

Cours module 4 DESC d'Allergologie et Immunologie Clinique

Jedi 25/03/21

09 h 00 – 12 h 30 F.Godesky

Allergie alimentaire adulte

13 h 30 – 17 h 00 P.Bierme -Anne-Karine Corréard ou A.Dupré la Tour

Allergie alimentaire pédiatrique

-

Vendredi 26/03/21

09 h 00 – 10 h 30 N. Freymond

Allergie et Asthme

10 h 30 – 12 h 30 G. Devouassoux

Asthme sévère

13 h 30 – 15 h 30 C. Dzviga

Pollution intérieure et impact sur les maladies allergiques

15 h 30 – 17 h 00 A. Bentaher

Protéases du système immunitaire : rôle pro-inflammatoire ou anti-inflammatoire ?

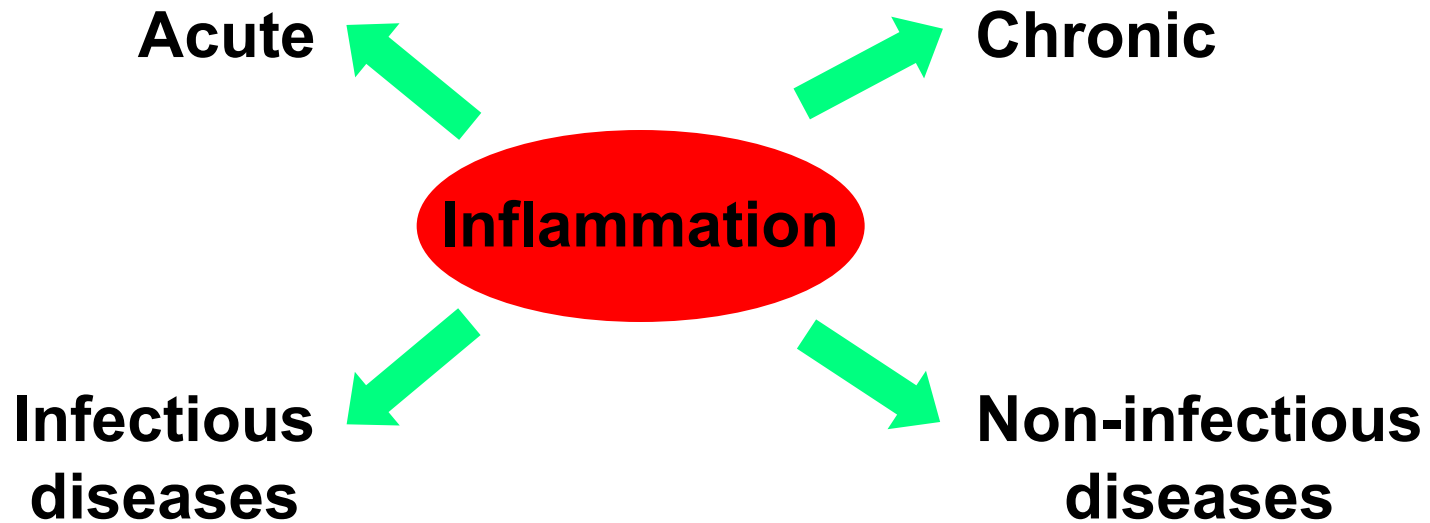
**PROTÉASES DU SYSTÈME IMMUNITAIRE:
RÔLE PRO- ou ANTI-INFLAMMATOIRE ?**

*A. Bentaher, Research Director, Inserm
Inflammation et Immunité de l'Épithélium Respiratoire*

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Leukocytes Recruitment : a Characteristic of Inflammation



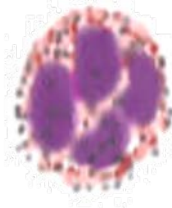
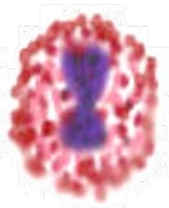
*Wherever inflammation occurs there are certain local mechanisms in common, despite differences in the precipitating factors,...: the recruitment of **leukocytes** from the circulation to the site of tissue damage.*

INFLAMMATION: ACUTE OR CHRONIC

Eosinophil

Basophil

Neutrophil

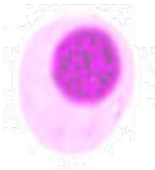


Monocytes

Lymphocyte

Dendritic ϕ

Plasma ϕ

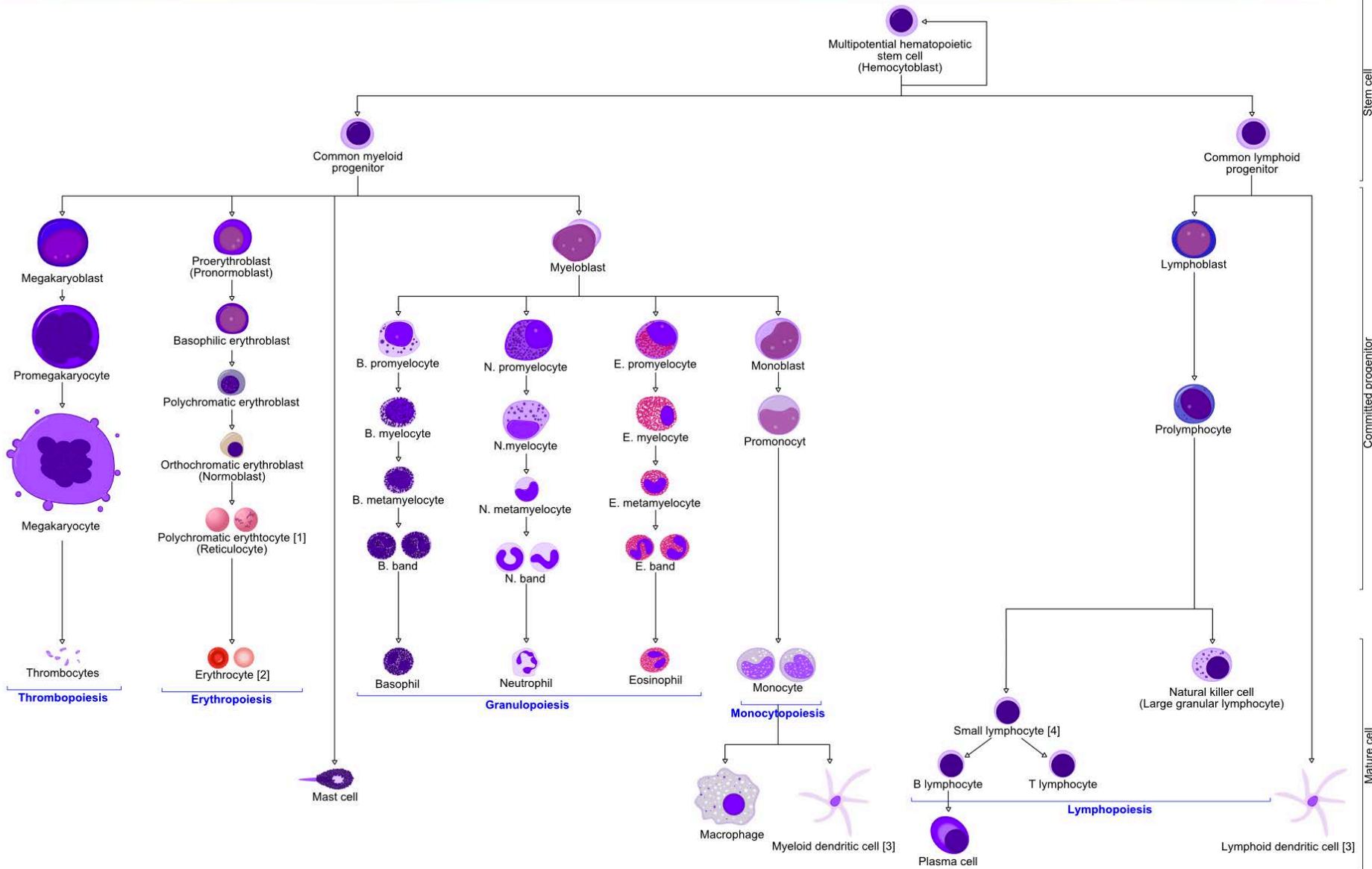


Granular leukocytes

Agranular leukocytes

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

Hematopoiesis in humans

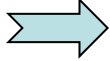


Bone marrow

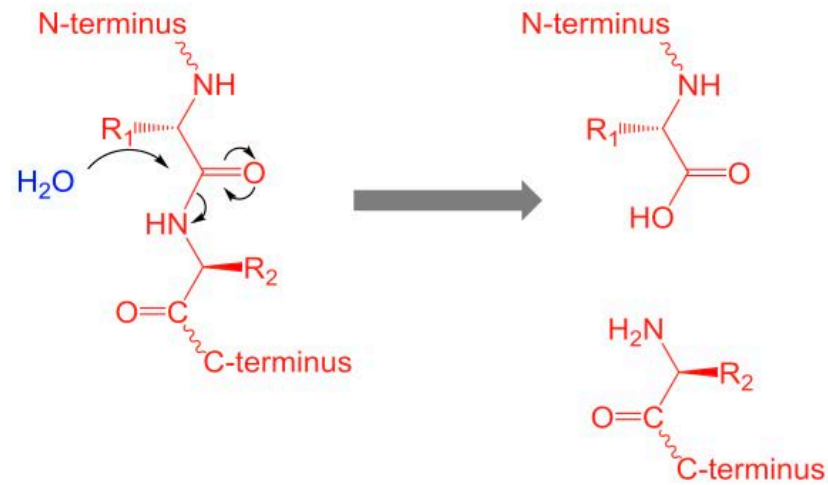
Blood

Tissue

Proteases :

- Enzyme that catalyzes (increases the rate of) proteolysis
- Breakdown of proteins  smaller polypeptides or amino acids
- Cleavage of peptide bonds within proteins
- Enzymes act on **substrates** to convert them into **products**

Proteases :



Proteases :

- **Classification into families based on catalytic residue, e.g.:**

Aspartic proteases*

Glutamic proteases*

Metalloproteases* metal zinc instead of residue

Serine proteases

Cysteine proteases

Threonine proteases

- **Classification fonctionnelle (en fonction du lieu de coupure) :**

Exopeptidases (ou exoprotéases) :

aminopeptidases - carboxypeptidases

Endopeptidases (ou endoprotéases) (à l'intérieur)

Proteases :

- Physiologic and Pathophysiologic consequences, e.g.:

Physiologic

- Gastrointestinal tract / Proteins in food.
- Implantation and Embryonic Development
- Blood serum / Blood- clotting & clot lysis
- Immune system.
- Lifetime of hormones, antibodies, or other enzymes.

Fastest "switching on" and "off" regulatory mechanisms
Cascade reactions

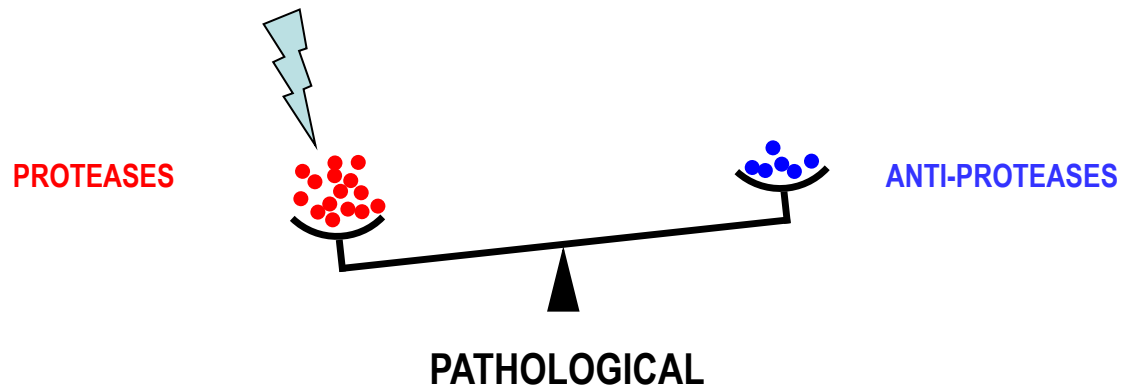
Pathophysiologic

- Matrix protein degradation
- Receptor cleavage
- Cytokine inactivation
- Cell lysis
- Tissue destruction
- Etc.....

Proteases :

- 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....
- Pathophysiologic roles:
Pulmonary diseases, arthritis, cancer.....

Proteases-antiproteases imbalance hypothesis



PROTEASES and ALLERGIC DISORDERS

Proteases-antiproteases balance



**Maintenance of epithelial barriers
(Skin and airways)**

Proteases-antiproteases **imbalance**

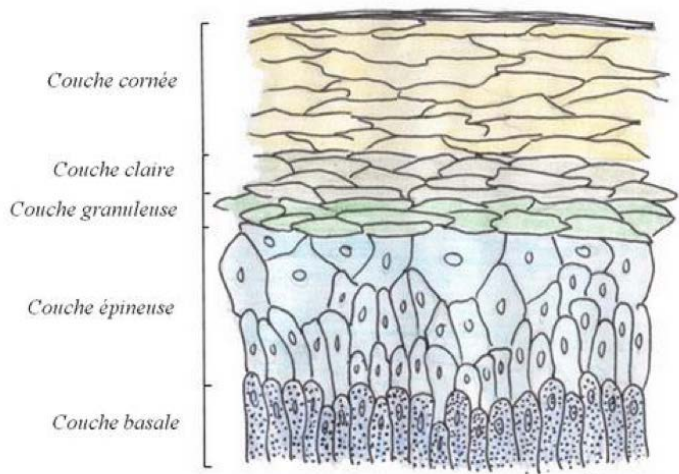


Disruption of epithelial barriers

**Allergic sensitization
inflammation**

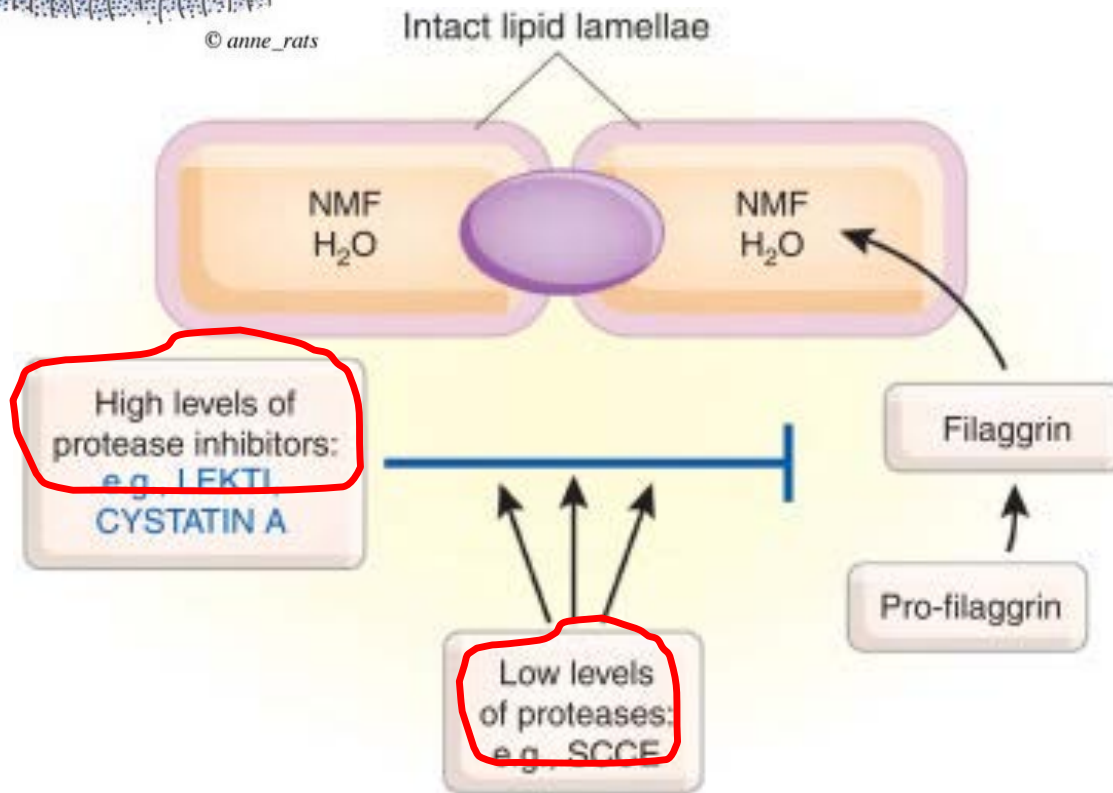
**airway pathophysiology and
remodeling in Asthma**

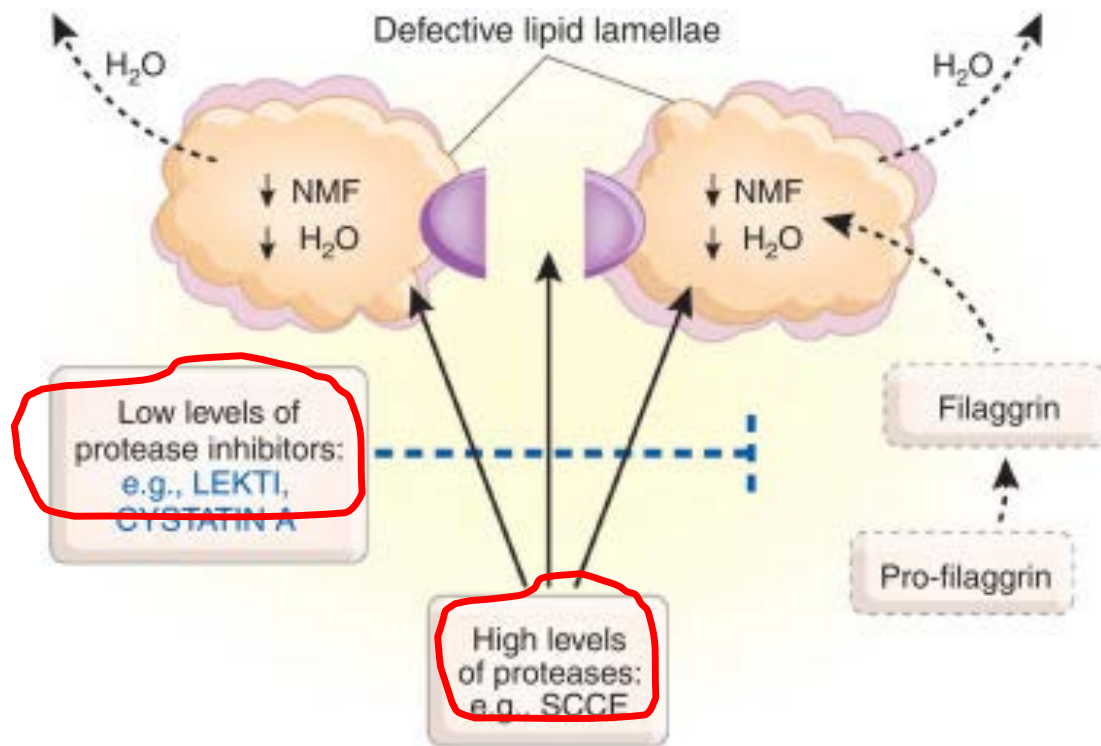
Immediate (mast cells) and late allergic response

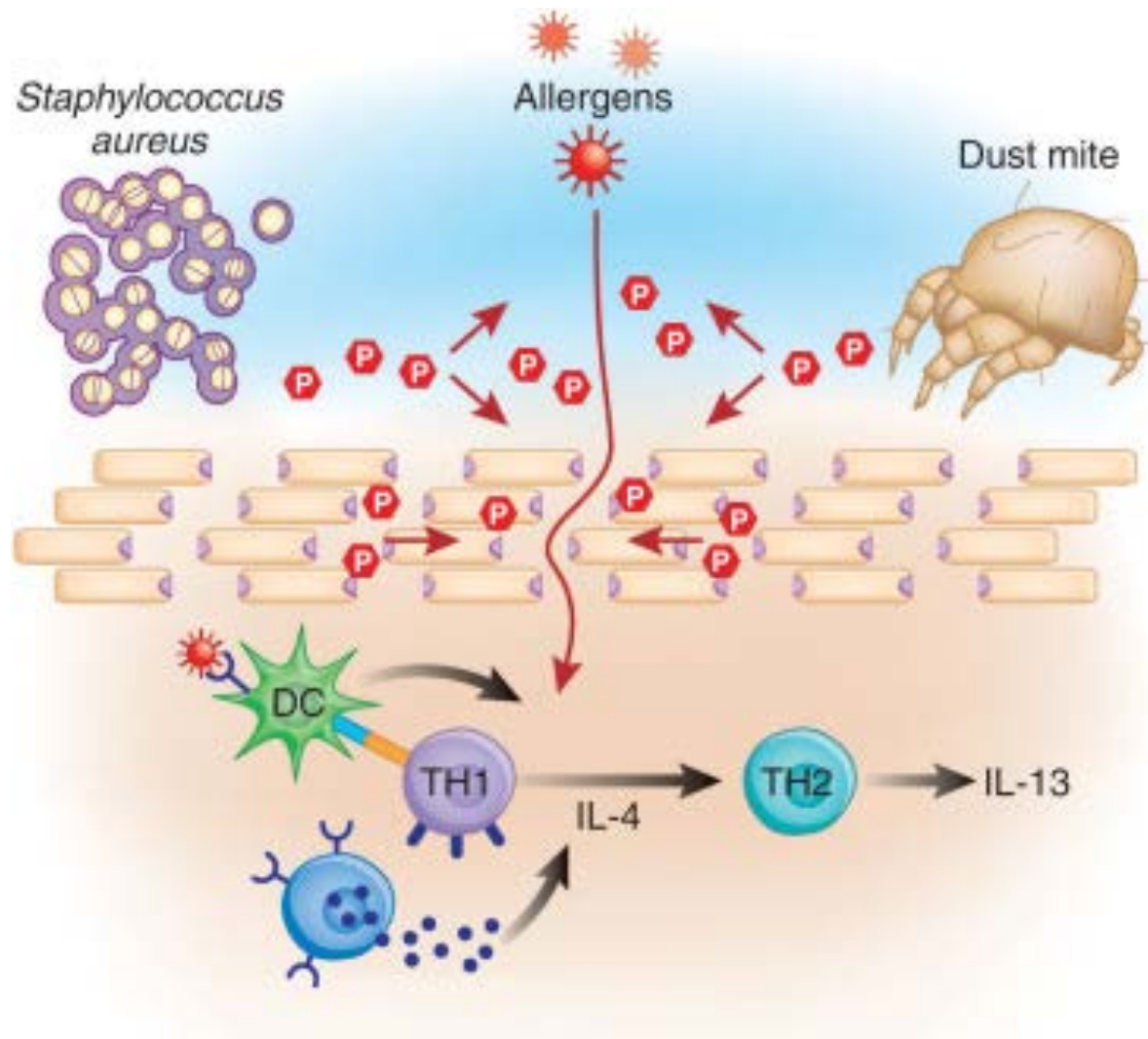


Traduction : © Agorot

© anne_rats

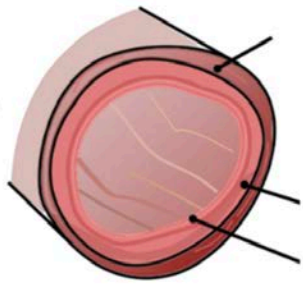






Comparison of (A) normal and (B, C) asthmatic airway wall showing epithelial damage, increased smooth muscle, inflammatory cell infiltration, and sub-basement membrane thickening.

healthy airway

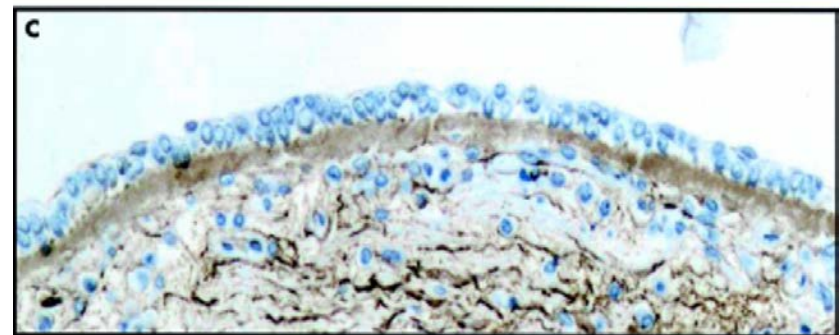
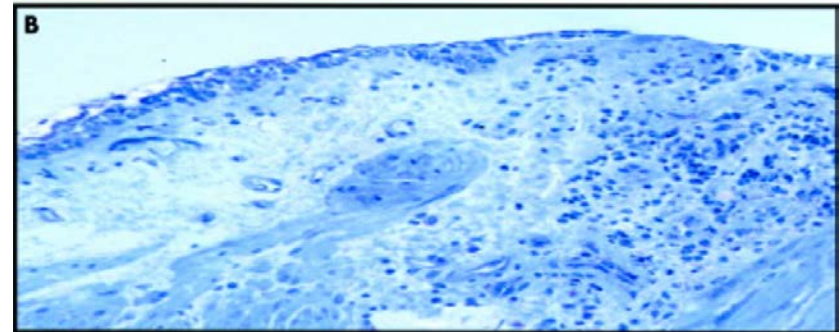
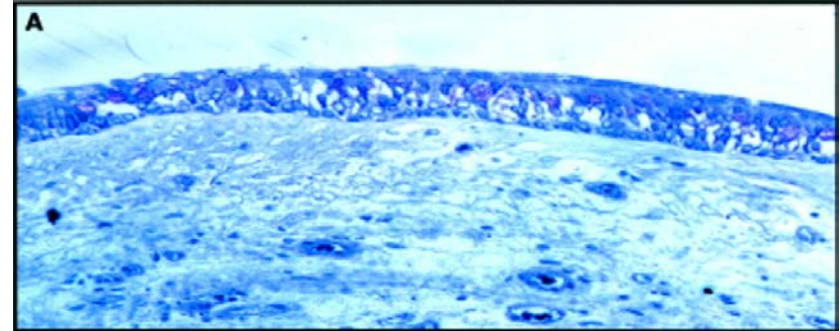
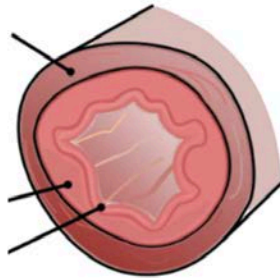


smooth muscle
muscle cells wrapped spirally around the wall

submucosa
fibroblasts embedded in connective tissue

mucosa
epithelium
basement membrane
subepithelial collagen layer

diseased airway



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

EVIDENCE FOR GENETIC LINKS

Single Nucleotide Polymorphism (SNP)

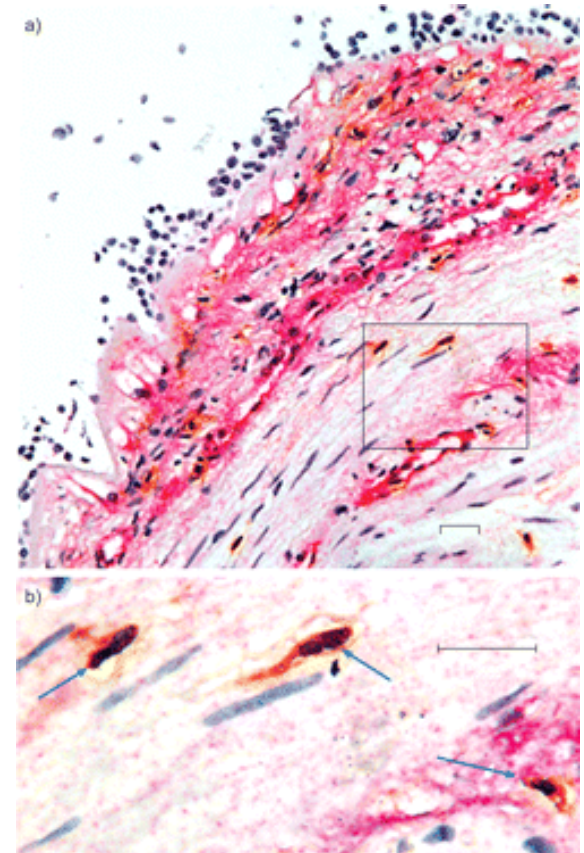
DPP10  **Exotaxin and CCL5 (RANTES) cleavage**

Chymase promoter  **Increase of IgE levels in AD**

α 1-antichymotrypsin  **Asthma,**

MAST CELLS: KEY PROTEASE SOURCE

- IgE-mediated type I hypersensitivity (e.g., asthma, rhinitis, and dermatitis)
- Within intraepithelial and smooth muscle cells
- Density correlates with bronchial hyperresponsiveness
- Central role in promoting airway remodeling and inflammation



MAST CELL-DERIVED PROTEASES: KEY PLAYER

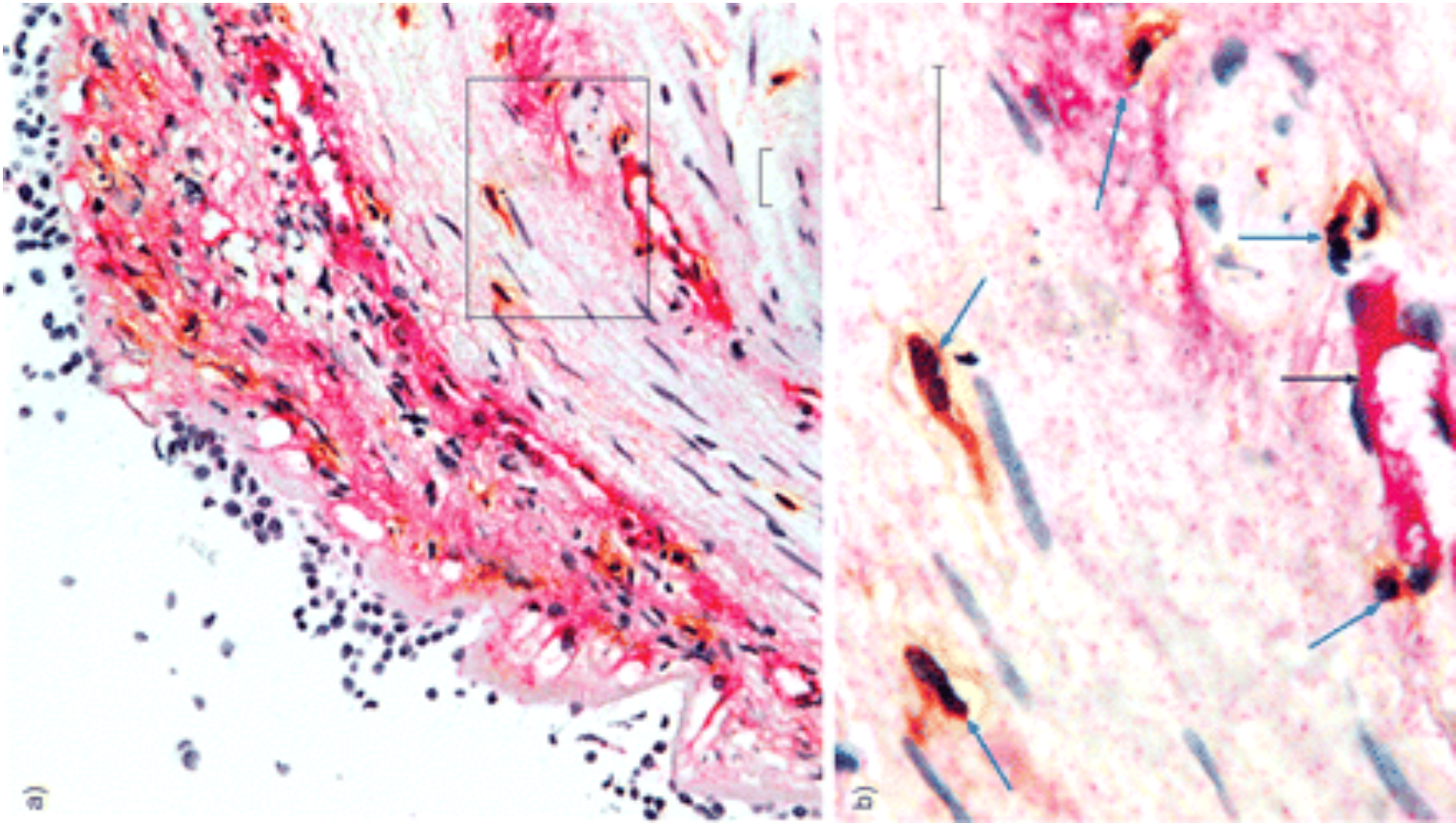
- Proteases account for around 25% of total MC protein
- MC-specific proteases: **tryptase** and **chymase**
- 10 to 35 pg of tryptase and chymase per one cell

**Central role
in promoting airway remodeling and inflammation**

TRYPTASE

- **Secretion of 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**
- **Méiateur 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**
- **Degrade vasoactive peptide (bronchodialating peptide)**
- **Cleaves extracellular matrix**
- **Activates matrix cleaving proteases**

TRYPTASE

- Can also act as mitogens (SM hyperplasia, fibrosis, ...)
- Causes degranulation of nearby MCs
- Cleaves to  interleukin IL-33 ( inflammation potency)
- Tryptase inhibition suppresses IL-33-dependent allergic airway inflammation

TRYPTASE

Deux formes moléculaires dans le plasma :

➤ **Tryptase α :**

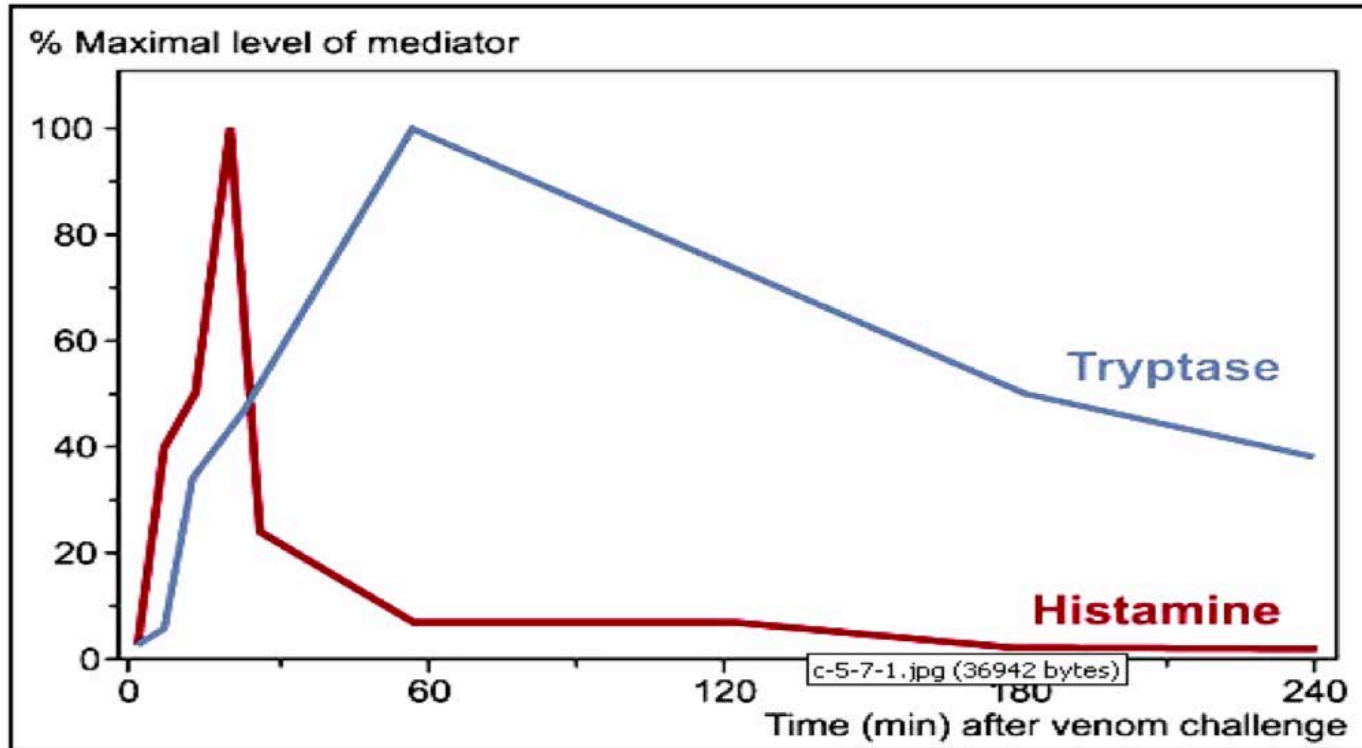
- **Forme physiologiquement sécrétée par les mastocytes**
- **Considérablement augmentée dans les mastocytoses systémiques**
- **Responsable du taux basal sérique**

➤ **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			Reference
	Elevated tryptase levels	Tryptase induces process	Tryptase inhibitor reduces response	
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:				
			Tryptase added to cell culture	
	Mixture of purified components	or tissue	<i>In vivo</i>	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

- More skin than lungs
- 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

- Expressed in PNNs, DCs, and monocytes.
- Cleave both tryptic and chymotryptic substrates.
- Functions as chymase
- Activates matrix metalloproteases

Cathepsin C

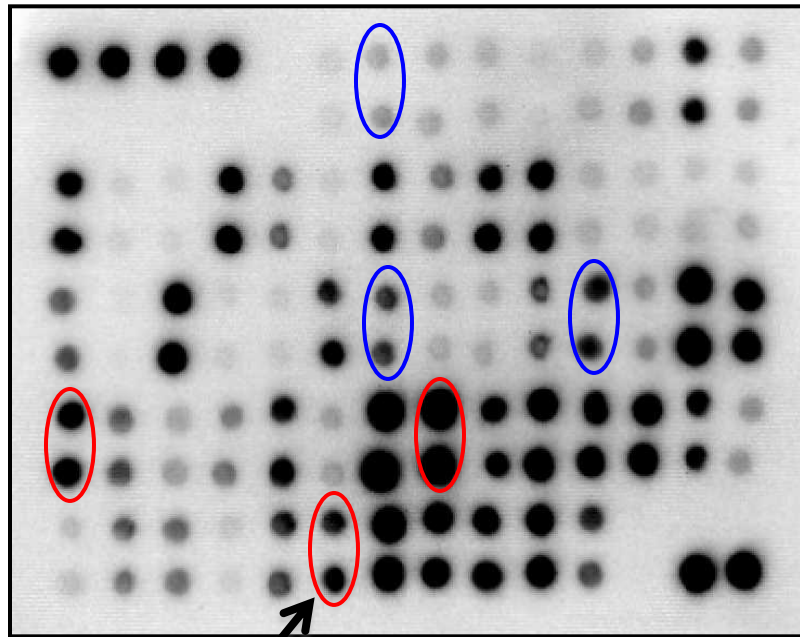
- Has endoproteolytic activity
- Activates of chymases, cathepsin G, and tryptases

Matrix metalloprotease 9

- activated by chymases,
- degradation of extracellular matrix

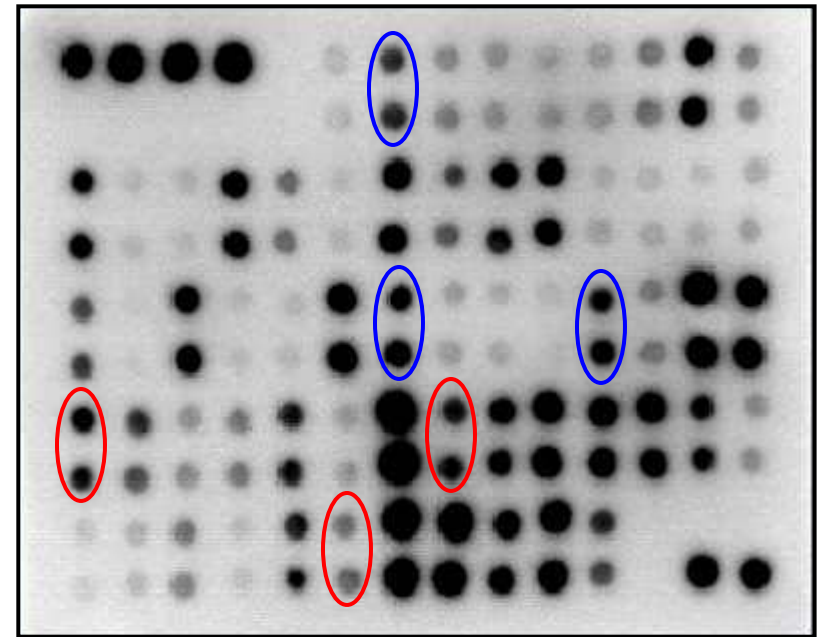
Altered cytokine levels in infected cell-free BALs in the absence of NE

Cytokine antibody microarray



WT

TNF- α



NE^{-/-}

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	<i>B. tropicalis</i> (mite)	Cysteine protease
Blo t 3	<i>B. tropicalis</i> (mite)	Trypsin
Blo t 6	<i>B. tropicalis</i> (mite)	Chymotrypsin
Der m 1	<i>D. microceras</i>	Cysteine protease
Der P 1	<i>D. pteronyssinus</i>	Cysteine protease
Der P 9	<i>D. pteronyssinus</i>	Collagenolytic serine protease
Eur m 1	<i>E. maynei</i>	Cysteine protease

Cockroach allergens

Bla g 2	<i>B. germanica</i>	Aspartic protease
Per a 2	<i>P. americana</i>	Aspartic protease-like
Per a 10	<i>P. americana</i>	Serine protease

Food allergens

Act d 1	<i>A. deliciosa</i> (kiwi fruit)	Cysteine protease
Cuc m 1	<i>C. melo</i> (muskmelon)	Alkaline serine protease

A. mellifera - *Apis mellifera*, *B. pensylvanicus* - *Bombus pensylvanicus*, *A. aegypti* - *Aedes aegypti*, *A. artemisiifolia* - *Ambrosia artemisiifolia*, *A. alternata* - *Alternaria alternata*, *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**
- **α 1-antitrypsin, secretory leukoprotease inhibitor (SLPI), and elafin**
- **Secreted in the lung lining fluids and protect the respiratory tract from proteolysis by proteases.**

PROTEASES-ANTI-PROTEASE 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 α 1-antitrypsin.**



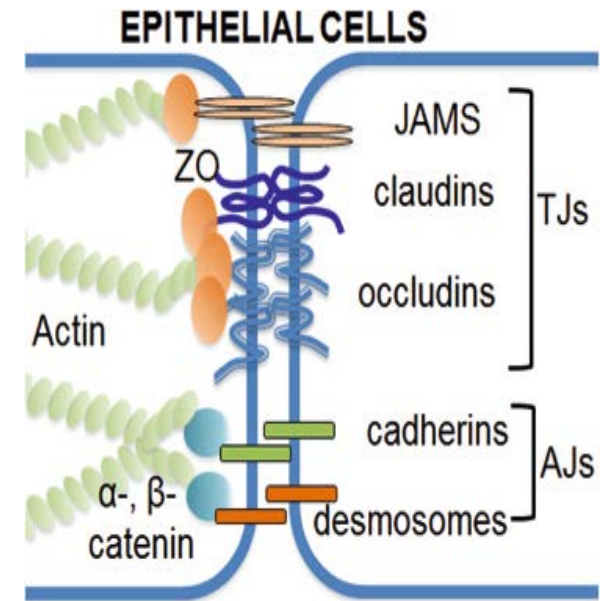
Inflammatory responses at the epithelial surfaces?

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.

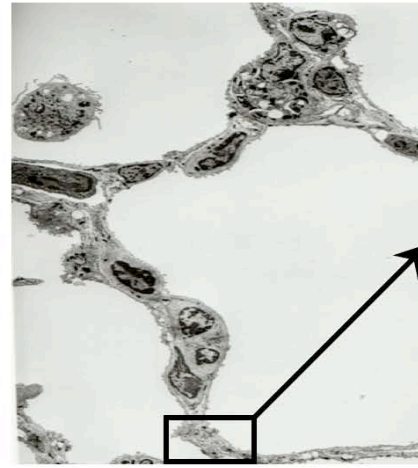
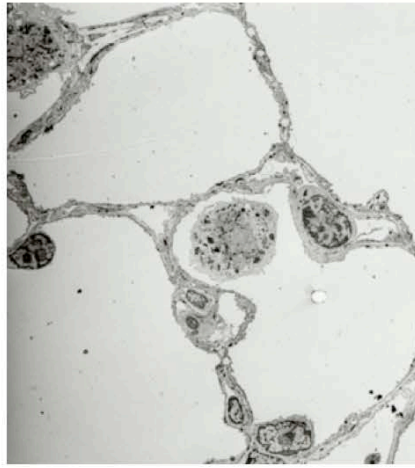
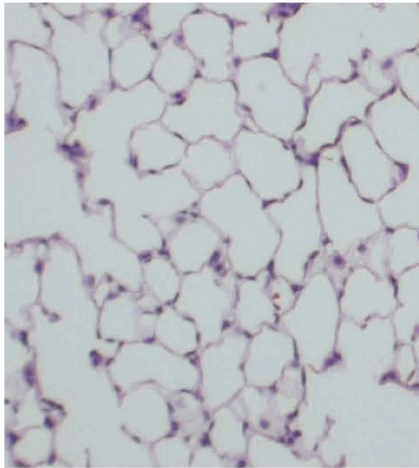


- Defective and disrupted epithelial barrier in allergic diseases such as asthma and dermatitis

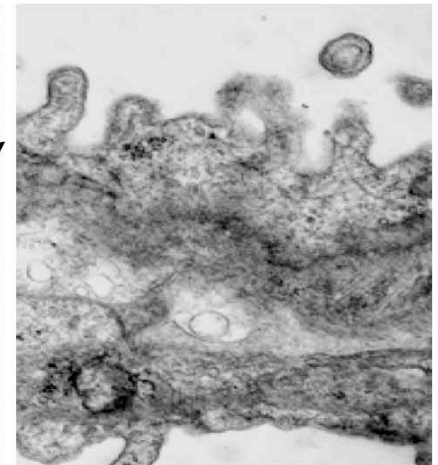
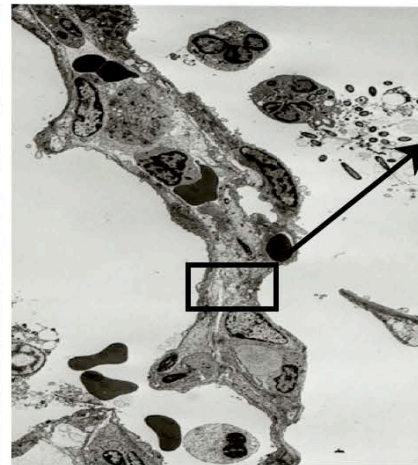
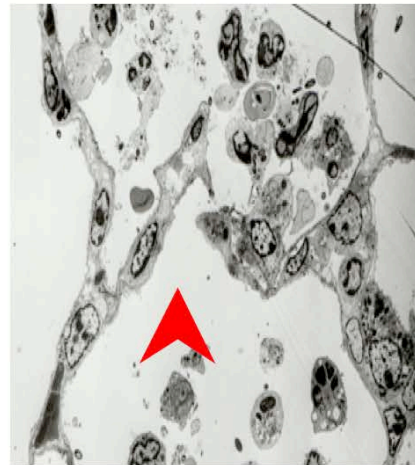
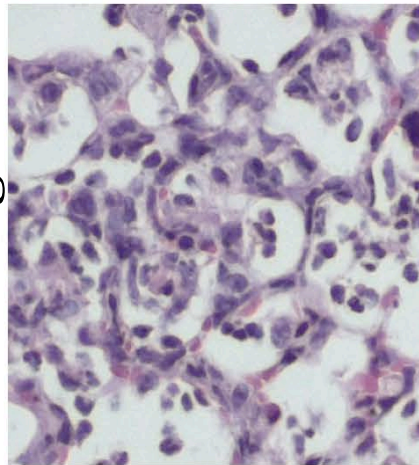
Pathogen-induced acute lung injury

WT Lung

Saline



Pathogen

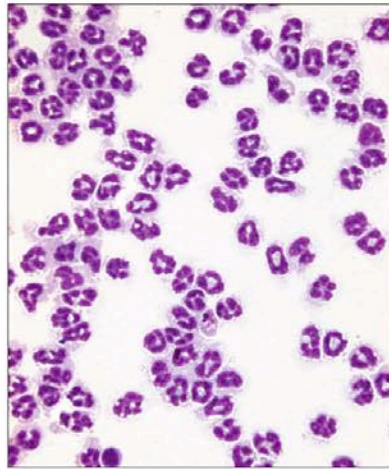


H&E

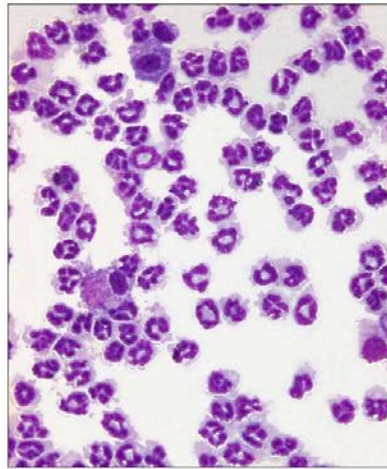
TEM

LPS-induced acute lung inflammation and injury

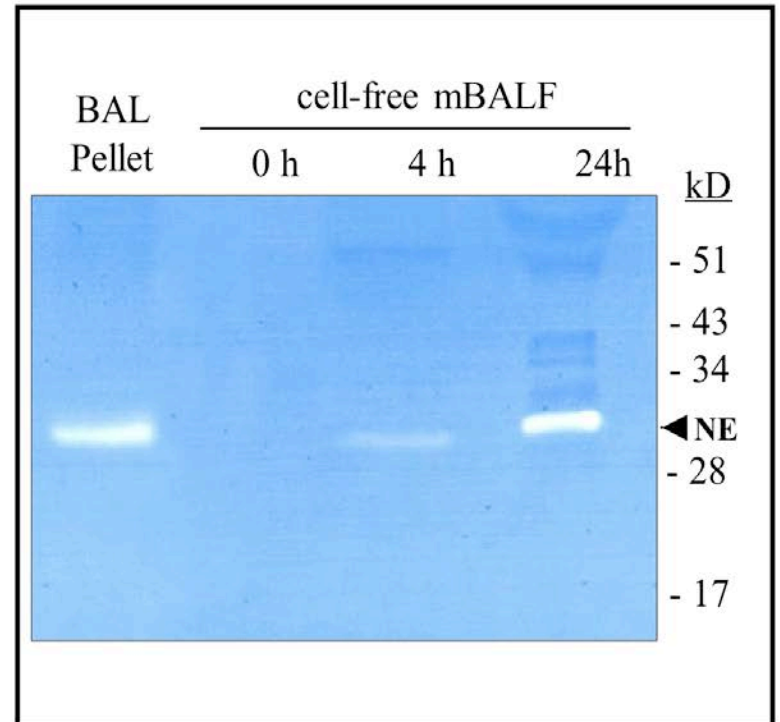
LPS - 24 h



NE^{-/-}

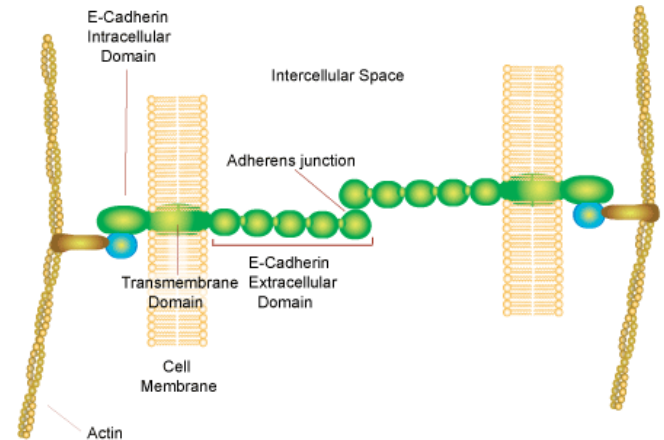


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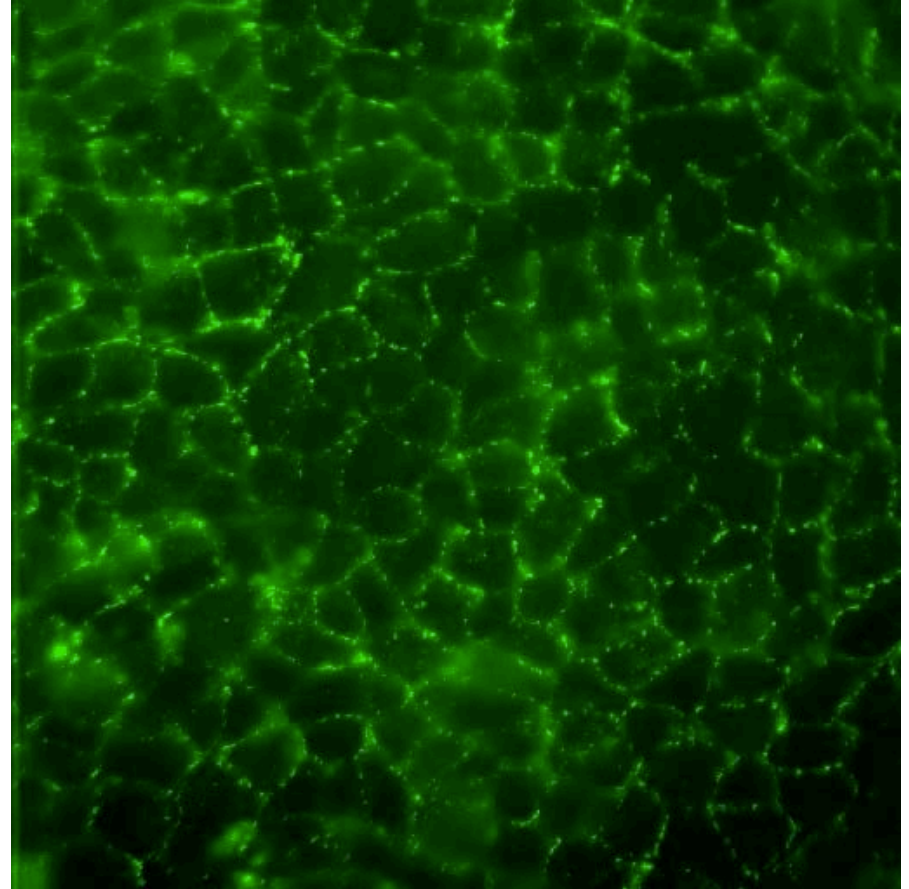
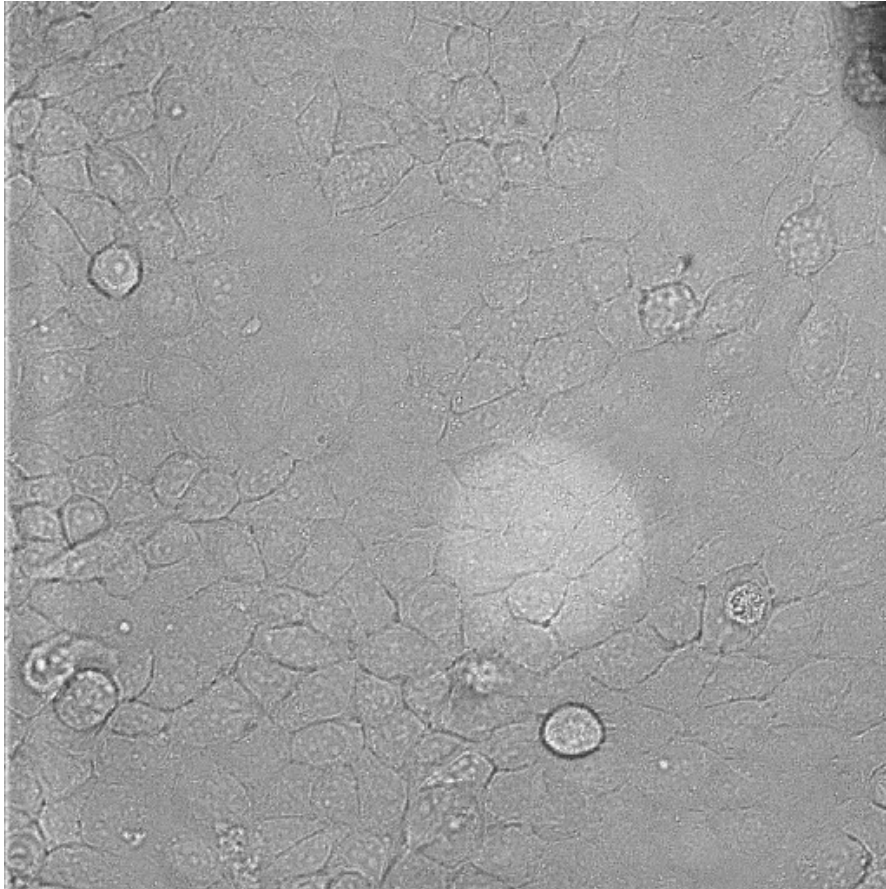


E-cadherin (E-cad)

- 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**
e.g. 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- κ B 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**

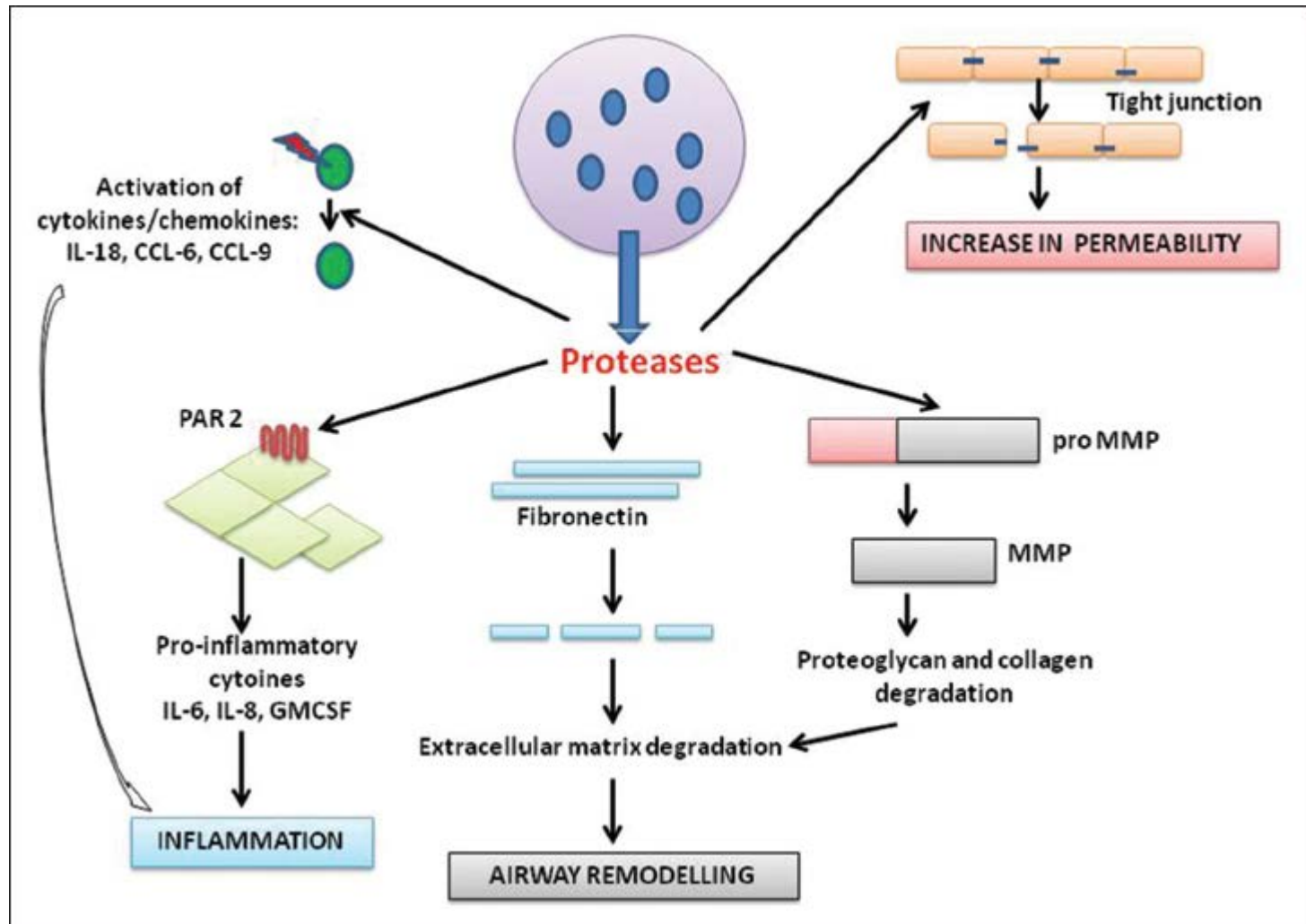
UTI, purified from a human source

 { **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