presentation*
    - ✓ *and direct effects on microbial growth*

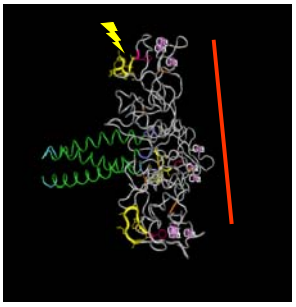


In vivo co-localization of CG and SP-D

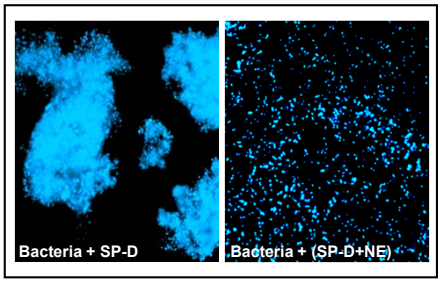


NE targets SP-D in vivo

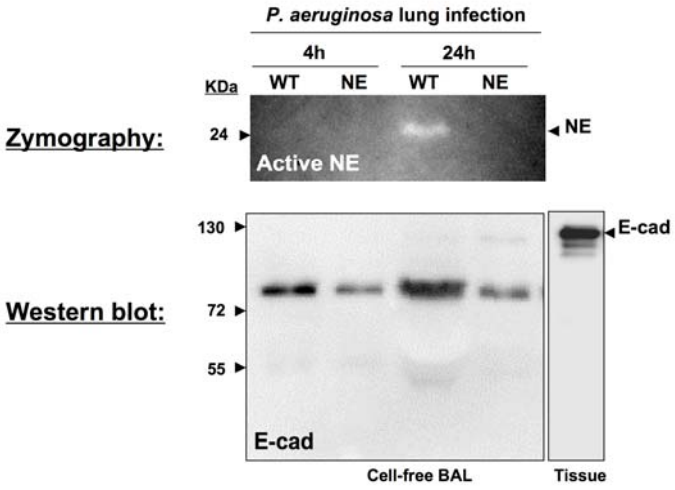




Aggregation assay:



NE-mediated E-cadherin degradation



R. Boxio et al., in revision

CONCLUSIONS

- If unopposed, neutrophil serine proteases are deleterious in the setting of both acute and chronic inflammation

Cigarette smoke model

Experimental Design:

WT & 3KO mice

2 cigarettes/day; 5 days/week

Exposure time (Months)

0 → 1 → 4 → 8

CIGARETTE SMOKE-INDUCED EMPHYSEMA

- **COPD: 3rd cause of mortality in the coming years / High economic burden**

- **Important component of COPD**

- **Cigarette smoke: Major etiologic cause**

- **Bacterial infections worsen the disease (Exacerbations)**

- ***Clinically:***

  Progressive and irreversible decline of lung function

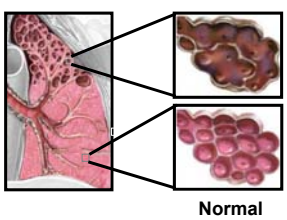
- ***Pathologically:***

  Airspace enlargment

  Alveolar wall destruction

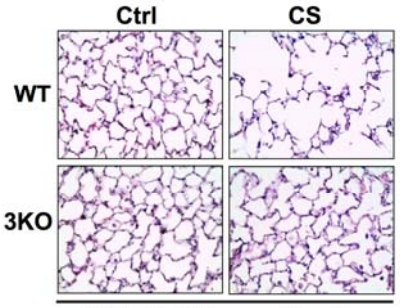
  Connective tissue breakdown (e.g. elastin degradation)

  Chronic inflammation



3KO mice are markedly protected from cigarette smoke-induced lung tissue destruction

Histology:



H&E - X 400 magnification

Mean Linear Intercept:

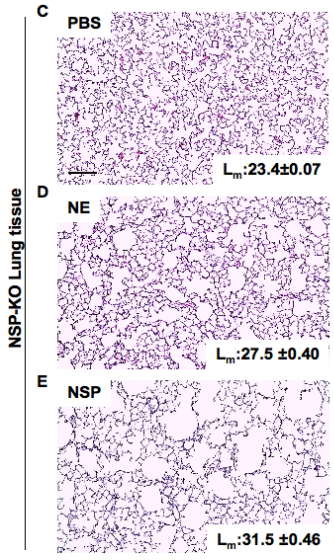
	WT	3KO
Ctrl	22.2 (±0.7)	22.3 (±0.6)
CS	33.5 (±2.1)*	24.1 (±0.8)

*Values are means (mm)±SD; * p<0.01*

Protection= ~ 85%

N. Guyot et al., A.J.P. 2014

Pooled NSPs cause a marked damage than NE alone



POTENTIAL THERAPEUTIC STRATEGIES

- ✓ ***Control of excessive neutrophil recruitment***
- ✓ ***Modulation of cell activation/degranulation (e.g., Protease release)***
- ✓ ***Protease inhibition, but with caution***

UE: Physiopathologie des Maladies Transmissible

Polynucléaires neutrophiles et défenses anti-infectieuses

Abderrazzak Bentaher, Research Director, Inserm
Inflammation et Immunité de l'Epithélium Respiratoire
EA7426

azzak.bentaher@inserm.fr

Surfactant protein D (SP-D)

- Member of the collectin family

- Expressed mainly in the lung

- Physiologic functions include:

  - Surfactant homeostasis

  - Innate host defense

    - ✓ *Microbial agglutination*
    - ✓ *Opsonization*
    - ✓ *Modulation of phagocyte function*
    - ✓ *Antigen presentation*
    - ✓ *and direct effects on microbial growth*

Trimer

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