Biomarkers in Bronchial Asthma

Yong Chul Lee, MD, PhD

Department of Internal Medicine and Research Center for Pulmonary Disorders,

Chonbuk National University Medical School, Jeonju, South Korea

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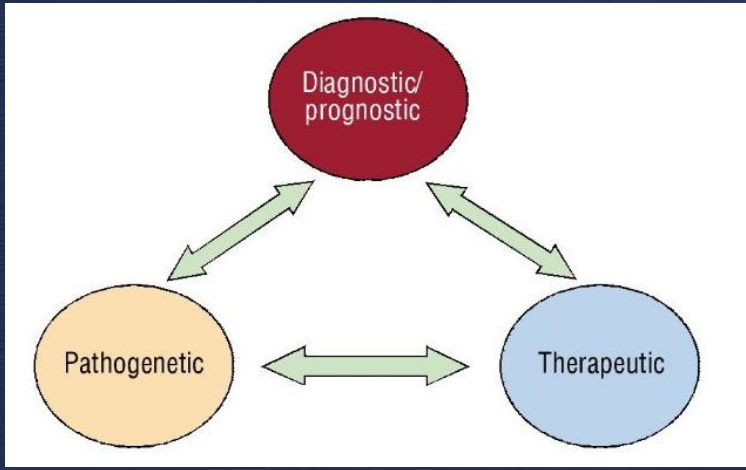
1. Overview
2. Current biomarkers
3. Biomarkers in Severe asthma
4. Organelle dysfunction and biomarkers
5. Summary and conclusion

Biomarker?

- The *National Institutes of Health* defines biomarker as a
  - "Characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"



De Gruttola VG *et al. Control Clin Trials* (2001)

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Why we search for biomarkers?

1. Define populations that will derive most benefit from a drug (*Pharmacogenetics*)
2. Improve drug development (*Pharmacokinetics*)
3. Predict disease course (*to justify more intense or prolonged treatments*) (*Diagnostics and prognostics*)
4. Monitor the effects of therapy (*Pharmacodynamics*)
5. Predict clinical outcomes (*Surrogate end-points*)
6. Monitor adverse events (*Safety biomarkers*)
7. Identify new biological pathways involved in the pathology of specific disorder and identify new treatment opportunities

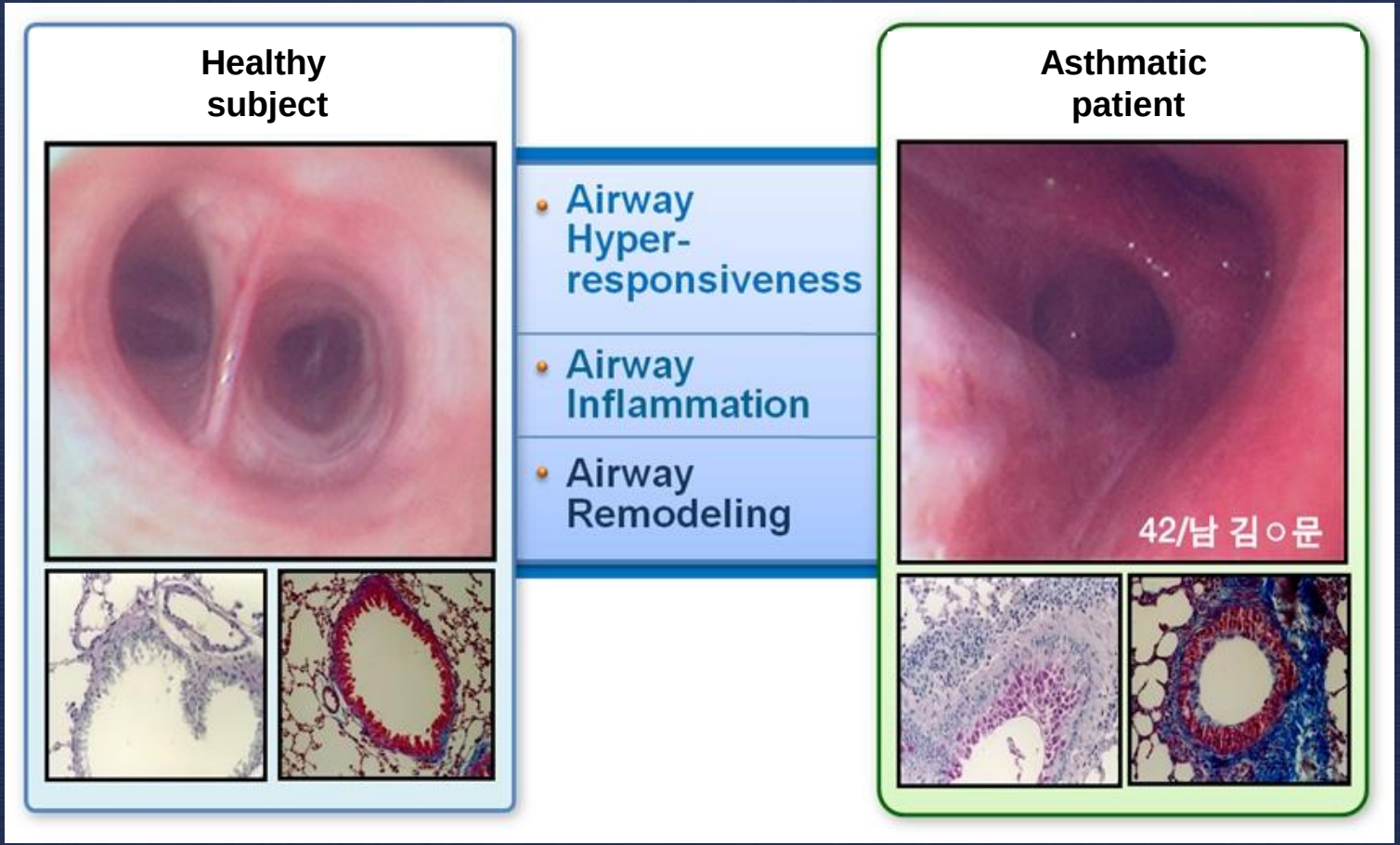
M. Cazzola, G. Novelli. *Pulmonary Pharmacology & Therapeutics* (2010)

An ideal biomarker?

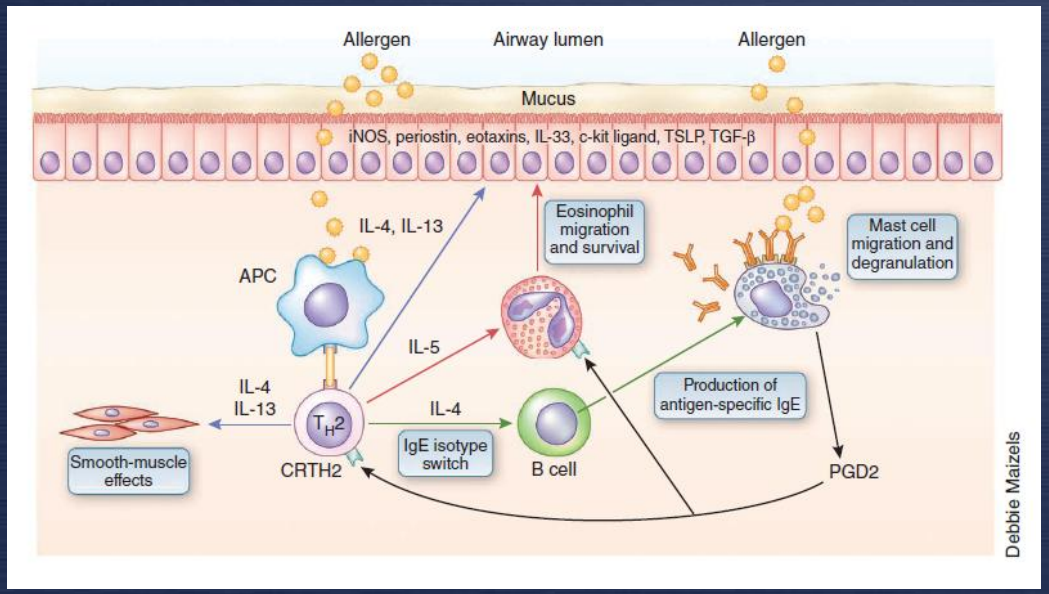
- *Clinical relevance*
  - Indicating a clear relationship between the biomarker and the pathophysiological events in a disorder, causing a clinical endpoint
- *Sensitivity and specificity*
  - For intervention effects
- *Reliability and repeatability*
  - The biomarker should be measured in a precise and reproducible way
- *Simplicity* of sampling methodology and measurement/detection technique
  - To promote widespread use

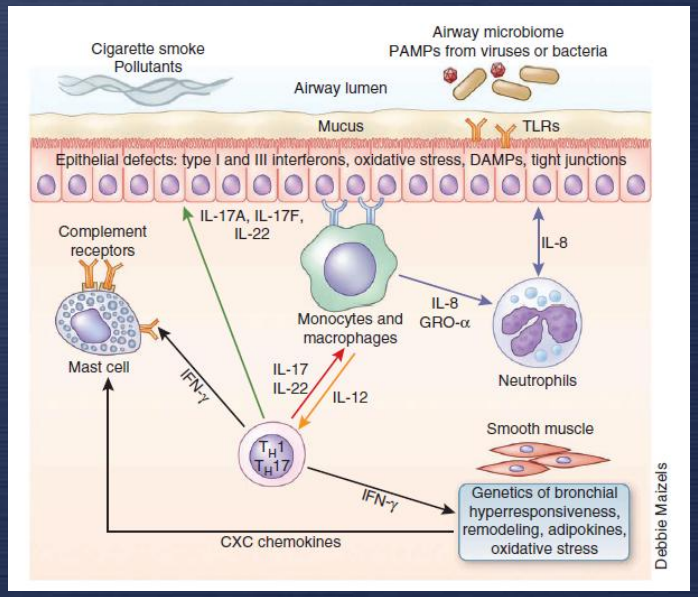
Lesko LJ, Atkinson AJ Jr. *Annu Rev Pharmacol Toxicol.* (2001)
Z. Diamant *et al. Pulmonary Pharmacology & Therapeutics* (2010)

Bronchial asthma



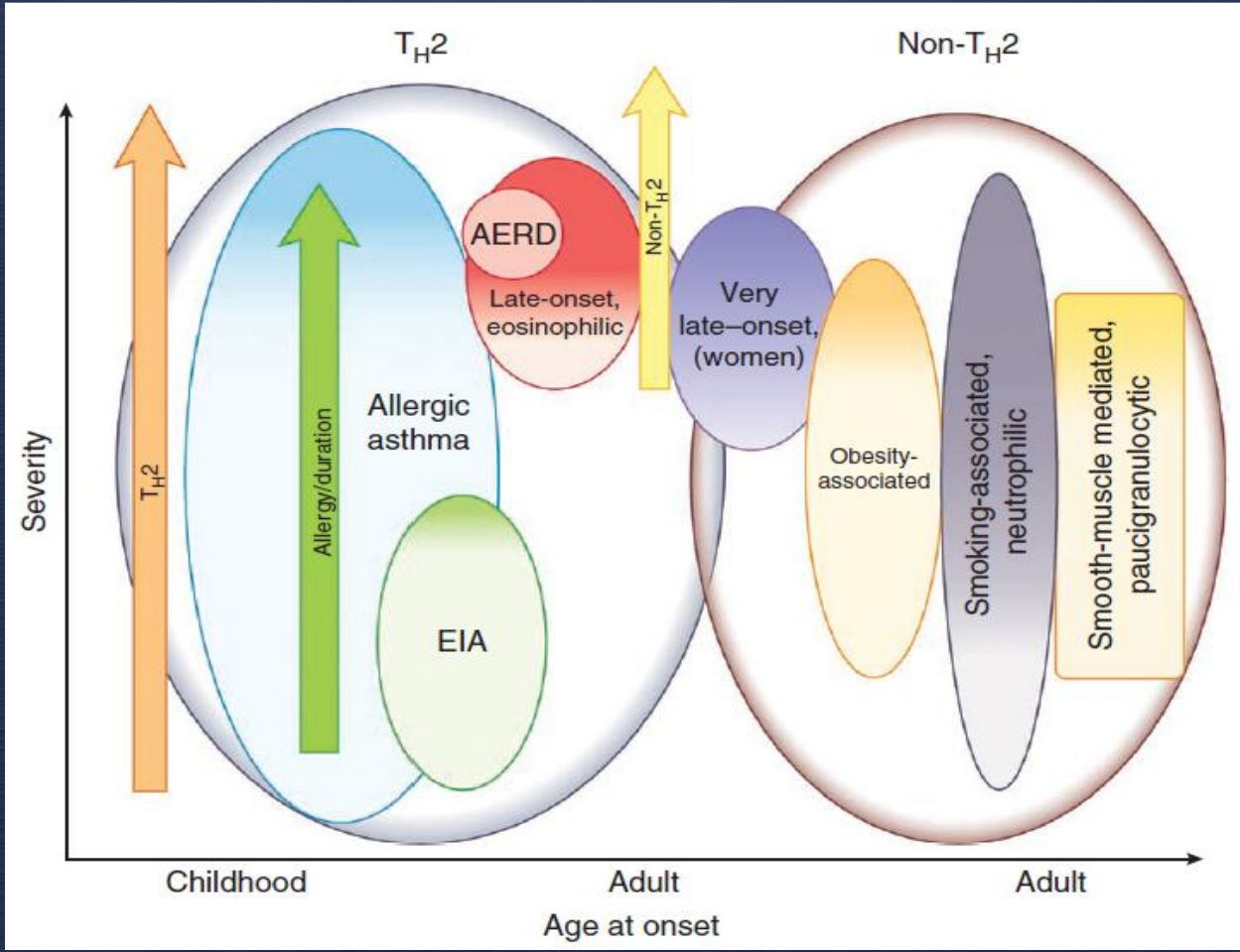
Th2 vs. non Th2 inflammation





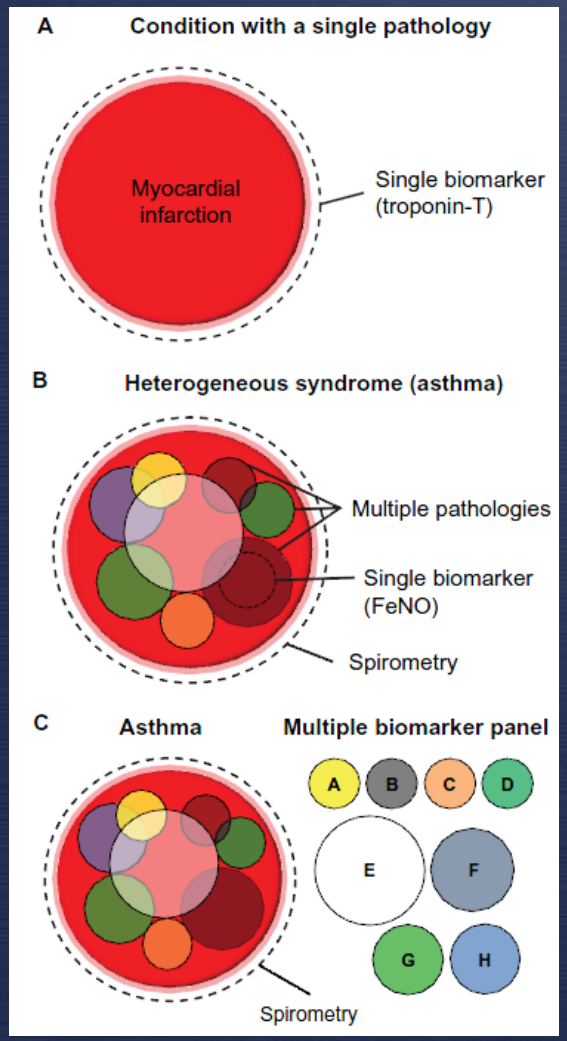
Wenzel S. Nature Medicine 2012;18:716-725

Bronchial asthma phenotypes



Sally E Wenzel. *Nat Med* (2012)

Heterogeneities of bronchial asthma



… in general, one single biomarker may capture only a small fraction of the intervention effect and, therefore, it is important to sample multiple biomarkers whenever possible…

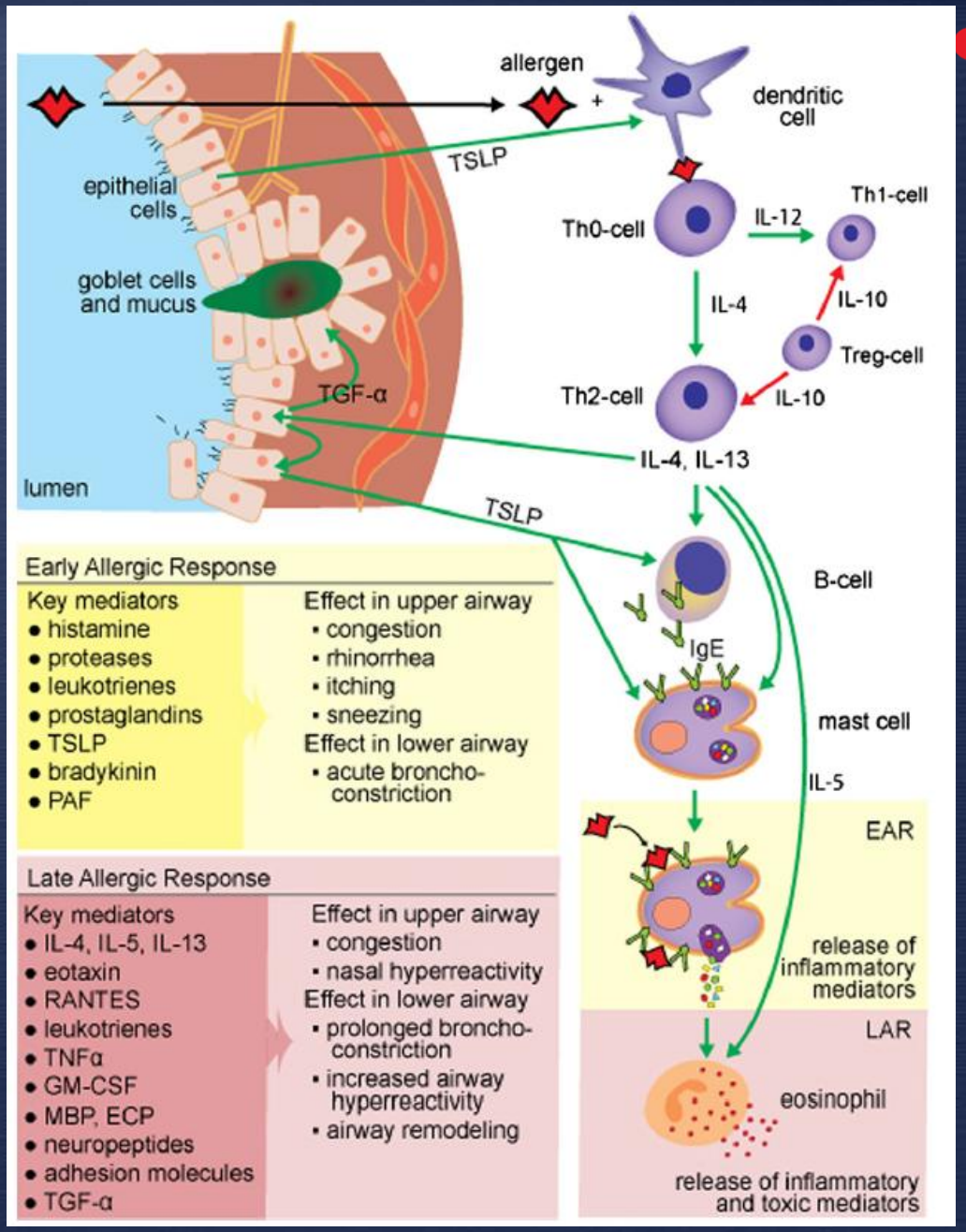
…A panel of biomarkers is required to diagnose the subtype of asthma accurately in an individual…

Wadsworth S, *et al. J Asthma Allergy* (2011)
Z. Diamant *et al. Pulmonary Pharmacology & Therapeutics* (2010)

Where to search for biomarkers?

- *Blood*
- *Urine*
- *BAL*
- *Bronchial Biopsies*
- *Induced Sputum*
- *Exhaled Air*
  - Fractional exhaled nitric oxide (FeNO)
  - Exhaled breath condensate (EBC)
  - Volatile organic compounds (VOCs), …

Current status of biomarkers in asthma and allergy



Z. Diamant *et al. Pulmonary Pharmacology & Therapeutics* (2010)

Current biomarkers in asthma

Table I Advantages and disadvantages of currently used asthma biomarkers

Biomarker	Advantage	Disadvantage
Pulmonary function tests (PFTs) (FEV_1, AHR)	Non-invasive, well validated, sensitive – ie, will detect all asthmatics, reproducible, PFTs will change rapidly with treatment.	Unable to identify sub-phenotypes of asthmatics, PFTs do not reflect pathology, PFTs cannot predict treatment response.
Tissue biopsy	Definitive measure of airway inflammation.	Highly invasive, disconnect between cell counts and symptoms, time consuming, requires high level of expertise.
Induced sputum (differential inflammatory cell counts)	Less invasive than tissue biopsy, reliable indicator of airway inflammation.	Very uncomfortable process, limited to children >8 yrs, requires expertise, reproducibility problems.
Exhaled nitric oxide (FeNO)	Non-invasive, simple measurement methods, indicates treatment (steroid) response.	Proven only for a sub-set of asthmatics, expensive equipment, a single biomarker reflects a single pathology.

Wadsworth S, *et al. J Asthma Allergy* (2011)

Current biomarkers in asthma

Table 2 Alternative biomarkers for asthma diagnosis and management

Biomarker source	Advantages	Disadvantages
Exhaled breath condensate (EBC) – pH & proteins (Inflammatory markers; IL-6, IL-8, TNF-α, H_2O_2, leukotrienes, 8-isoprostane. Non-inflammatory markers; actin, cytokeratins, albumin)	Non-invasive, multiple biomarkers in sample therefore sub-phenotyping possible.	Collection techniques drastically affect proteins in sample, reproducibility a problem, salivary contamination, unproven clinical effectiveness.
Serum proteins (Leptin/adiponectin, eosinophillic cationic protein, chemokines, chitinases)	Less invasive, multiple biomarkers = sub-phenotyping, standardised collection and processing techniques.	Less sensitive and slower response to airway changes, unproven clinical effectiveness.
Urinary metabolites (>70) endpoints	Non-invasive, multiple biomarkers = sub-phenotyping, standarised collection and processing, good sensitivity and specificity.	Unproven clinical effectiveness (although preliminary data is positive), limited access to NMR equipment.

Wadsworth S, *et al. J Asthma Allergy* (2011)

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Which biomarkers should be assessed as standardized asthma outcomes in future clinical research studies?

Asthma outcomes: Biomarkers

- National Institutes of Health institutes and federal agencies convened an expert group
  - Conducted a comprehensive search of the literature to identify studies that developed and/or tested asthma biomarkers
  - Classified the biomarkers as either
    - Core (required in future studies),
    - Supplemental (used according to study aims and standardized), or
    - Emerging (requiring validation and standardization)

And discussed at an NIH–organized workshop convened in *March 2010* and finalized in *September 2011*

Szefler SJ, *et al. J Allergy Clin Immunol.* (2012)

Recommendations

TABLE I. Recommendations for classifying asthma biomarker outcome measures for NIH-initiated clinical research for adults and children

	Characterization of study population for prospective clinical trials (ie, baseline information)	Prospective clinical trial efficacy/effectiveness outcomes	Observational study outcomes*
Core outcomes	Serologic multiallergen screen (IgE) to define atopic status (also for observational studies)	None	None
Supplemental outcomes	1. FENO 2. Sputum eosinophils 3. CBC (total eosinophils) 4. Total IgE 5. Allergen-specific IgE 6. Urinary LTE_4	1. FENO 2. Sputum eosinophils 3. CBC (total eosinophils) 4. Total IgE 5. Allergen-specific IgE 6. Urinary LTE_4	1. FENO 2. Sputum eosinophils 3. CBC (total eosinophils) 4. Total IgE 5. Allergen-specific IgE 6. Urinary LTE_4
Emerging outcomes	1. Allergen skin prick testing 2. Sputum neutrophils and analytes† 3. Airway imaging 4. Exhaled breath condensate markers 5. Discovery through genetics and genomics	1. Allergen skin prick testing 2. Sputum neutrophils and analytes 3. Airway imaging 4. Cortisol measures 5. Exhaled breath condensate markers 6. Discovery through genetics and genomics	1. Sputum neutrophils and analytes 2. Airway imaging 3. Discovery through genetics and genomics

Szefler SJ, *et al. J Allergy Clin Immunol.* (2012)

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Emerging Biomarkers

- *Cortisol measures*
- *High-resolution CT scanning*
- *Sputum neutrophils and analytes*
- *Exhaled breath condensate*
- *Biomarker discovery through genetics and genomic profiling*

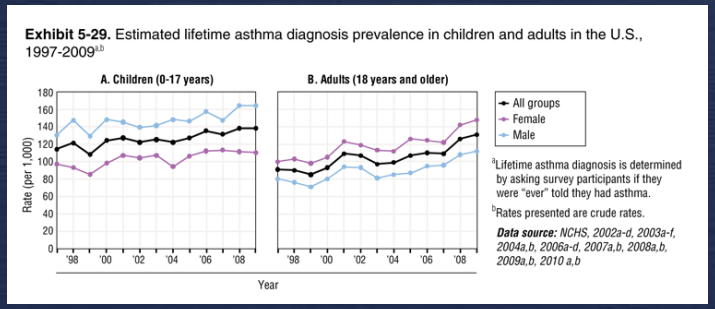
What is a severe asthma?

- **UNIFORM DEFINITION OF SEVERE ASTHMA**

  - Severe asthma is defined by the **level of current clinical control** and **risks**
  - as *"Uncontrolled asthma which can result in risk of frequent severe exacerbations (or death) and/or adverse reactions to medications and/or chronic morbidity (including impaired lung function or reduced lung growth in children)."*

  – Using this definition, both interventional studies and surveys suggest that at least 5–10% of the asthma population has **"severe" asthma.**

  – An estimated 300 million people worldwide suffer from asthma, with 250,000 annual deaths attributed to the disease. It is estimated that the number of people with asthma will grow by more than 100 million by 2025.



Uniform definition of asthma severity, control, and exacerbations: Document presented for the World Health Organization Consultation on Severe Asthma
J Allergy Clin Immunol 2010;126:926-38

What is a severe asthma?

- Severe asthma includes 3 groups, each carrying different public health messages and challenges:

1. Untreated severe asthma.
2. Difficult-to-treat severe asthma.
3. Treatment-resistant severe asthma. This group includes the following:
   - Asthma for which control is not achieved despite the highest level of recommended treatment: refractory asthma and corticosteroid-resistant asthma.

   - Asthma for which control can be maintained only with the highest level of recommended treatment.

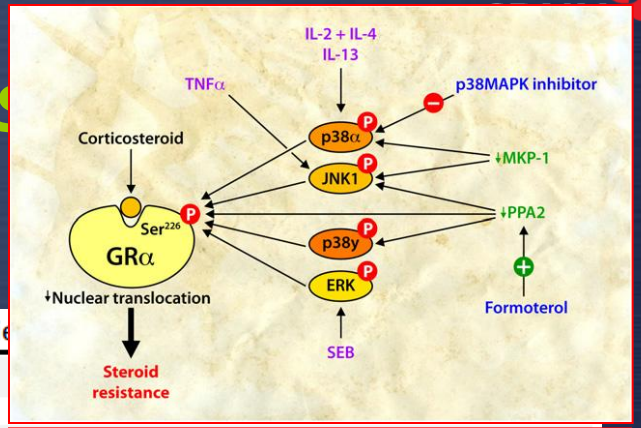
Uniform definition of asthma severity, control, and exacerbations: Document presented for the World Health Organization Consultation on Severe Asthma
J Allergy Clin Immunol 2010;126:926-38

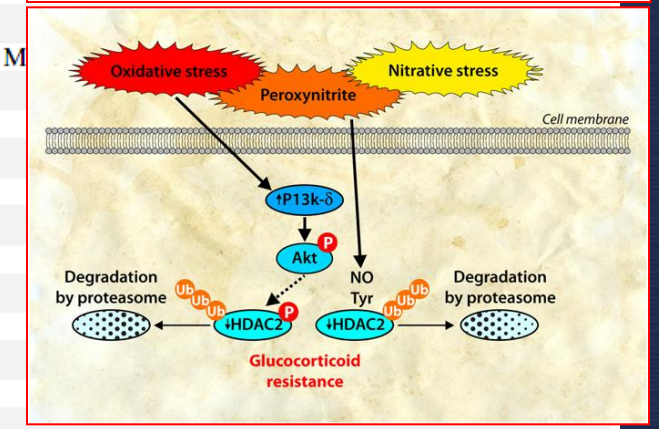
Corticosteroid Resis

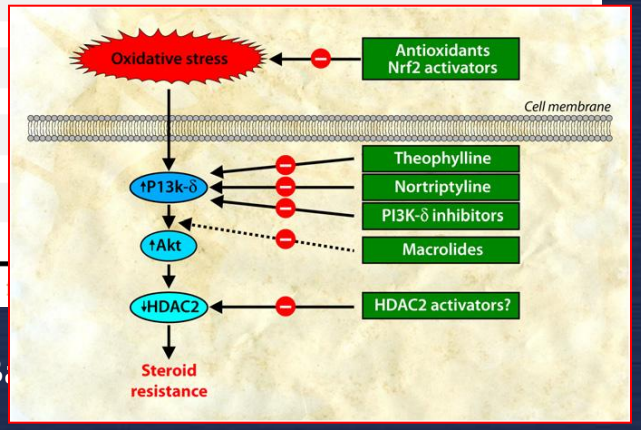
TABLE I. Molecular mechanisms of steroid resistance in asthmatic patients and patie

- Familial glucocorticoid resistance
- GR modification
  - ↑ Phosphorylation: ↓ nuclear translocation
    - ↑ p38MAPKα caused by IL-2 plus IL-4 or IL-13 in patients with severe asthma or caused by M
    - ↑ p38MAPKγ in patients with severe asthma
    - ↑ JNK1 caused by proinflammatory cytokines in patients with severe asthma
    - ↑ ERK caused by microbial superantigens in patients with severe nonallergic asthma
    - ↓ MKP-1 in patients with severe asthma
    - ↓ PP2A in patients with severe asthma
  - Nitrosylation: ↑ NO from inducible NO synthase
  - Ubiquitination: ↑ degradation by proteasome
- Increased GRβ expression
- Increased proinflammatory transcription factors
  - AP-1, JNK
- Immune mechanisms
  - ↓ Treg cells (↓IL-19, ↓vitamin D_3)
  - ↑ T_H17 (IL-17)
- Defective histone acetylation
  - ↓ Acetylation of lysine-5 on histone 4 in patients with severe asthma
  - ↓ HDAC-2 in patients with COPD and patients with severe asthma and smokers with asthma
  - ↑ Oxidative stress
  - ↑ PI3Kδ activation

ERK, Extracellular signal-regulated kinase; *MAP*, mitogen-activated protein; *MIF*, macrophage migration inhibitory

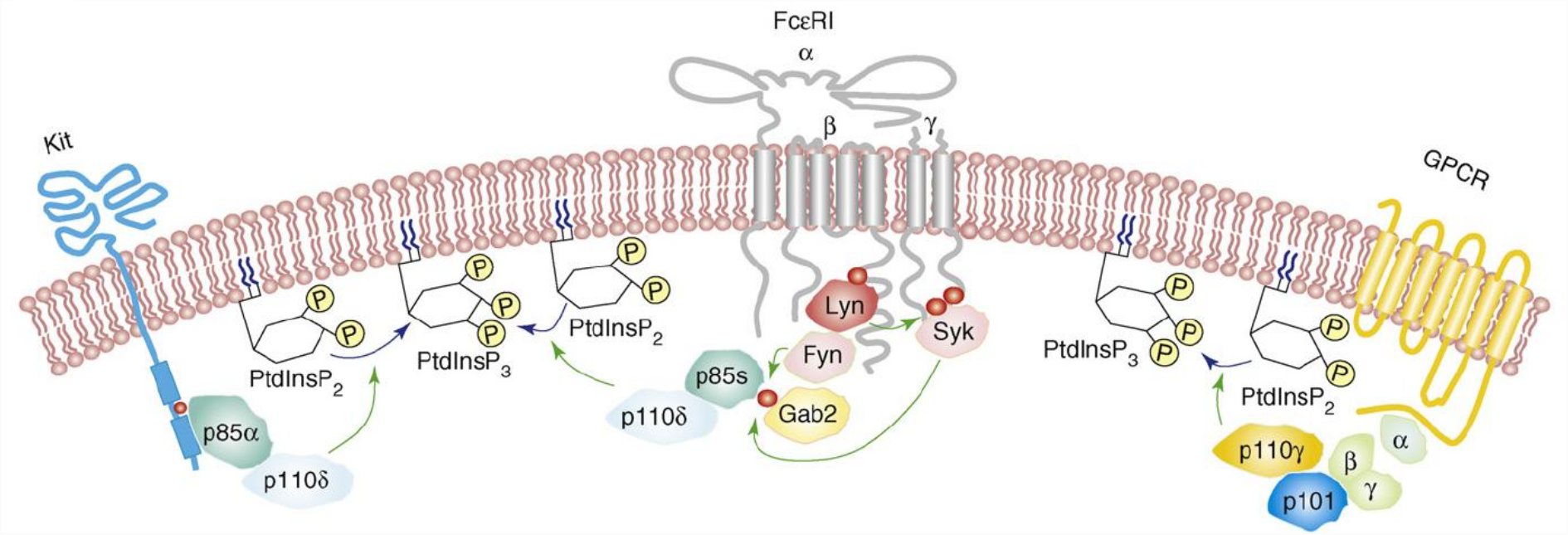






PI3K

phosphorylate inositol lipids within cell membranes as an early step in the signaling cascades initiated by many ligand-receptor interactions.



Trends in Immunology 2008;29:493-501

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Phosphoinositide 3-kinase family

PI3K activity has been found in all eukaryotic cell types and is linked to an incredibly diverse set of key cellular functions, including

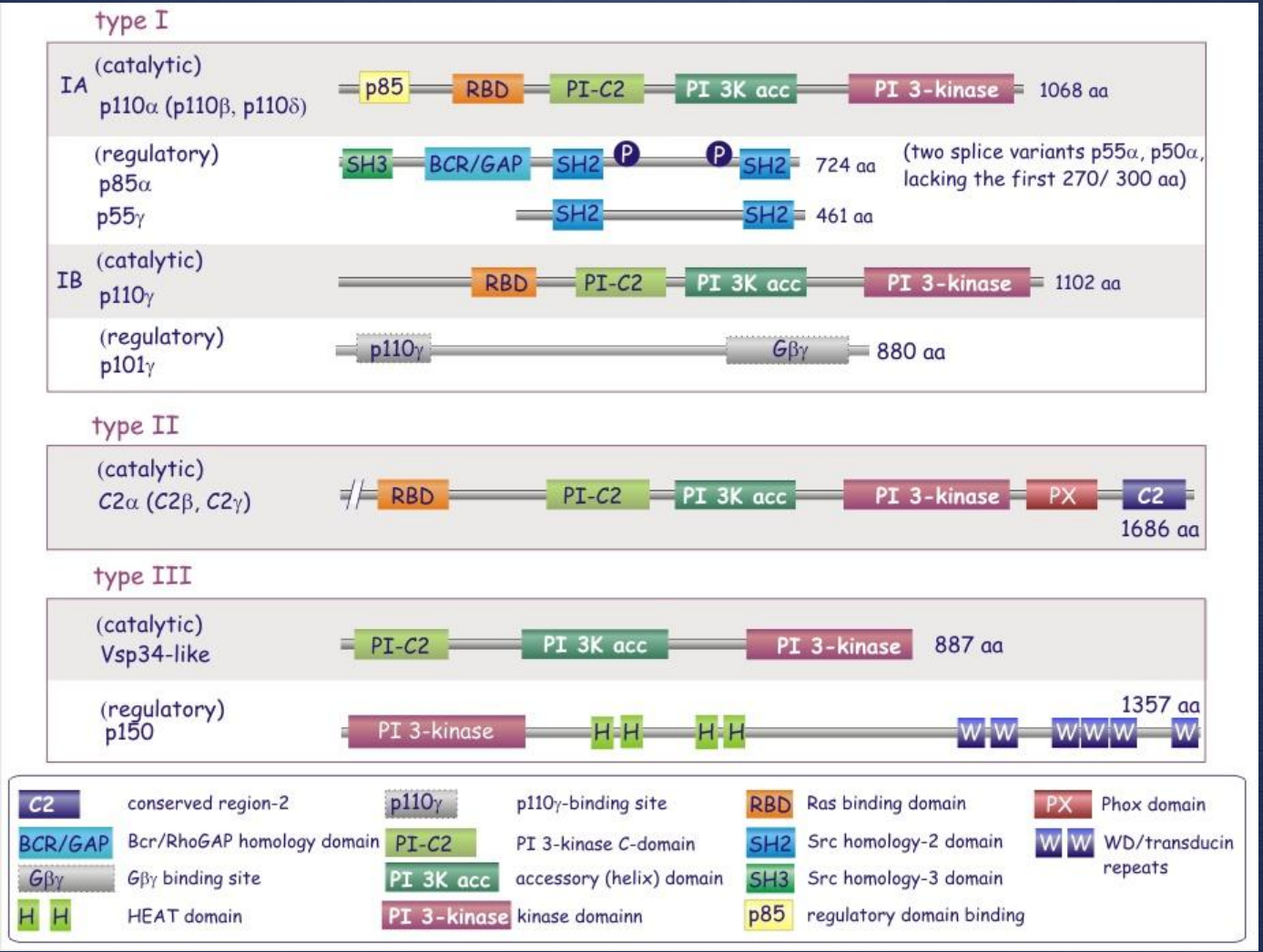
cell growth, proliferation, motility, differentiation, survival and intracellular trafficking

Table 1 Characteristics of the members of PI3K family

PI3K class	Isoform	Catalytic subunit	Regulatory subunit	Substrate
Class IA	PI3Kα	p110α	p85α, p85β, p55α, p55γ, p50α	(*In vitro*) PtdIns, PtdIns 4-phosphate, PtdIns (4,5)-bisphosphate
	PI3Kβ	p110β		
	PI3Kδ	p110δ		(*In vivo*) PtdIns (4,5)-bisphosphate
Class IB	PI3Kγ	p110γ	p101, p84	(*In vitro*) PtdIns, PtdIns 4-phosphate
Class II	PI3K-C2α	C2α	Clathrin	(*In vitro*) PtdIns, PtdIns 4-phosphate
	PI3K-C2β	C2β		
	PI3K-C2γ	C2γ		(*In vivo*) no clearly defined
Class III		Vps34	Vps15p	(*In vitro, in vivo*) PtdIns

SJ Park et al. Phosphoinositide 3-kinase δ inhibitor as a novel therapeutic agent in asthma. *Respirology (2008)*

PI3K isoforms



Involvement of PTEN in Asthma;

First description of effects of PI3K inhibitors on asthma

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Involvement of PTEN in airway hyperresponsiveness and inflammation in bronchial asthma

Yong-Geun Kwak,[1] Chang H. Song,[2] Ho K. Yi,[3] Pyoung H. Hwang,[3] Jong-Suk Kim,[4] Kyung S. Lee,[5] and Yong C. Lee[5]

[1]Department of Pharmacology, Institute of Cardiovascular Research,
[2]Department of Anatomy,
[3]Department of Pediatrics,
[4]Department of Biochemistry, and
[5]Department of Internal Medicine, Research Center for Allergic Immune Diseases, Chonbuk National University Medical School, Chonju, South Korea

Phosphatase and tensin homologue deleted on chromosome ten (PTEN) is part of a complex signaling system that affects a variety of important cell functions. PTEN blocks the action of PI3K by dephosphorylating the signaling lipid phosphatidylinositol 3,4,5-triphosphate. We have used a mouse model for asthma to determine the effect of PI3K inhibitors and PTEN on allergen-induced bronchial inflammation and airway hyperresponsiveness. PI3K activity increased significantly after allergen challenge. PTEN protein expression and PTEN activity were decreased in OVA-induced asthma. Immunoreactive PTEN localized in epithelial layers around the bronchioles in control mice. However, this immunoreactive PTEN dramatically disappeared in allergen-induced asthmatic lungs. The increased IL-4, IL-5, and eosinophil cationic protein levels in bronchoalveolar lavage fluids after OVA inhalation were significantly reduced by the intratracheal administration of PI3K inhibitors or adenoviruses carrying PTEN cDNA (AdPTEN). Intratracheal administration of PI3K inhibitors or AdPTEN remarkably reduced bronchial inflammation and airway hyperresponsiveness. These findings indicate that PTEN may play a pivotal role in the pathogenesis of the asthma phenotype.

J. Clin. Invest. 111:1083–1092 (2003). doi:10.1172/JCI200316440.

Journal of Clinical Investigation
2003;111:1083-92.(IF:16.915)

REUTERS HEALTH INFORMATION

PTEN Levels Tied to Asthma Symptoms in Mice

By David Douglas

NEW YORK (Reuters Health) Apr 24 - The intracellular signaling regulator PTEN appears to play a "pivotal role" in the pathogenesis of asthma, according to South Korean researchers.

Reporting in the April issue of the Journal of Clinical Investigation, Dr. Yong C. Lee of Chonbuk National University Medical Center, Chonju, and colleagues note that PTEN (phosphatase and tensin homologue deleted on chromosome ten) may serve as a hub linking signaling pathways. Among such pathways are those involving the enzyme P13K, which is implicated in inflammation.

Studies of ovalbumin-sensitized mice, a model of allergic asthma, showed that P13K activity increased significantly after allergen challenge. In controls, immunoreactive PTEN was found in the epithelial layers around the bronchioles. However, "it dramatically disappeared in allergen-induced asthmatic lungs."

In addition, intratracheal administration of P13K inhibitors or adenoviruses carrying PTEN cDNA (AdPTEN) caused a significant drop in the elevated levels of interleukin (IL) -4, IL-5 and eosinophil cationic protein seen after ovalbumin challenge.

P13K inhibitors or AdPTEN also "remarkably reduced" bronchial inflammation and airway hyperresponsiveness.

These results, the researchers conclude, "strongly indicate that alterations in PTEN levels are implicated in the pathogenesis of bronchial asthma."

Furthermore, Dr. Lee told Reuters Health that as part of their ongoing investigations in this field, "we have also demonstrated that PTEN expression is diminished in airway inflammatory cells of patients with bronchial asthma."

REUTERS, NEW YORK by David Douglas

Role of PTEN in Asthma

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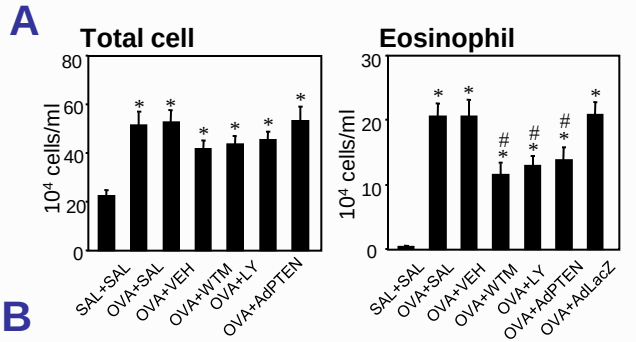
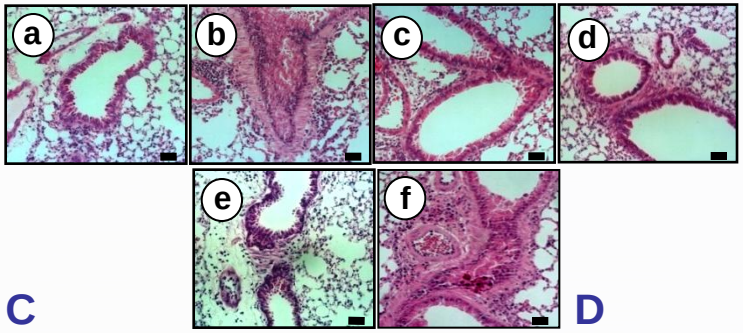
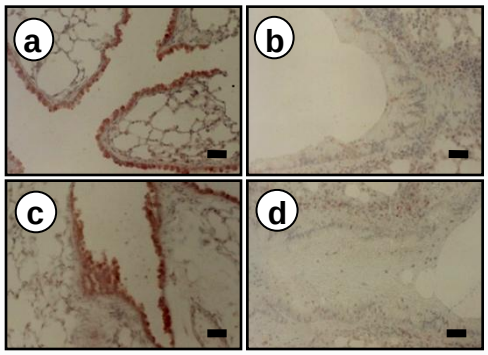
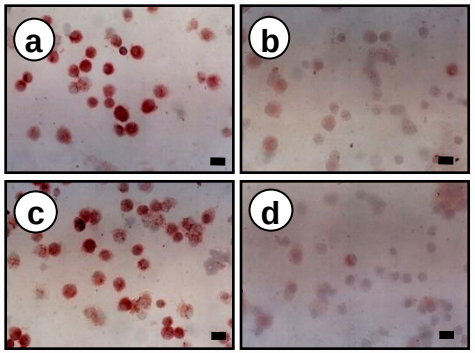
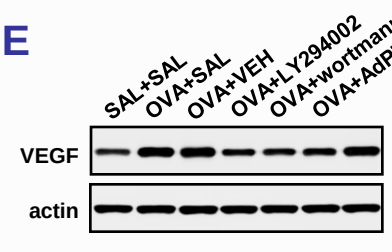
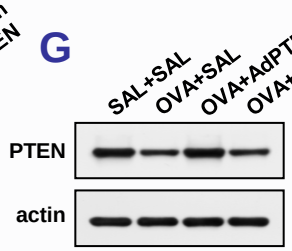


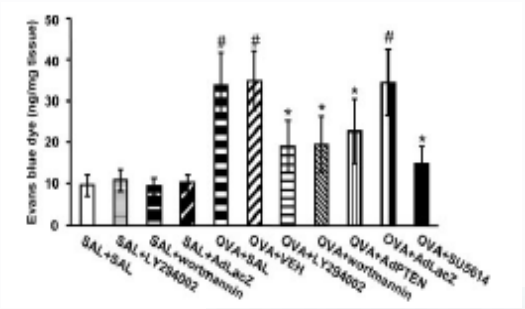


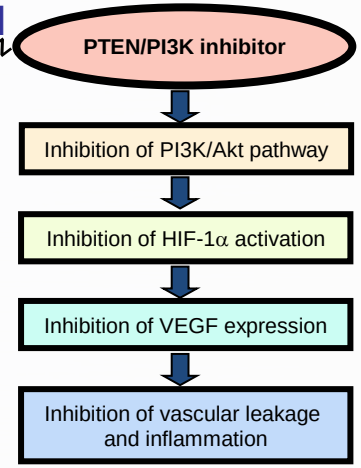


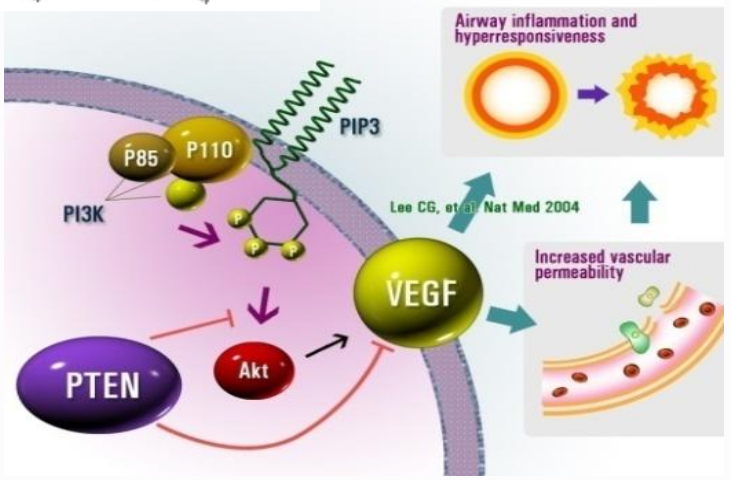






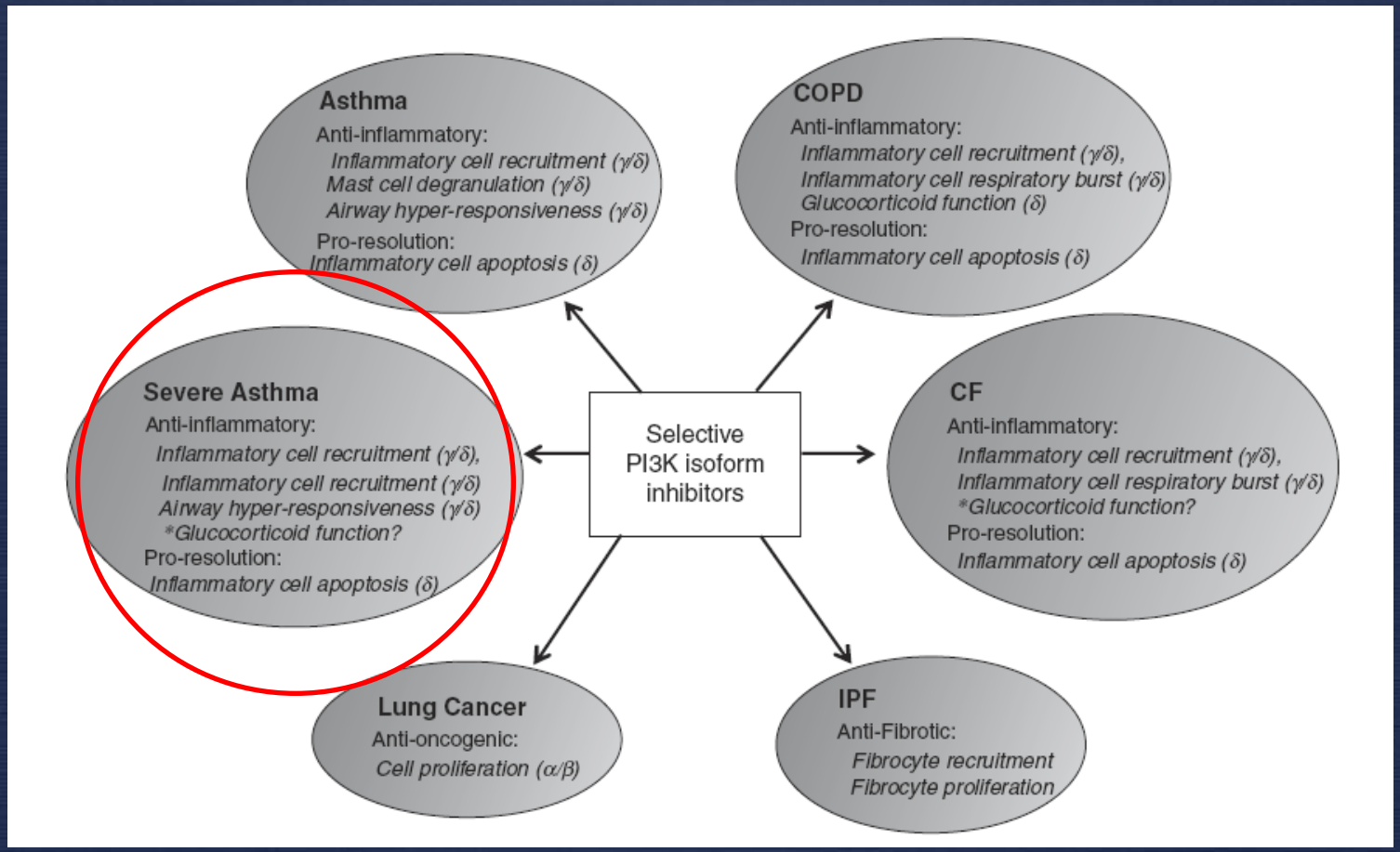






J Clin Invest 2003;111:1083-92, J Immunol 2006;179:6820-8, Mol Pharmacol 2006;69:1829-39, Immunopharmacol Immunotoxicol 2008

PI3K isoforms in pulmonary disorders



Ther Adv Respir Dis 2010; 4: 19-34

Involvement of PI3Kδ in Asthma

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Inhibition of phosphoinositide 3-kinase δ attenuates allergic airway inflammation and hyperresponsiveness in murine asthma model

Kyung S. Lee,* Ho K. Lee,* Joel S. Hayflick,† Yong C. Lee,*,[1] and Kamal D. Puri†,[1]

*Department of Internal Medicine, Research Center for Allergic Immune Diseases, Chonbuk National University Medical School, Jeonju, South Korea; and †ICOS Corporation, Bothell, Washington, USA

ABSTRACT P110δ phosphoinositide 3-kinase (PI3K) plays a pivotal role in the recruitment and activation of certain inflammatory cells. Recent findings revealed that the activity of p110δ also contributes to allergen-IgE-induced mast cell activation and vascular permeability. We investigated the role of p110δ in allergic airway inflammation and hyperresponsiveness using IC87114, a selective p110δ inhibitor, in a mouse asthma model. BALB/c mice were sensitized with OVA and, upon OVA aerosol challenge, developed airway eosinophilia, mucus hypersecretion, elevation in cytokine and chemokine levels, up-regulation of ICAM-1 and VCAM-1 expression, and airway hyperresponsiveness. Intratracheal administration of IC87114 significantly ($P<0.05$) attenuated OVA-induced influx into lungs of total leukocytes, eosinophils, neutrophils, and lymphocytes, as well as levels of IL-4, IL-5, IL-13, and RANTES in a dose-dependent manner. IC87114 also significantly ($P<0.05$) reduced the serum levels of total IgE and OVA-specific IgE and LTC_4 release into the airspace. Histological studies show that IC87114 inhibited OVA-induced lung tissue eosinophilia, airway mucus production, and inflammation score. In addition, IC87114 significantly ($P<0.05$) suppressed OVA-induced airway hyperresponsiveness to inhaled methacholine. Western blot analyses of whole lung tissue lysates shows that IC87114 markedly attenuated the OVA-induced increase in expression of IL-4, IL-5, IL-13, ICAM-1, VCAM-1, RANTES, and eotaxin. Furthermore, IC87114 treatment markedly attenuated OVA-induced serine phosphorylation of Akt, a downstream effector of PI3K signaling. Taken together, our findings implicate that inhibition of p110δ signaling pathway may have therapeutic potential for the treatment of allergic airway inflammation.—Lee, K. S., Lee, H. K., Hayflick, J. S., Lee, Y. C., Puri, K. D. Inhibition of phosphoinositide 3-kinase δ attenuates allergic airway inflammation and hyperresponsiveness in murine asthma model. *FASEB J.* 20, 455–465 (2006)

CHRONIC INFLAMM
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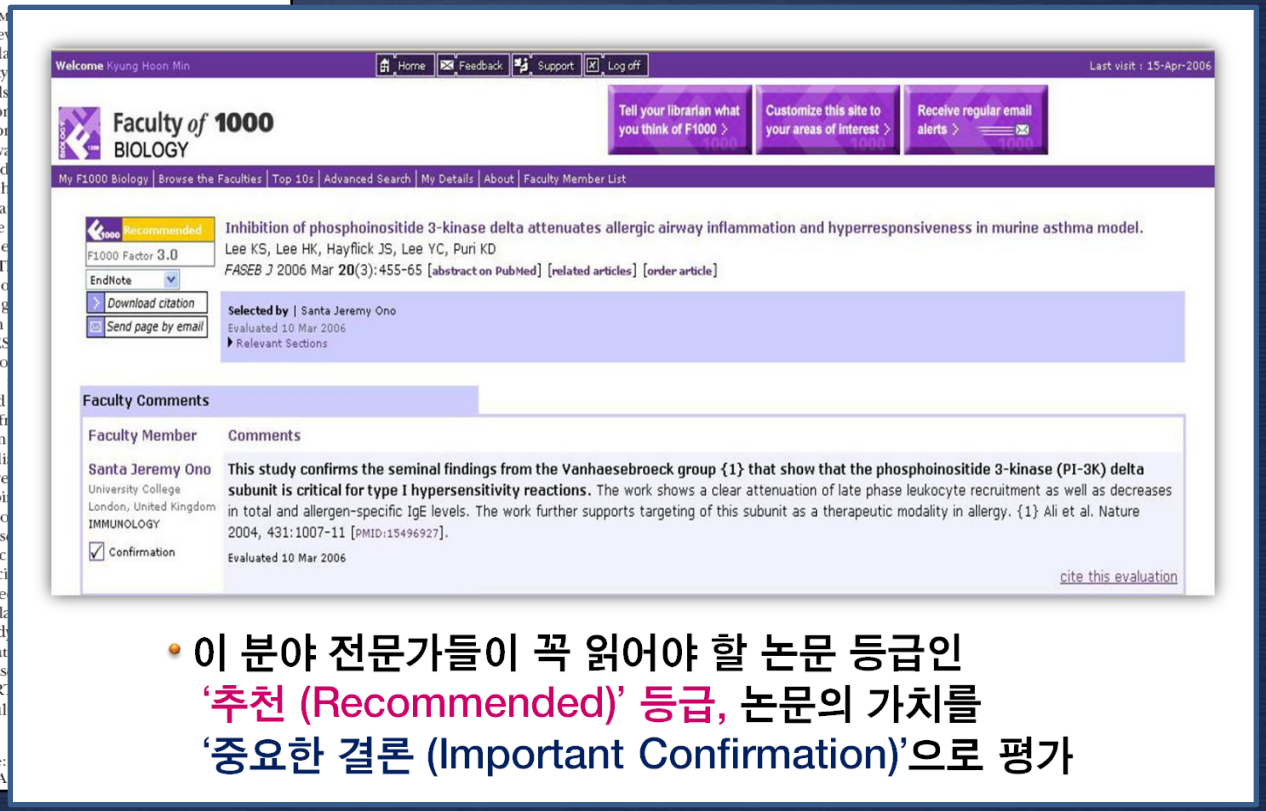
[1] Correspondence:
Ave. SE, Bothell, WA



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FASEB J 2006;20:455-465

전북대학교병원 CHONBUK NATIONAL UNIVERSITY HOSPITAL 전북대학교

PI3Kδ and VEGF in Asthma

CBNU

Phosphoinositide 3-kinase-δ inhibitor reduces vascular permeability in a murine model of asthma

Kyung Sun Lee, PhD,[a,b,c] Seoung Ju Park, MD,[a,b,c] So Ri Kim, MD,[a,b,c] Kyung Hoon Min, MD,[a,b,c] Sun Mi Jin, BS,[b,c] Kamal D. Puri, PhD,[d] and Yong Chul Lee, MD, PhD[a,b,c] *Jeonju, South Korea, and Bothell, Wash*

Background: Bronchial asthma is characterized by inflammation of the airways, which is usually accompanied by increased vascular permeability, resulting in plasma exudation. Vascular endothelial growth factor (VEGF) has been implicated in contributing to asthmatic tissue edema through its effect on vascular permeability. Many cellular responses of VEGF are regulated by the lipid products of phosphoinositide 3-kinase (PI3K). However, the effect of PI3K catalytic subunit p110δ on VEGF-mediated signaling is unknown. Recently, an isoform-specific small molecule inhibitor, IC87114, which is selective for p110δ catalytic activity, has been identified.

Objective: We have sought to investigate the role of PI3K-δ, more specifically in the increase of vascular permeability.

Methods: Female BALB/c mice were sensitized and challenged with ovalbumin. We have investigated the effect of IC87114 on airway inflammation, T_H2 cytokines expression, airway hyperresponsiveness, plasma extravasation, hypoxia-inducible factor 1α expression, and VEGF expression in a murine model of asthma.

Results: Our current study has revealed that IC87114 reduces antigen-induced airway infiltration of inflammatory cells, secretion of T_H2 cytokines in lungs, airway hyperresponsiveness, and vascular permeability. Moreover, we have found that inhibition of p110δ reduces ovalbumin-induced upregulation of VEGF level.

Conclusion: These results suggest that PI3K-δ inhibitor attenuates antigen-induced airway inflammation and hyperresponsiveness by preventing vascular leakage in mice.

Clinical implications: These findings provide a crucial molecular mechanism for the potential role of PI3K-δ in asthma and other airway inflammatory disorders. (J Allergy Clin Immunol 2006;118:403-9.)

Key words: *Airway, PI3K-δ, vascular permeability, VEGF*

Mechanisms of asthma and allergic inflammation

Bronchial asthma is an increasingly prevalent and occasionally severe disease characterized by chronic inflammation of the airways, which is usually accompanied by increased vascular permeability, resulting in plasma exudation.[1] Changes in vascular permeability are crucial to the development and perpetuation of the inflammatory response. Increased vascular permeability causes secretion of intravascular components, which contribute to the airway obstruction and hyperresponsiveness.[2,3]

Phosphoinositide 3-kinase (PI3K) is a signal transduction enzyme that catalyzes the phosphorylation of phosphatidylinositol (4,5)-bisphosphate to form phosphatidylinositol (3,4,5)-trisphosphate (PIP3) in response to activation of either receptor tyrosine kinase, G-protein coupled receptors, or cytokine receptors, which ultimately regulate cell growth, differentiation, survival, proliferation, migration, and cytokine production.[4-7] The class IA PI3K consists of a heterodimer composed of a 110-kd (p110α, β, δ) catalytic subunit and an adaptor protein (p85α, p85β, p55α, p55γ, p50α).[8] Previous studies suggest that PI3K contributes to the pathogenesis of asthma by effecting the recruitment, activation, and apoptosis of inflammatory cells.[9,10] PI3K plays a key role in induction of the T_H2 response.[9-13] This enzyme is

J Allergy Clin Immunol 2006;118:403-409

PI3Kδ and VEGF in Asthma

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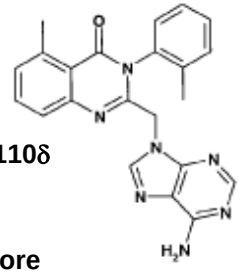
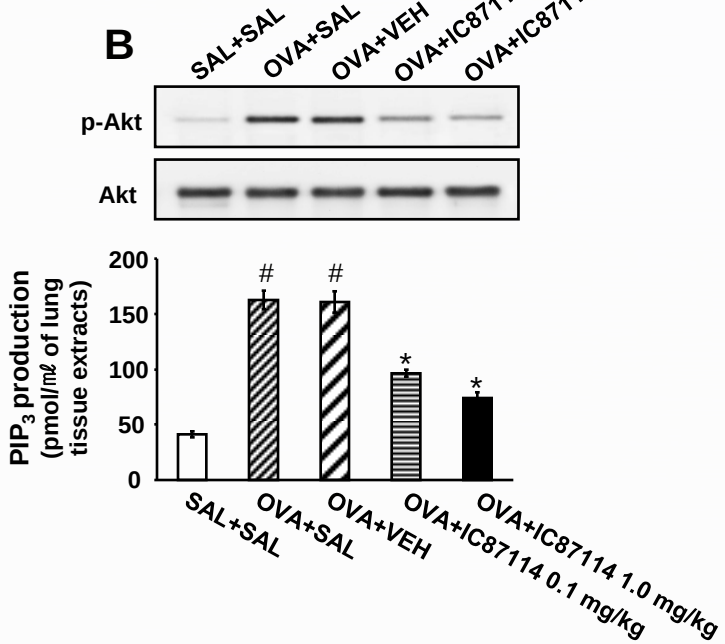
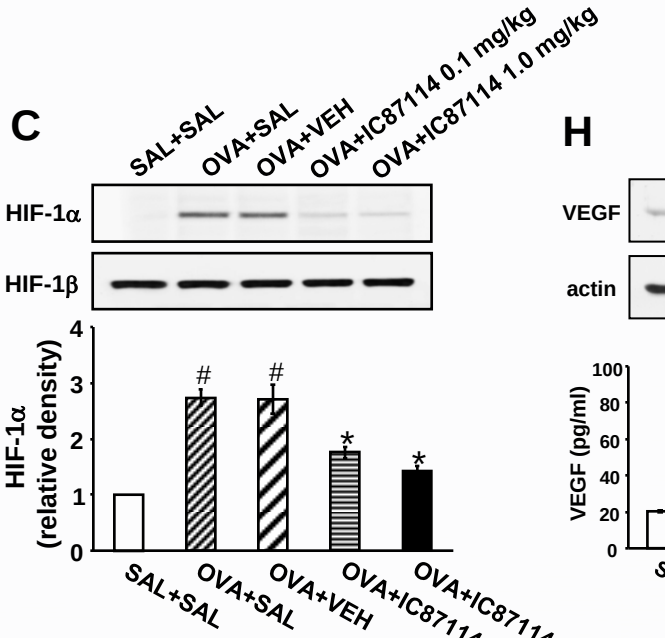
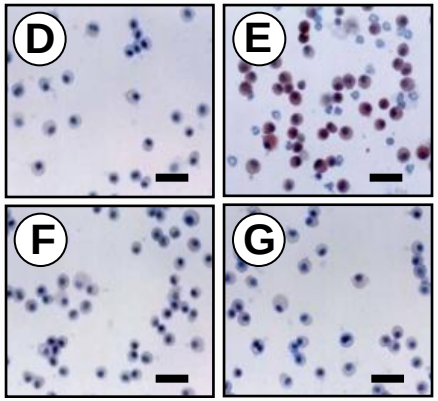


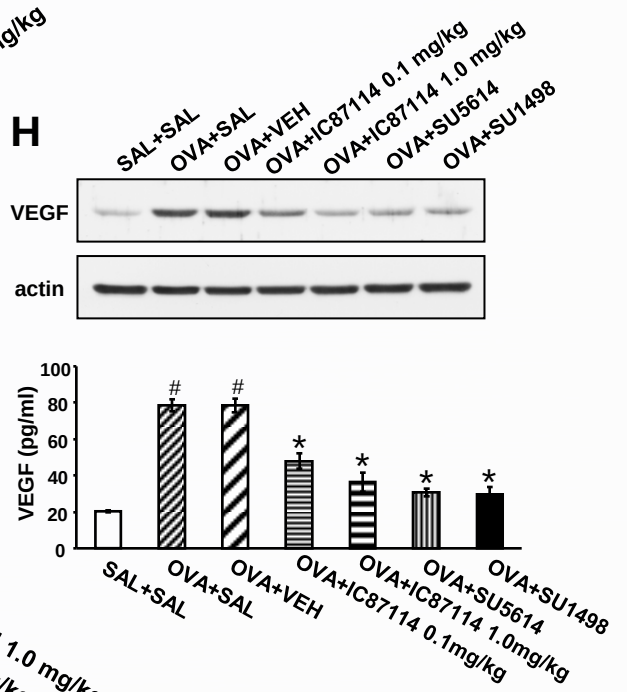
8 - IC87114

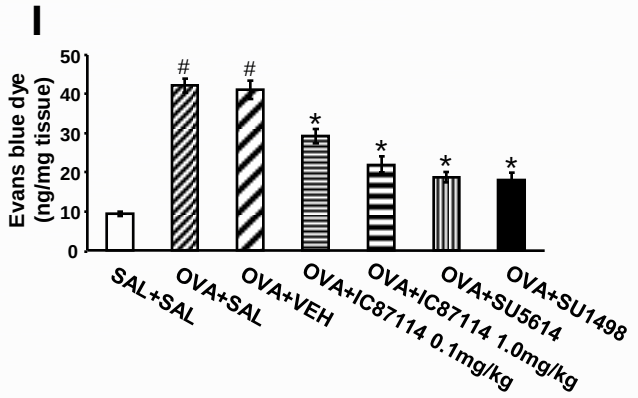
A selective p110δ inhibitor containing a quinazoline core structure.











J Allergy Clin Immunol 2006;118:403-409

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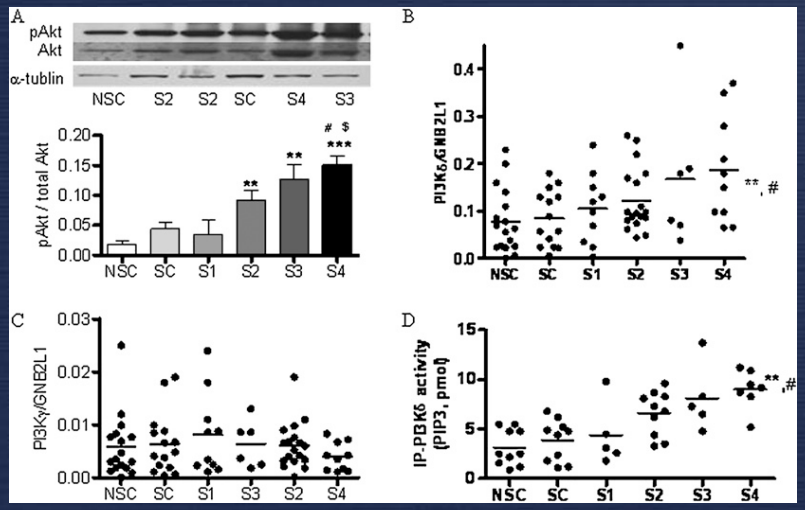
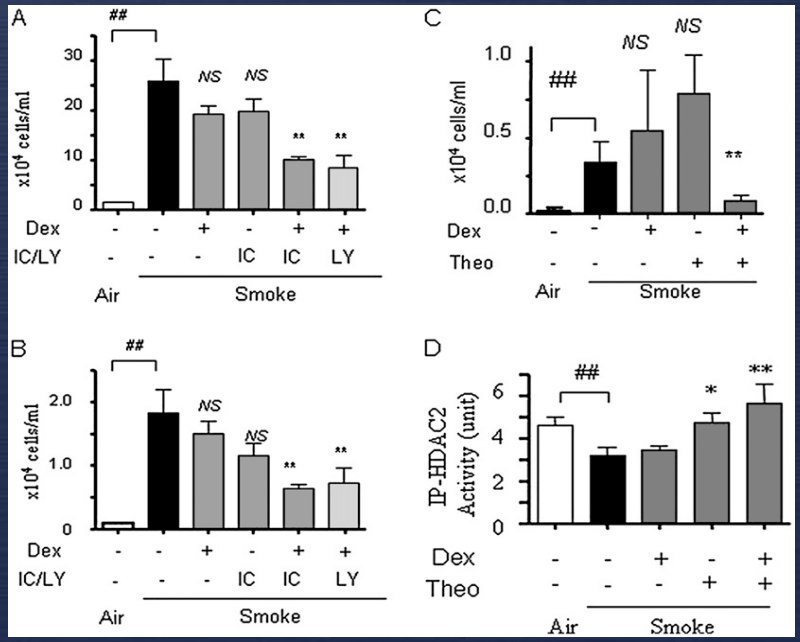
Targeting Phosphoinositide-3-Kinase-δ with Theophylline Reverses Corticosteroid Insensitivity in Chronic Obstructive Pulmonary Disease

Yasuo To[1]*, Kazuhiro Ito[1]*, Yasuo Kizawa[1,2], Marco Failla[1], Misako Ito[1], Tadashi Kusama[2], W. Mark Elliott[3], James C. Hogg[3], Ian M. Adcock[1], and Peter J. Barnes[1]

[1]Airway Disease Section, NHLI Imperial College, London, United Kingdom; [2]Department of Physiology and Anatomy, Nihon University College of Pharmacy, Chiba, Japan; [3]University of British Columbia and the James Hogg iCAPTURE Center for Cardiovascular and Pulmonary Research, St. Paul's Hospital, Vancouver, British Columbia, Canada





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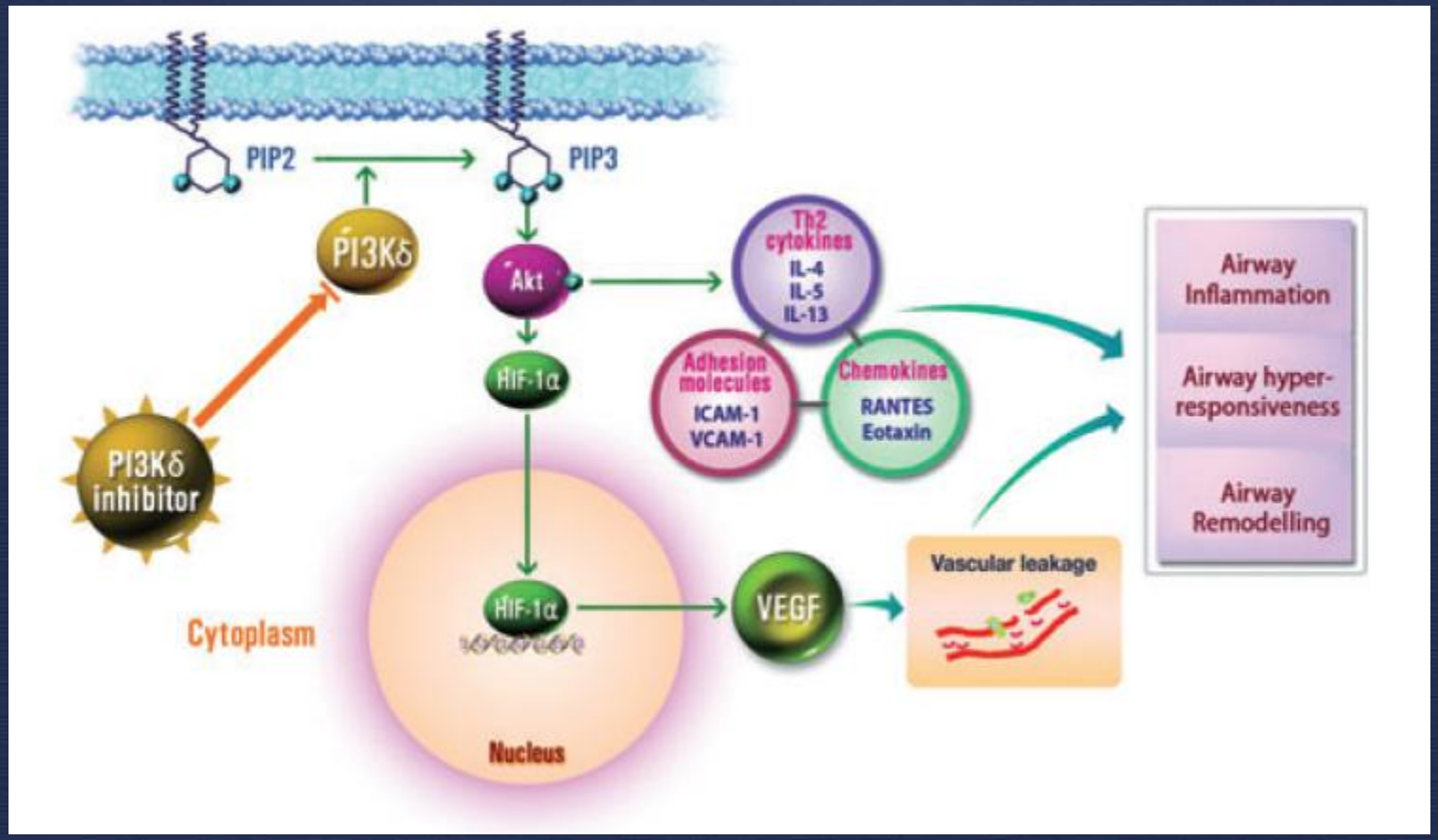
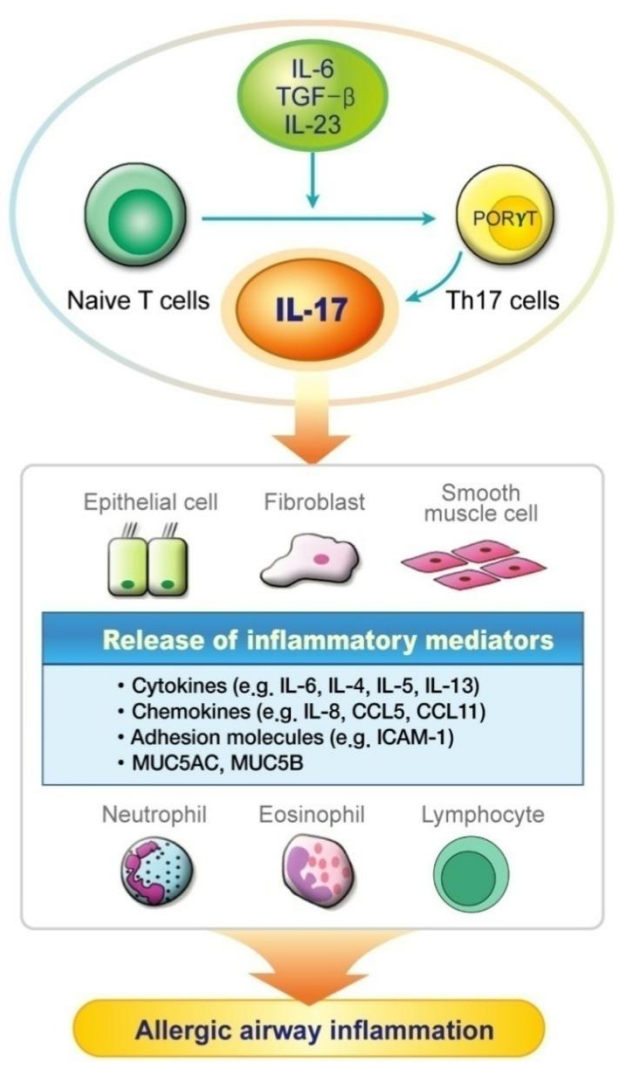
Role of PI3K- δ inhibition in asthma



SJ Park et al. Phosphoinositide 3-kinase δ inhibitor as a novel therapeutic agent in asthma. *Respirology (2008)*

IL-17 in asthma



- Under investigation

- Increased IL-17A and IL-17F levels are positively correlated to disease severity, suggesting an important role for IL-17A and IL-17F in severe asthma.

Al-Ramli W et al. J Allergy Clin Immunol 2009;123:1185–7
Chakir J et al. J Allergy Clin Immunol 2003;111:1293–8

- Elevated IL-17A levels also correlate to increased neutrophilic inflammation, a characteristic of severe and steroid-resistant asthma.

- ***IL-17 in the pathogenesis of asthma, particularly severe, neutrophilic asthma.***

Lee YC et al. Respir Res 2010;11:78

IL-17 in asthma

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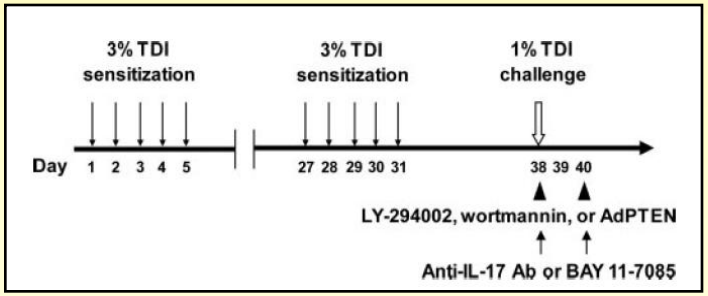
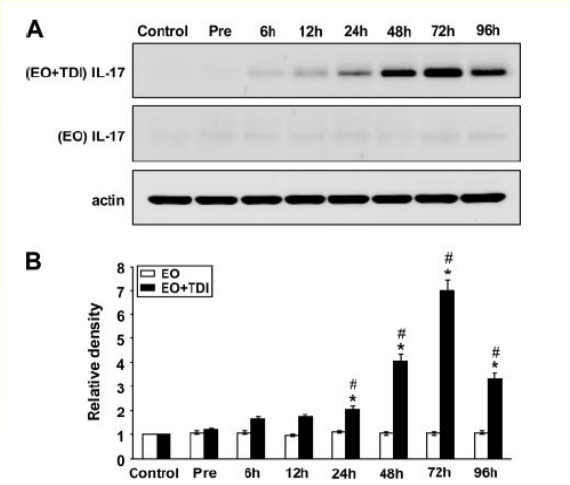
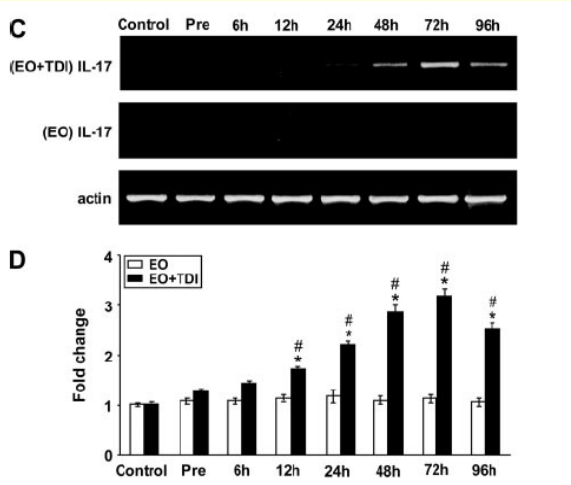
PTEN Down-Regulates IL-17 Expression in a Murine Model of Toluene Diisocyanate-Induced Airway Disease[1]

So Ri Kim,[2*†] Kyung Sun Lee,[2*†] Seoung Ju Park,[*†] Kyung Hoon Min,[*†] Ka Young Lee,[*†] Yeong Hun Choe,[*†] Young Rae Lee,[‡] Jong Suk Kim,[‡] Soo Jong Hong,[§] and Yong Chul Lee[3*†]







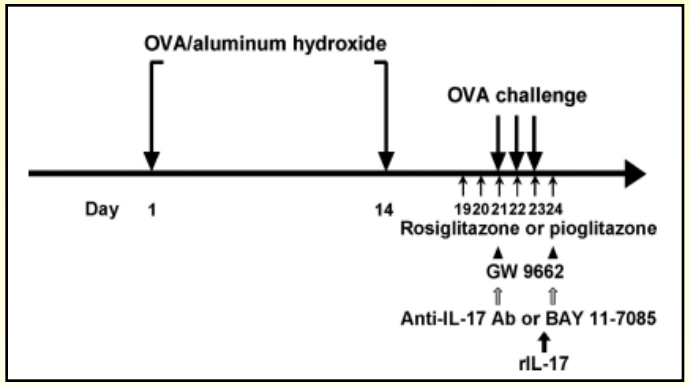
Lee YC et al. J Immunol 2007; 79: 6820–6829.

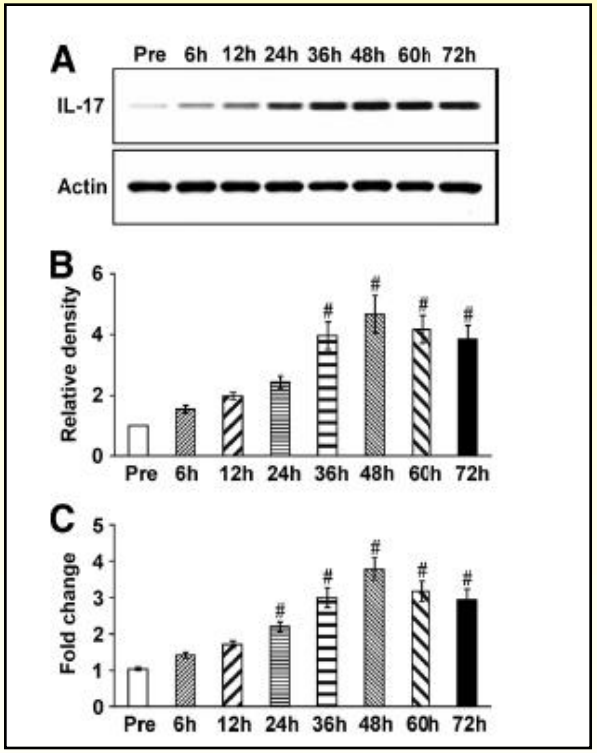
IL-17 in asthma

CBNU

Peroxisome Proliferator-Activated Receptor γ Agonist Down-Regulates IL-17 Expression in a Murine Model of Allergic Airway Inflammation[1]

Seoung Ju Park,[2*†] Kyung Sun Lee,[2*†] So Ri Kim,[*†] Kyung Hoon Min,[*†] Yeong Hun Choe,[*†] Hee Moon,[*†] Han Jung Chae,[†‡] Wan Hee Yoo,[*†] and Yong Chul Lee[3*†]





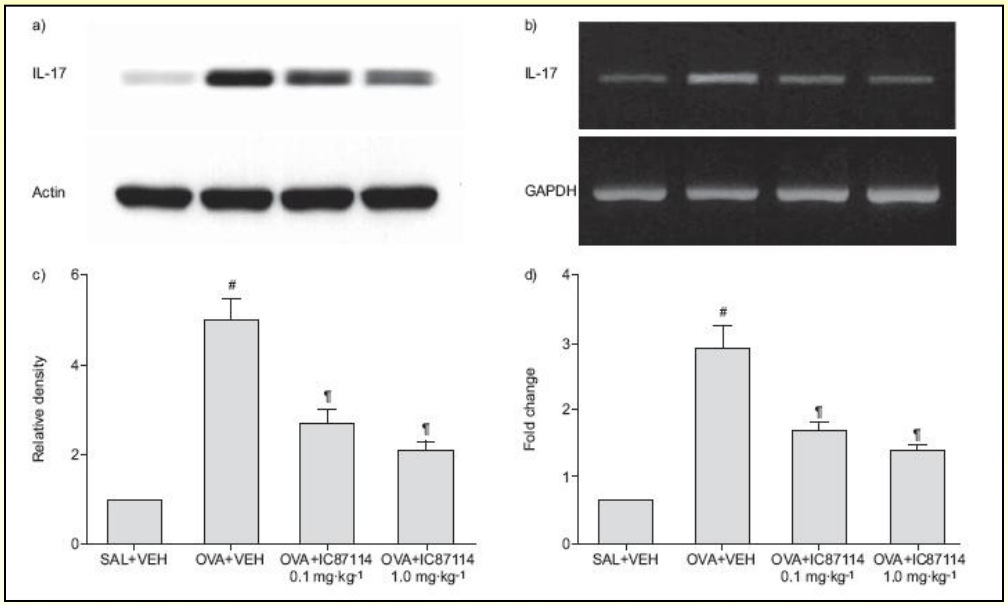
Lee YC et al. J Immunol 2009;183: 3259–3267.

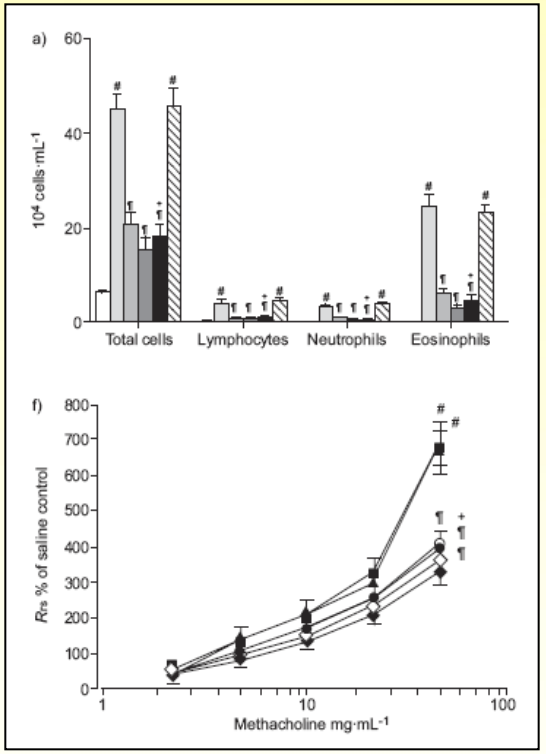
IL-17 in asthma

CBNU

Phosphoinositide 3-kinase δ inhibitor suppresses interleukin-17 expression in a murine asthma model

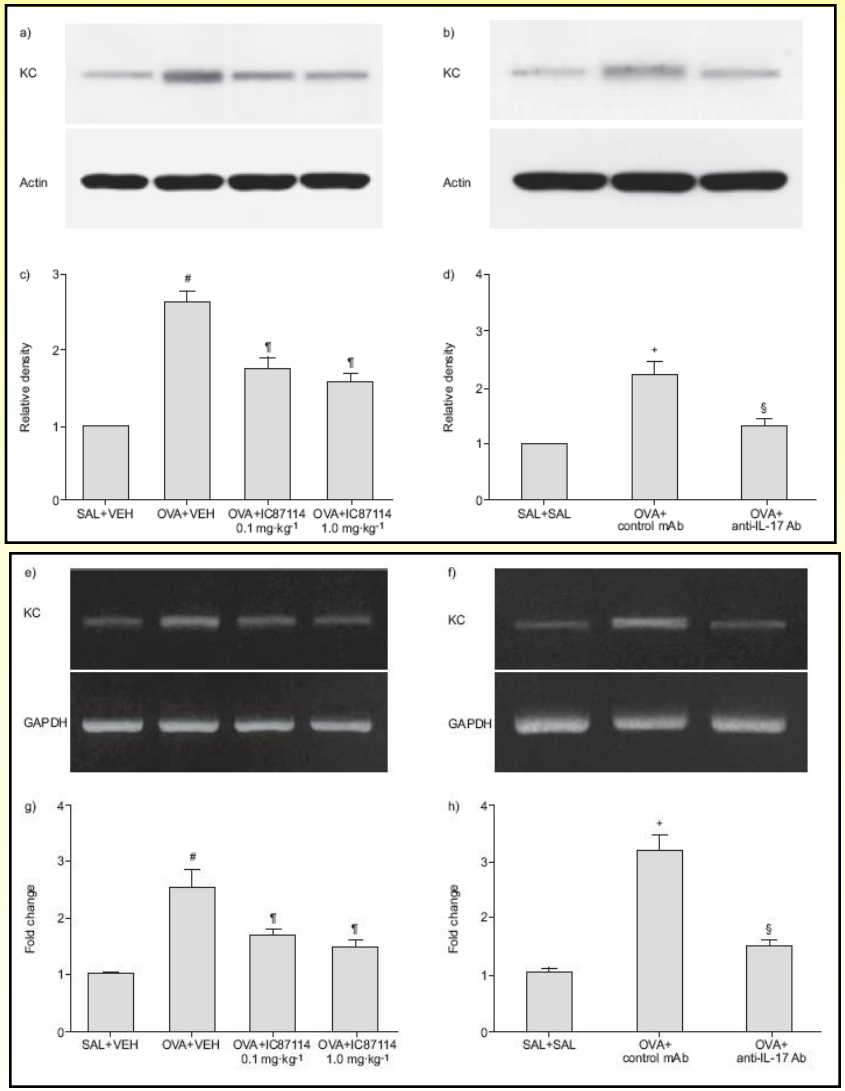
S.J. Park*,¶, K.S. Lee*,¶, S.R. Kim*, K.H. Min*, H. Moon*, M.H. Lee*, C.R. Chung*, H.J. Han*, K.D. Puri# and Y.C. Lee*

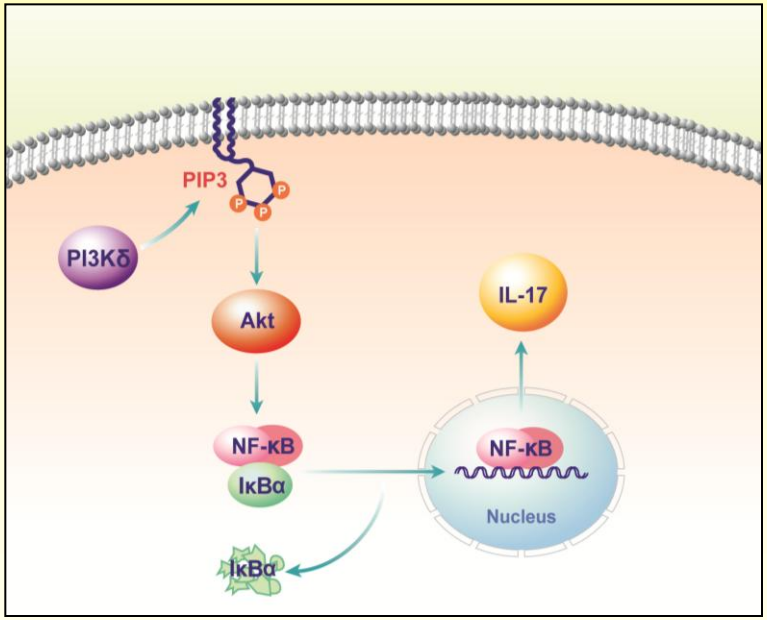




Lee YC et al. Eur Respir J 2010; 36: 1448–1459

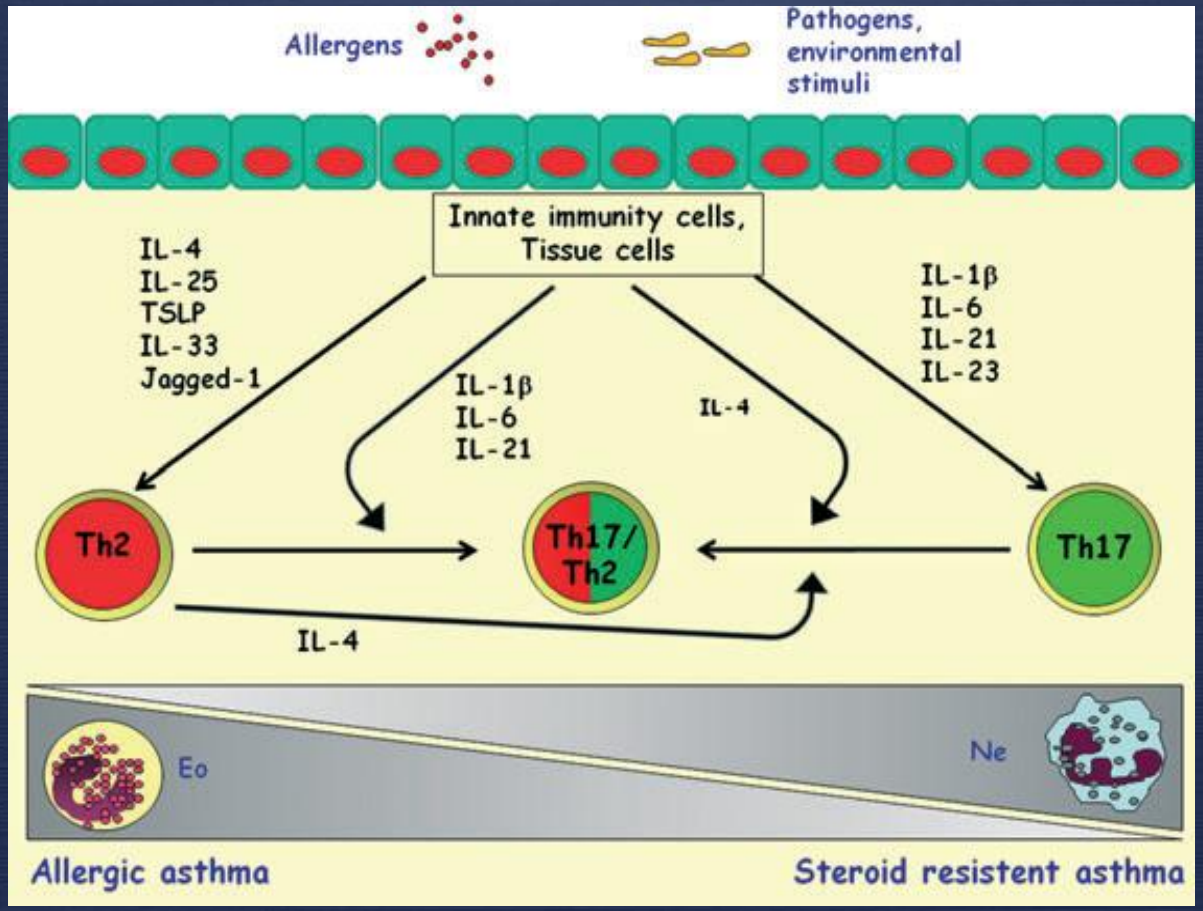
IL-17 in asthma





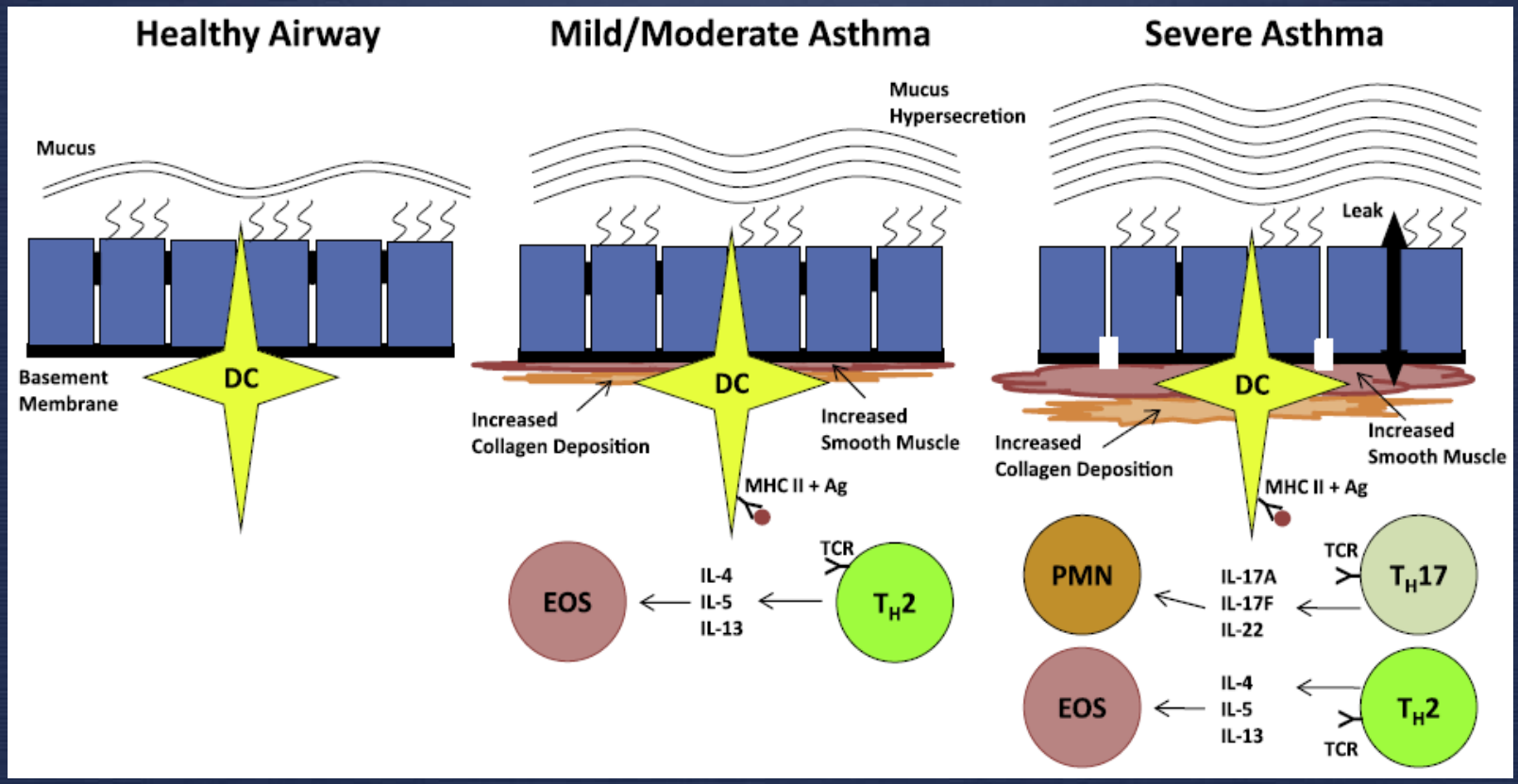
Lee YC et al. Eur Respir J 2010; 36: 1448–1459

Th2/Th17 cells in asthma



Cosmi L et el. Allergy 2011

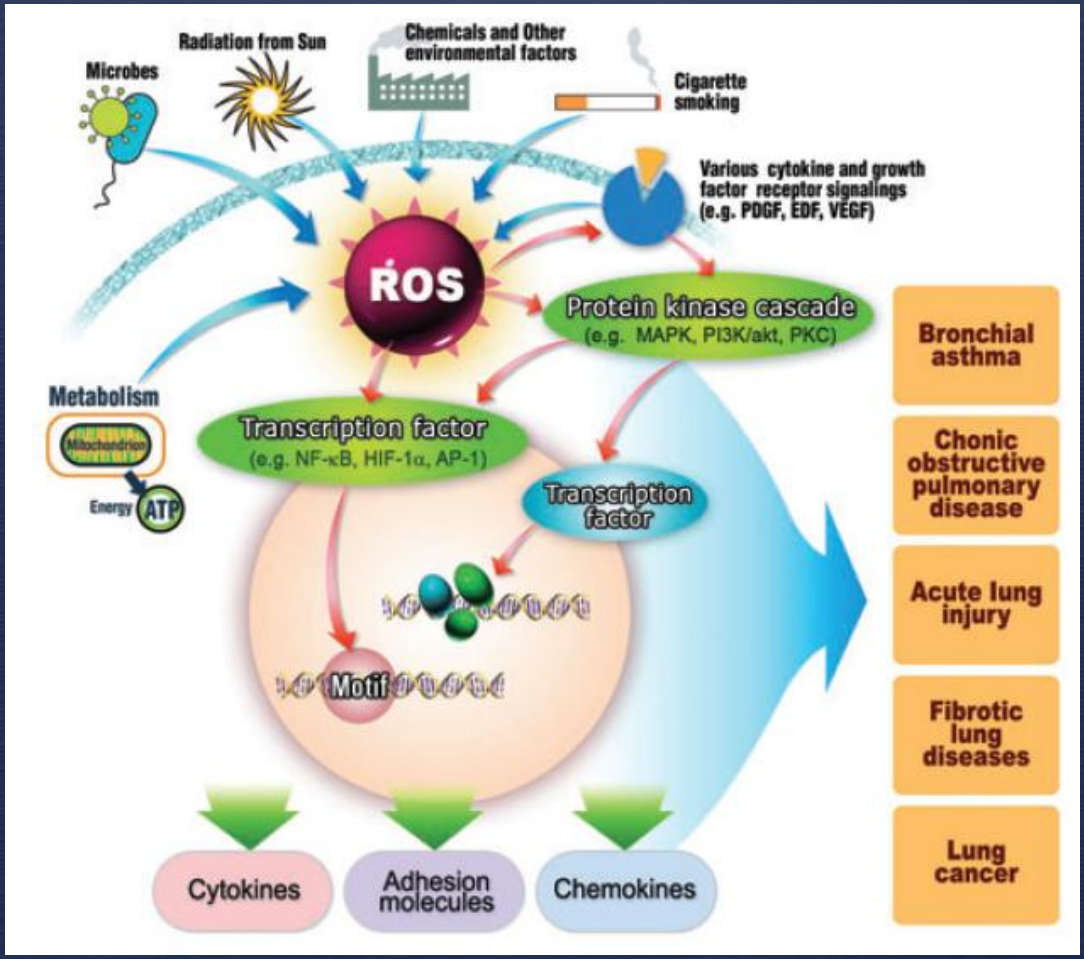
Th17 cells in asthma; summary



Aujlia SJ et al. Biochimica et Biophysica Acta 2011

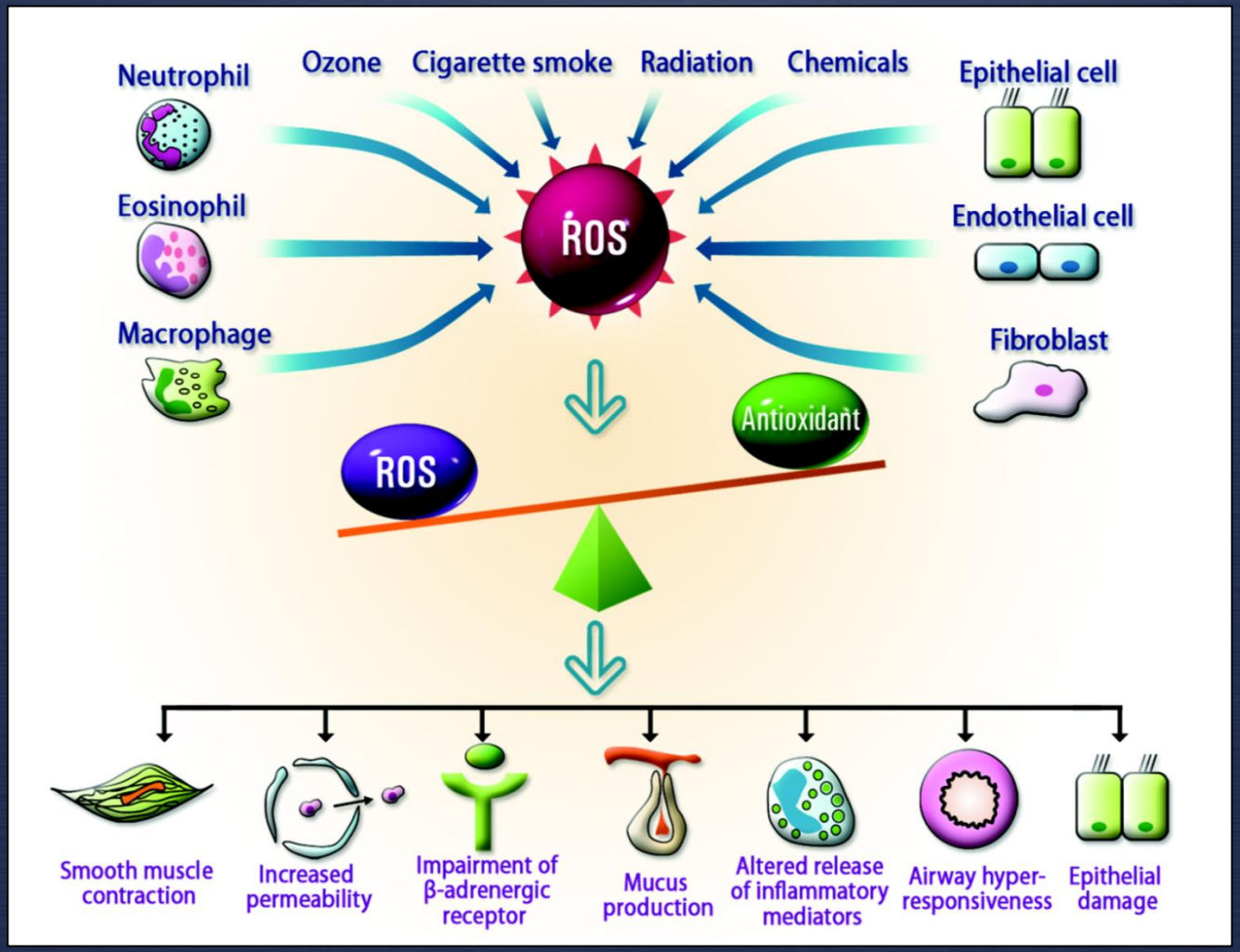
CBNU

Oxidative Stress in various pulmonary disorders



Lee YC, et al. Respirology (2009)

Reactive oxygen species (ROS) in airways



Reactive oxygen species (ROS) in asthma

CBNU

[Antioxidant therapy for asthma]

Blockade of airway hyperresponsiveness and inflammation in a murine model of asthma by a prodrug of cysteine, L-2-oxothiazolidine-4-carboxylic acid

Yong Chul Lee,*,† Kyung Sun Lee,*,† Seoung Ju Park,*,† Hee Sun Park,*,† Jae Sung Lim,*,† Kwang-Hyun Park,‡ Mie-Ja

ABSTRACT

Oxidative stress plays an i production of reactive oxy mechanisms may be presen reducing agents against carboxylic acid (OTC), a mouse model for asthma inflammation and airway h inflammation and airway hy was increased by ovalbu administration of OTC. The lungs after OVA inhalation the increased expression o inhalation was significantly increased NF-κB levels in were decreased by the adr airway inflammation and hy

Key words: eosinophilia • N

A Prodrug of Cysteine, L-2-Oxothiazolidine-4-carboxylic Acid, Regulates Vascular Permeability by Reducing Vascular Endothelial Growth Factor Expression in Asthma

Kyung Sun Lee, Hee Sun Sun Mi Jin, Kwang-Hyun

Department of Internal Medicine, Ai H.S.P., S.J.P., S.R.K., K.H.M., S.M. Research (U.-H.K.), and Departmen

Received June 22, 2005; accepted Augu

ABSTRACT

Inflammation of the asthmatic airw by increased vascular permeability idative stress plays critical roles i though reactive oxygen species (R vascular leakage, the mechanisms creased vascular permeability are r have used a murine model of asthm L-2-oxothiazolidine-4-carboxylic aci teine that acts as an antioxidant, r crease of vascular permeability. Th lowing typical pathophysiological

A novel thiol compound, *N*-acetylcysteine amide, attenuates allergic airway disease by regulating activation of NF-κB and hypoxia-inducible factor-1α

Kyung Sun Lee[1]*, So Ri Kim[1]*, Hee Sun Park[2], Seoung Ju Park[1], Kyung Hoon Min[1], Ka Young Lee[1], Yeong Hun Choe[1], Sang Hyun Hong[1], Hyo Jin Han[1], Young Rae Lee[3], Jong Suk Kim[3], Daphne Atlas[4] and Yong Chul Lee[1,5]

Abstract

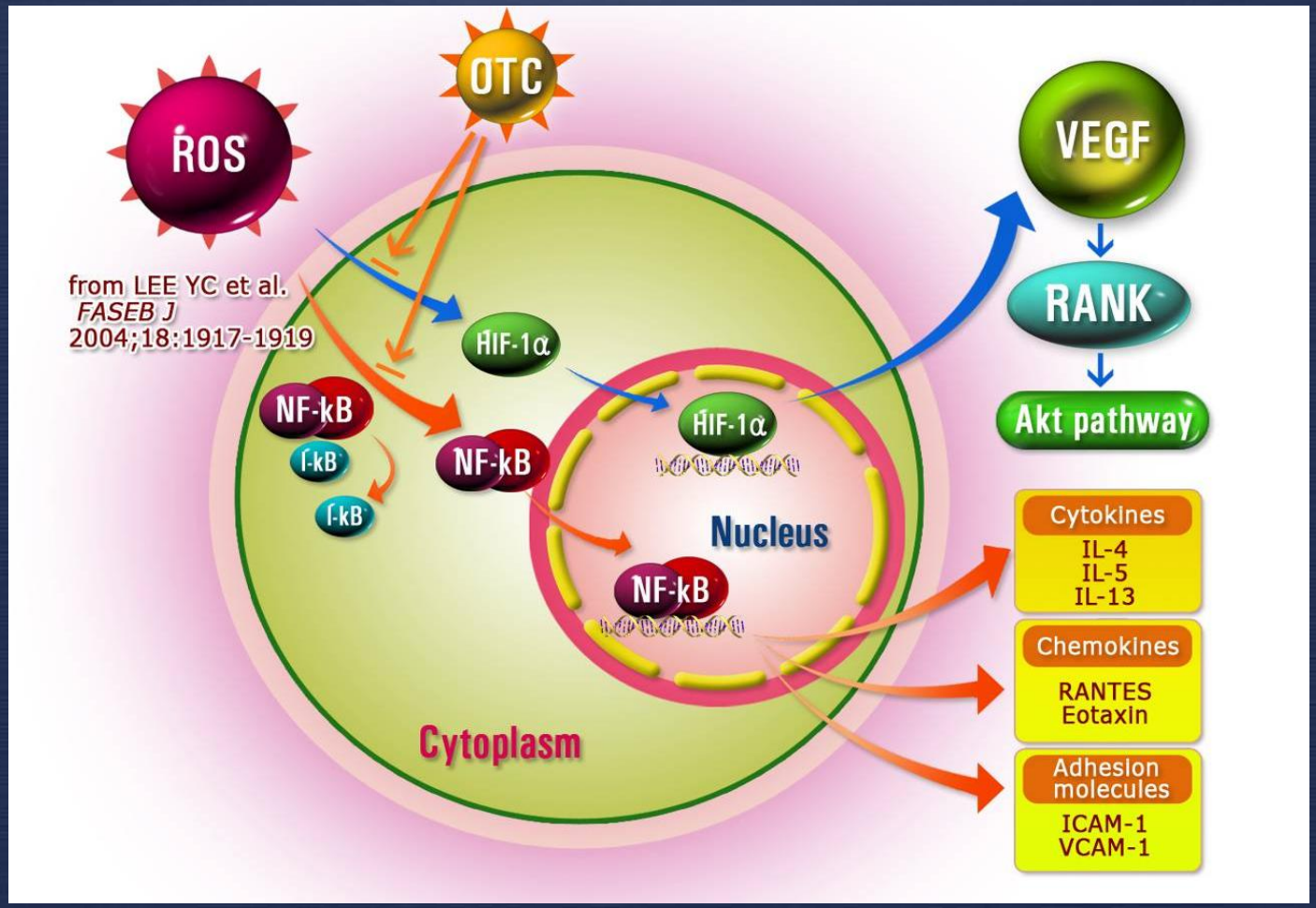
Reactive oxygen species (ROS) play an important role in the pathogenesis of airway inflammation and hyperresponsiveness. Recent studies have demonstrated that antioxidants are able to reduce airway inflammation and hyperreactivity in animal models of allergic airway disease. A newly developed antioxidant, small molecular weight thiol compound, *N*-acetylcysteine amide (AD4) has been shown to increase cellular levels of glutathione and to attenuate oxidative stress related disorders such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis. However, the effects of AD4 on allergic airway disease such as asthma are unknown. We used ovalbumin (OVA)-inhaled mice to evaluate the role of AD4 in allergic airway disease. In this study with OVA-inhaled mice, the increased ROS generation, the increased levels of Th2 cytokines and VEGF, the increased vascular permeability, the increased mucus production, and the increased airway resistance in the lungs were significantly reduced by the administration of AD4. We also found that the administration of AD4 decreased the increases of the NF-κB and hypoxia-inducible factor-1α (HIF-1α) levels in nuclear protein extracts of lung tissues after OVA inhalation. These results suggest that AD4 attenuates airway inflammation and hyperresponsiveness by regulating activation of NF-κB and HIF-1α as well as reducing ROS generation in allergic airway disease.

Keywords: hypoxia-inducible factor-1, α subunit; lung inflammation; *N*-acetylcysteinamide; NF-κB; oxidative stress; respiratory hypersensitivity

Introduction

Lee YC et al. FASEB J 2004;18(15):1917-9
Lee YC et al. Mol Pharmacol 2005;68(5):1281-90
Lee YC et al. EMM 2007;39:756-768

ROS enhances asthmatic inflammation including Th2 response

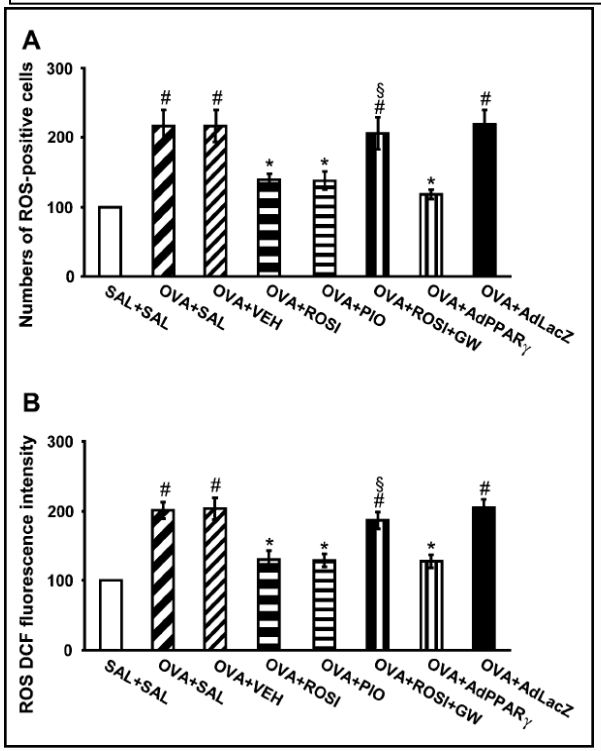


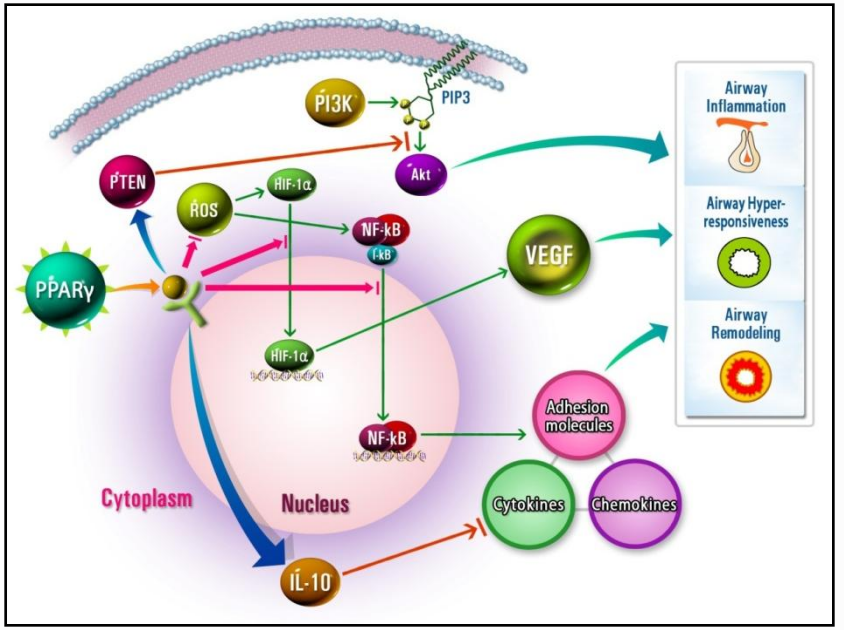
PPAR γ as a ROS modulator

CBNU

Peroxisome proliferator activated receptor-γ modulates reactive oxygen species generation and activation of nuclear factor-κB and hypoxia-inducible factor 1α in allergic airway disease of mice

Kyung Sun Lee, PhD,[a,b,c*] So Ri Kim, MD,[a,b,c*] Seoung Ju Park, MD,[a,b,c] Hee Sun Park, MD, PhD,[a,b,c] Kyung Hoon Min, MD,[a,b,c] Sun Mi Jin, BS,[b,c] Moon Kyu Lee, MD, PhD,[f] Uh Hyun Kim, MD, PhD,[d,e] and Yong Chul Lee, MD, PhD[a,b,c] *Jeonju and Seoul, Korea*





Lee YC et al. J Allergy Clin Immunol 2006;118:120-7

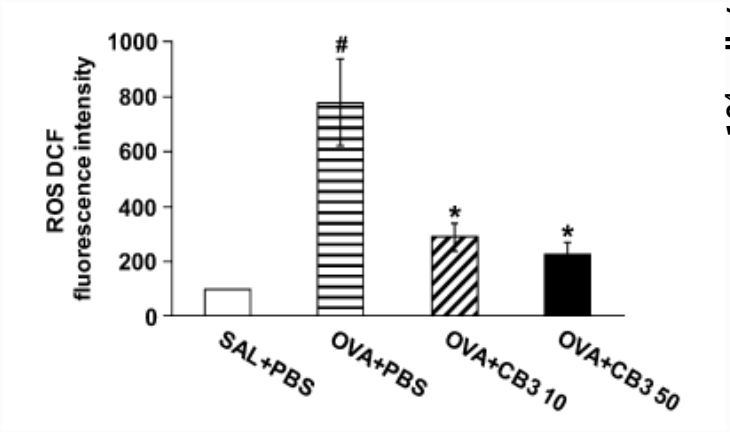
ROS and p38 signaling pathway

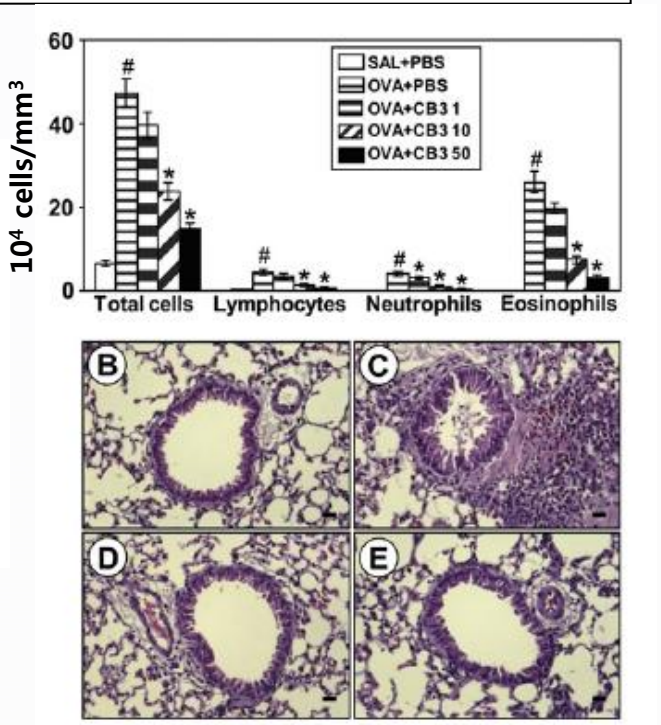
CBNU

A Novel Dithiol Amide CB3 Attenuates Allergic Airway Disease through Negative Regulation of p38 Mitogen-activated Protein Kinase

So Ri Kim[1]*, Kyung Sun Lee[1]*, Seoung Ju Park[1], Kyung Hoon Min[1], Min Hee Lee[1], Kyung Ae Lee[1], Orit Bartov[2], Daphne Atlas[2]*, and Yong Chul Lee[1]

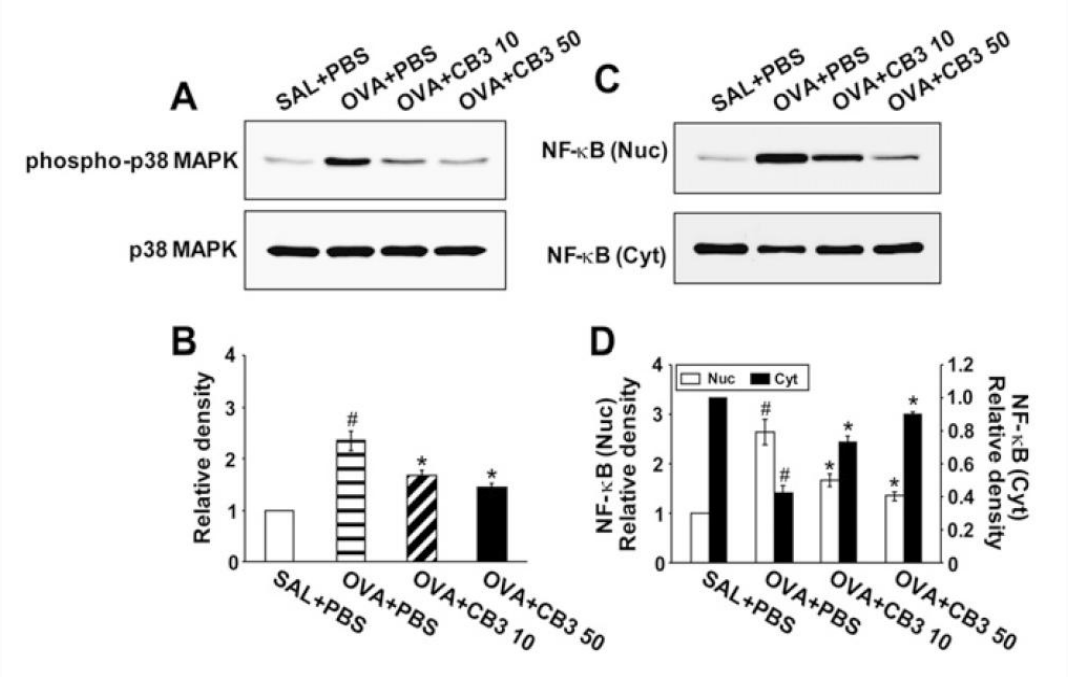
[1]Department of Internal Medicine and Research Center for Pulmonary Disorders, Chonbuk National University Medical School, Jeonju, South Korea; and [2]Department of Biological Chemistry, Silverman Institute of Life Sciences, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

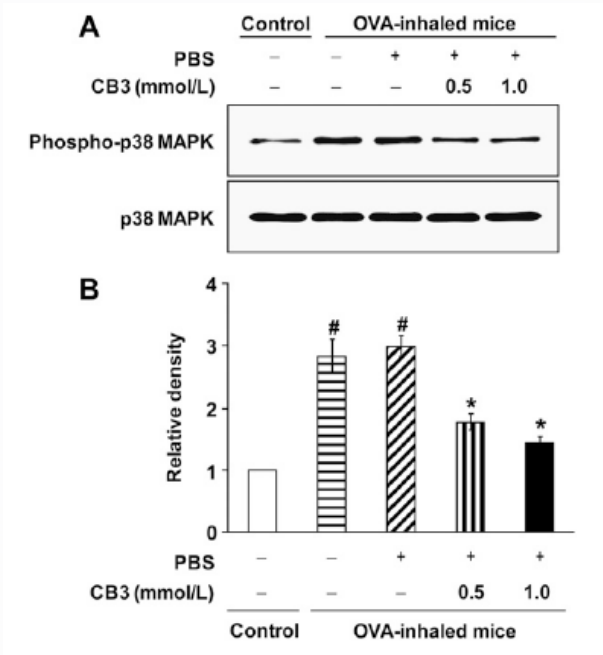




Lee YC et al. Am J Respir Crit Care Med 2011;183:1015–1024

ROS and p38 signaling pathway

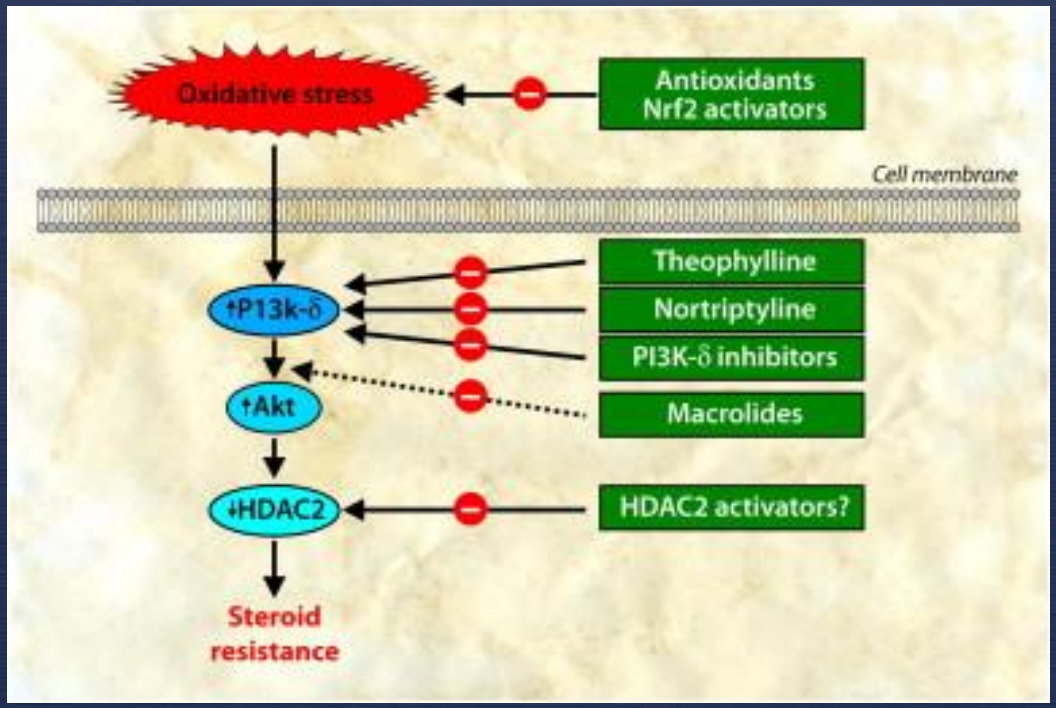




Lee YC et al. Am J Respir Crit Care Med 2011;183:1015–1024

Oxidative stress/ROS and steroid resistance

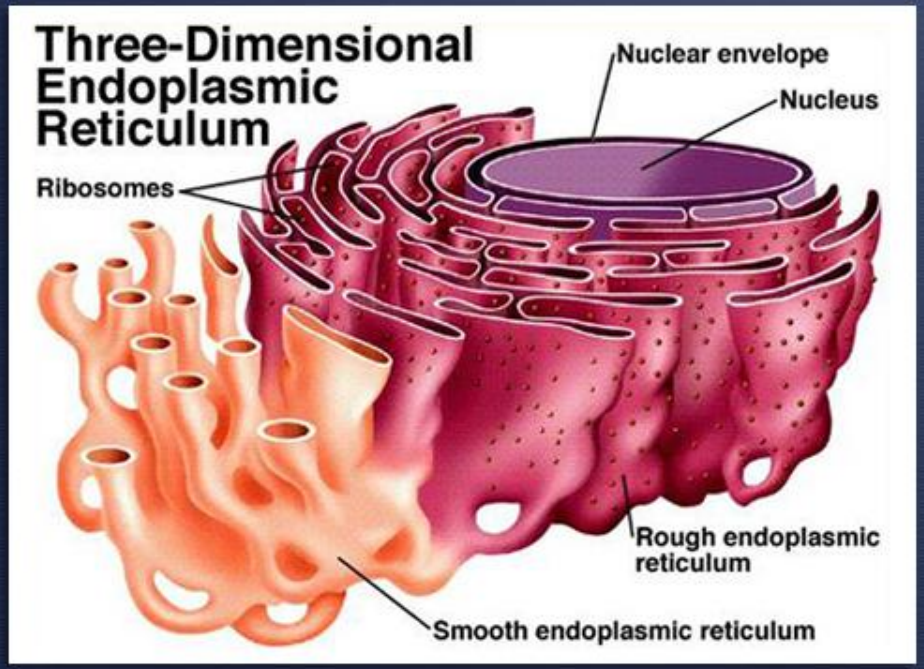
: reduction of HDAC2 expression



Barnes PJ. J Allergy Clin Immunol 2013;131:636-645

WHAT IS AN ER?

CBNU



Endoplasmic reticulum (ER)

*A specialized organelle that plays a central role in the biosynthesis, correct folding, and post-translational modifications of secretory and membrane proteins

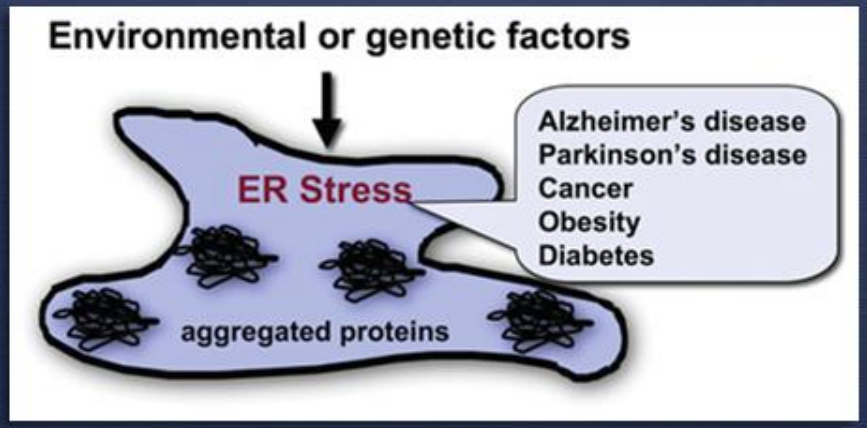
*Highly sensitive to stresses that perturb cellular energy levels, redox state, or Ca^{2+} concentration.

Gaut JR, Hendershot LM. Curr Opin Cell Biol 1993:5: 589–595

WHAT IS AN ER STRESS?

CBNU

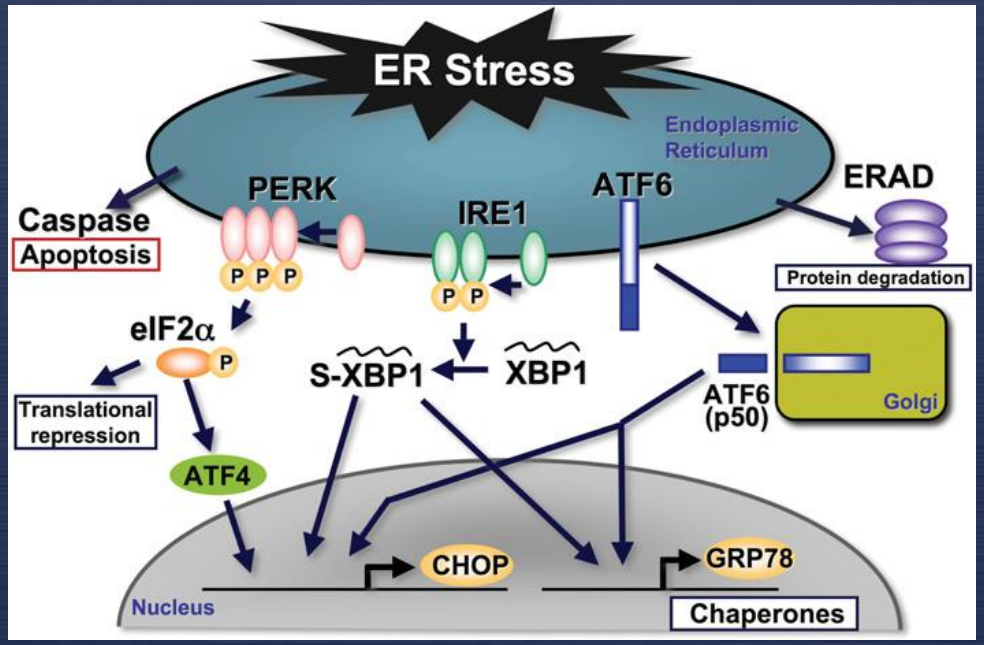
* Stress reduces protein folding capacity of the ER, resulting in the accumulation and aggregation of unfolded proteins, which is referred to as "ER stress".



* Protein aggregation is toxic to cells and, consequently, numerous pathophysiological conditions are associated with ER stress, including ischemia, neurodegenerative diseases and diabetes.

HOSOI T and OZAWA K. Clinical Science 2010:118, 19–29
Kaufman RJ. J Clin Invest 2002:110:1389–1398

Unfolded protein response?



Three different classes of ER stress sensors have been identified

- Inositol-requiring protein-1 (IRE1)
- Activating transcription factor-6 (ATF6)
- Protein kinase RNA (PKR)-like ER kinase (PERK)

HOSOI T and OZAWA K. Clinical Science 2010:118, 19–29

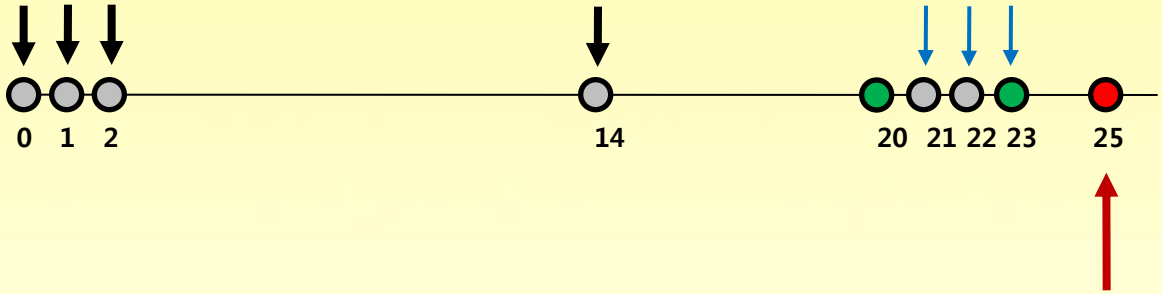
CBNU

Endoplasmic reticulum (ER) stress is implicated in the pathogenesis of severe asthma

Methods (1)

C57BL/6 mice were sensitized intranasally with 10μg of OVA and 1μg of LPS on days 0, 1, 2 and 14.

3 % OVA Challenge by nebulization on days 21, 22, and 23.

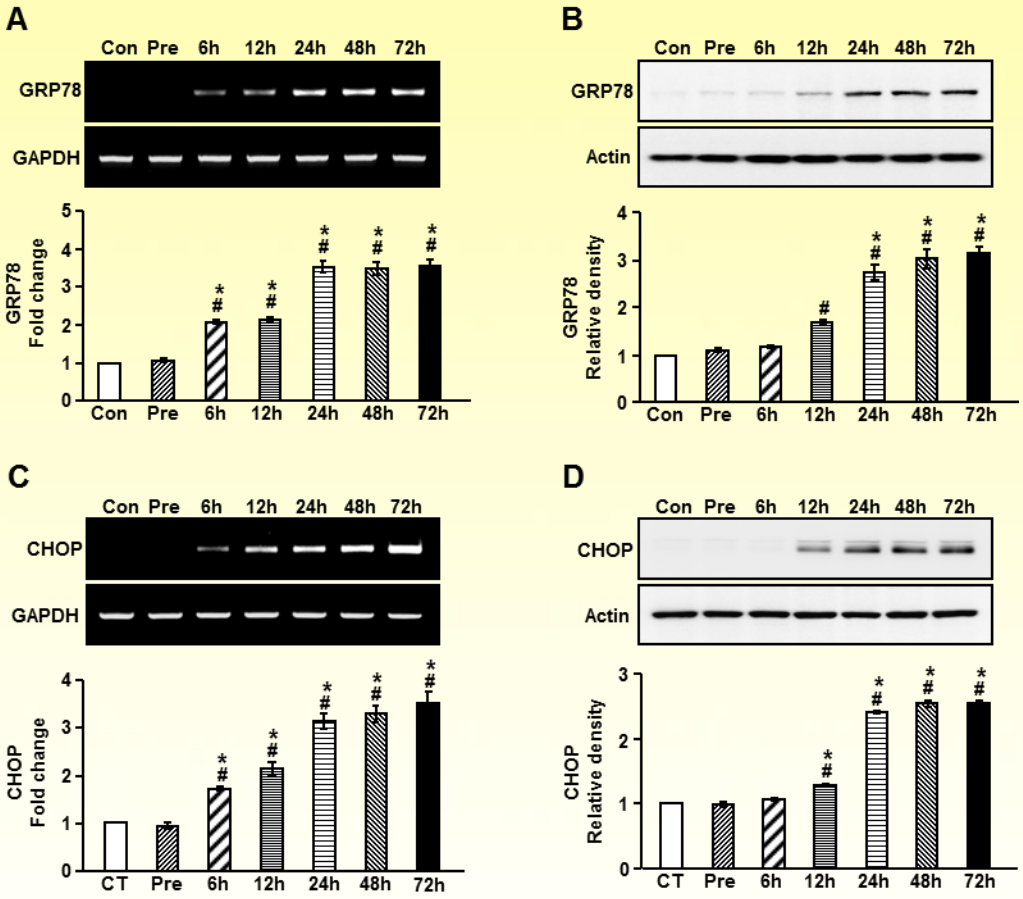


: drug (PBA 80 mg/kg)

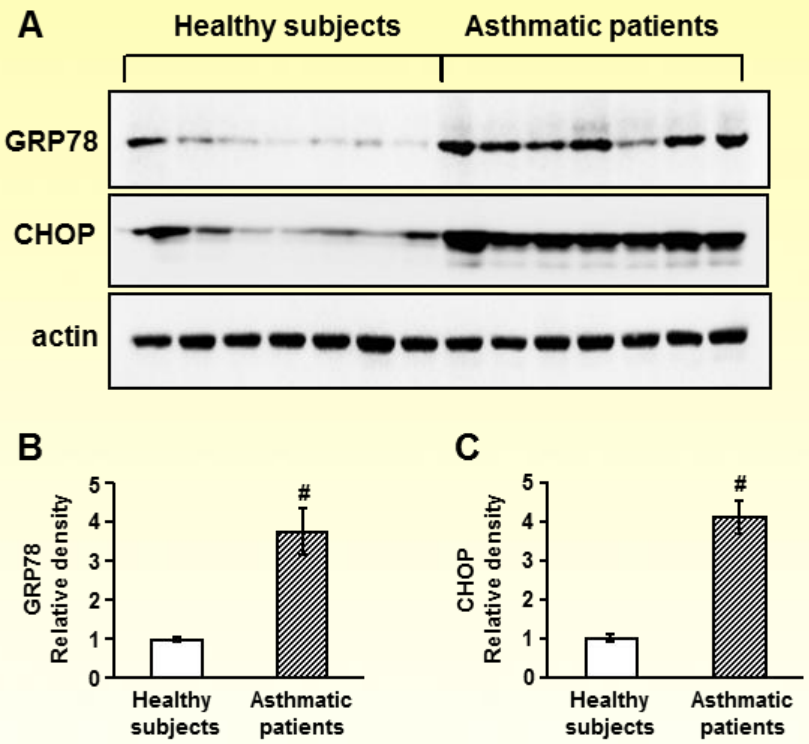
PBA was given intraperitoneally at 1 hour before the first challenge and at 6 hours after the last challenge.

: sacrificed at 48 hours after last challenge

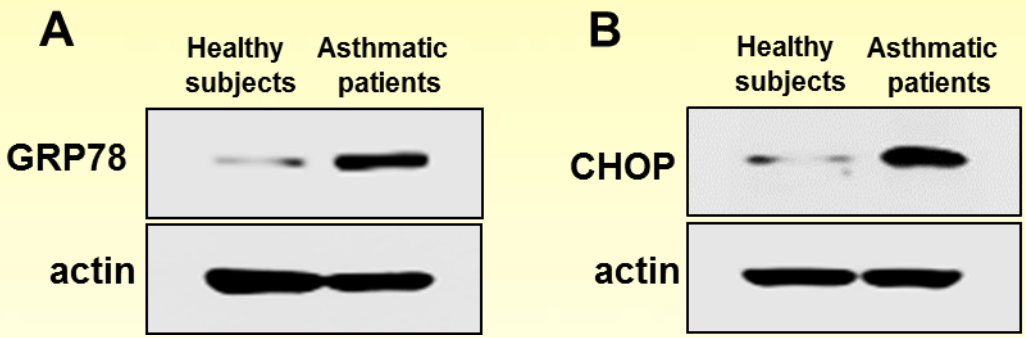
ER stress is increased in bronchial asthma



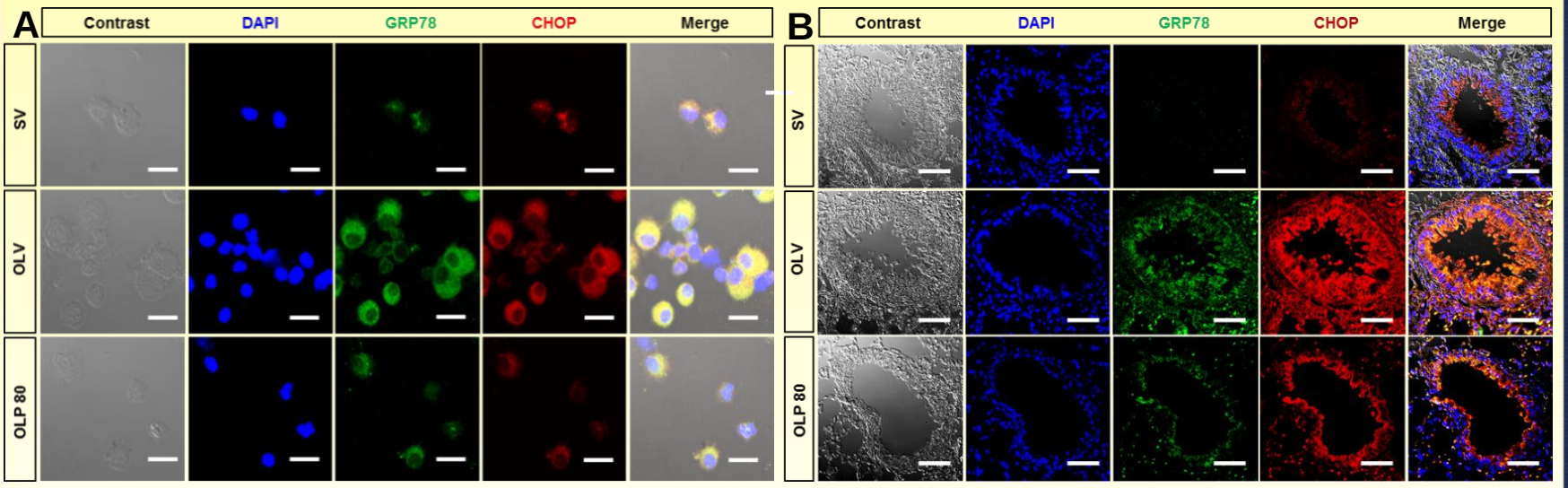
ER stress is increased in bronchial asthma



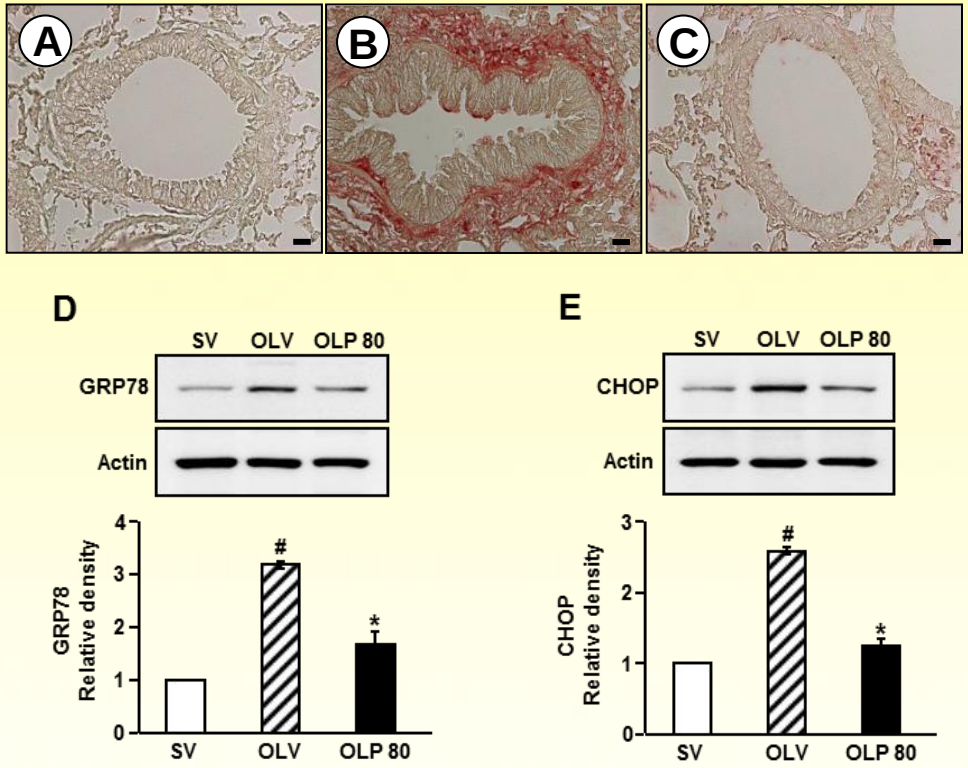
ER stress is increased in bronchial asthma



ER stress is increased in bronchial asthma



4-PBA can regulate ER stress in bronchial asthma



4-PBA attenuates the airway inflammation and hyperreponsiveness in bronchial asthma

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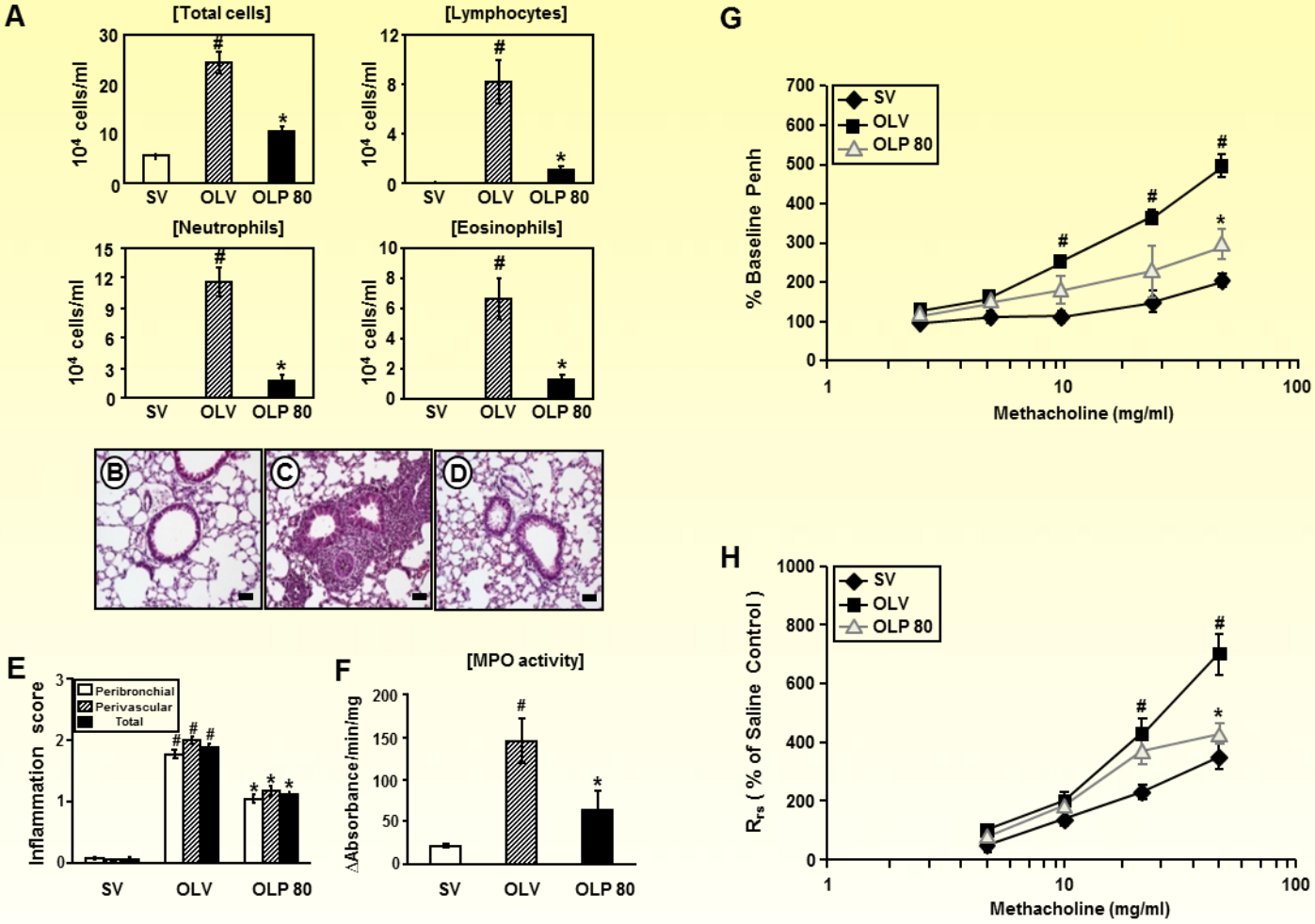
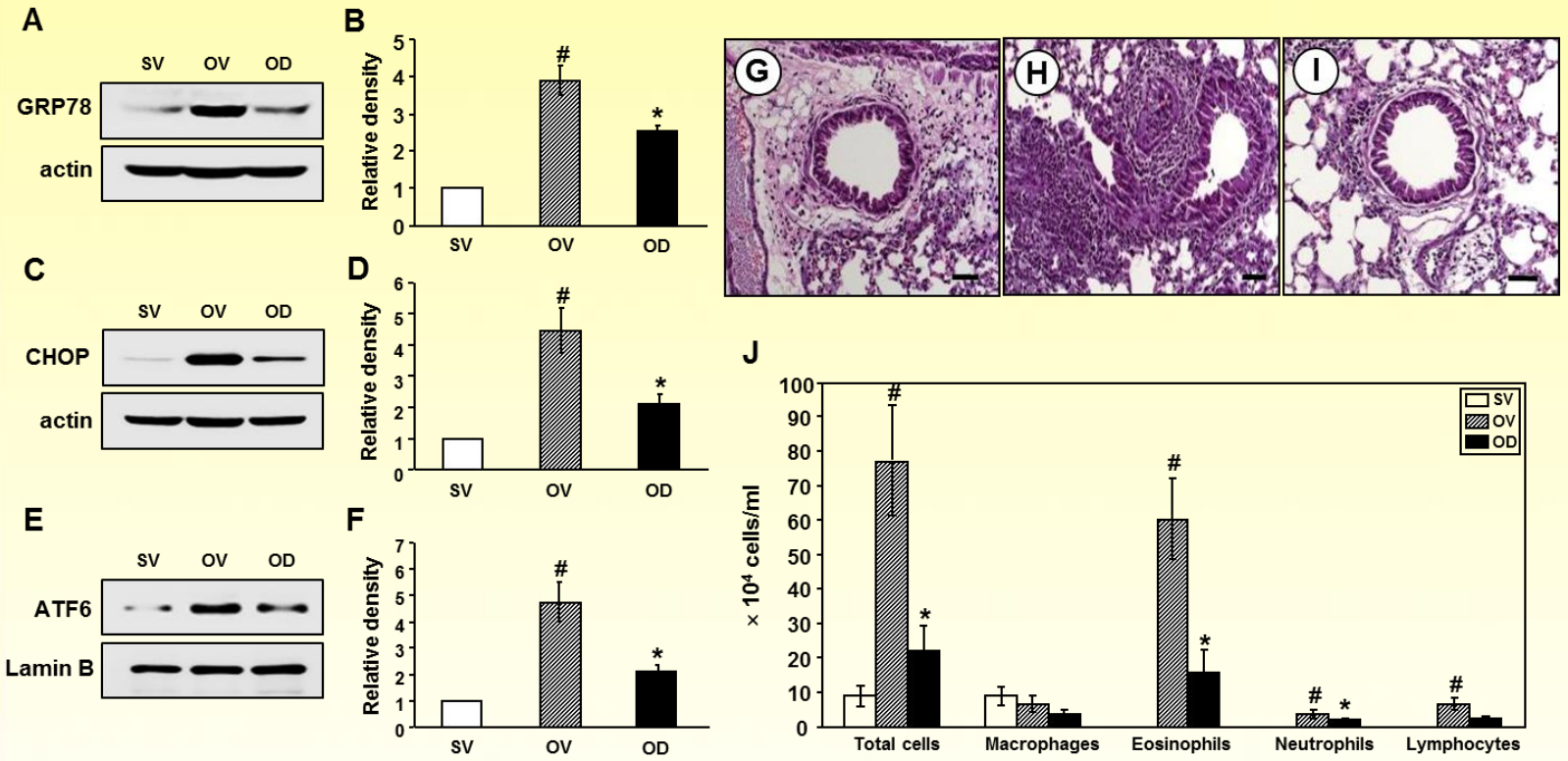
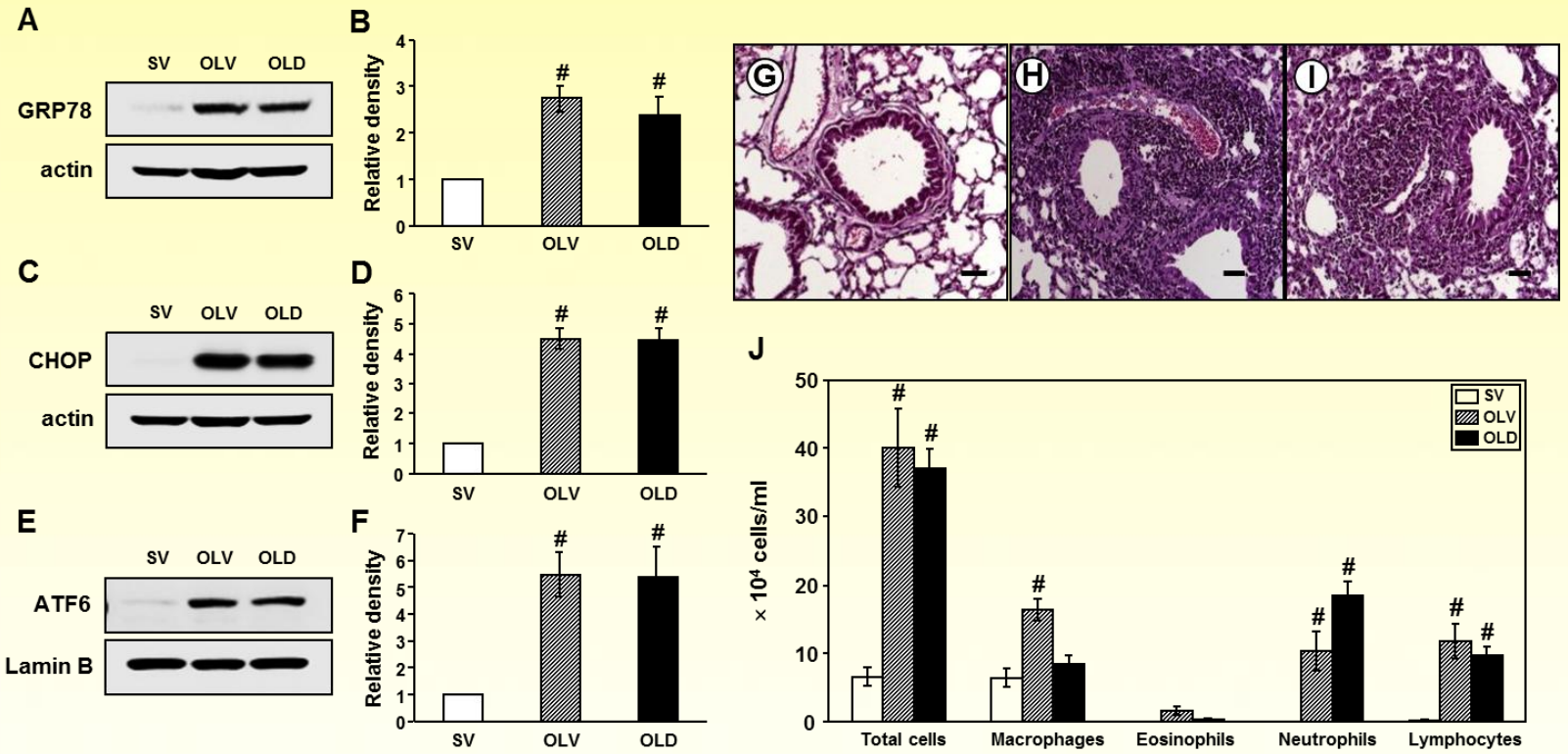


Effects of dexamethasone on bronchial asthma



Effects of dexamethasone on severe asthma



ER stress in LPS-induced lung inflammation

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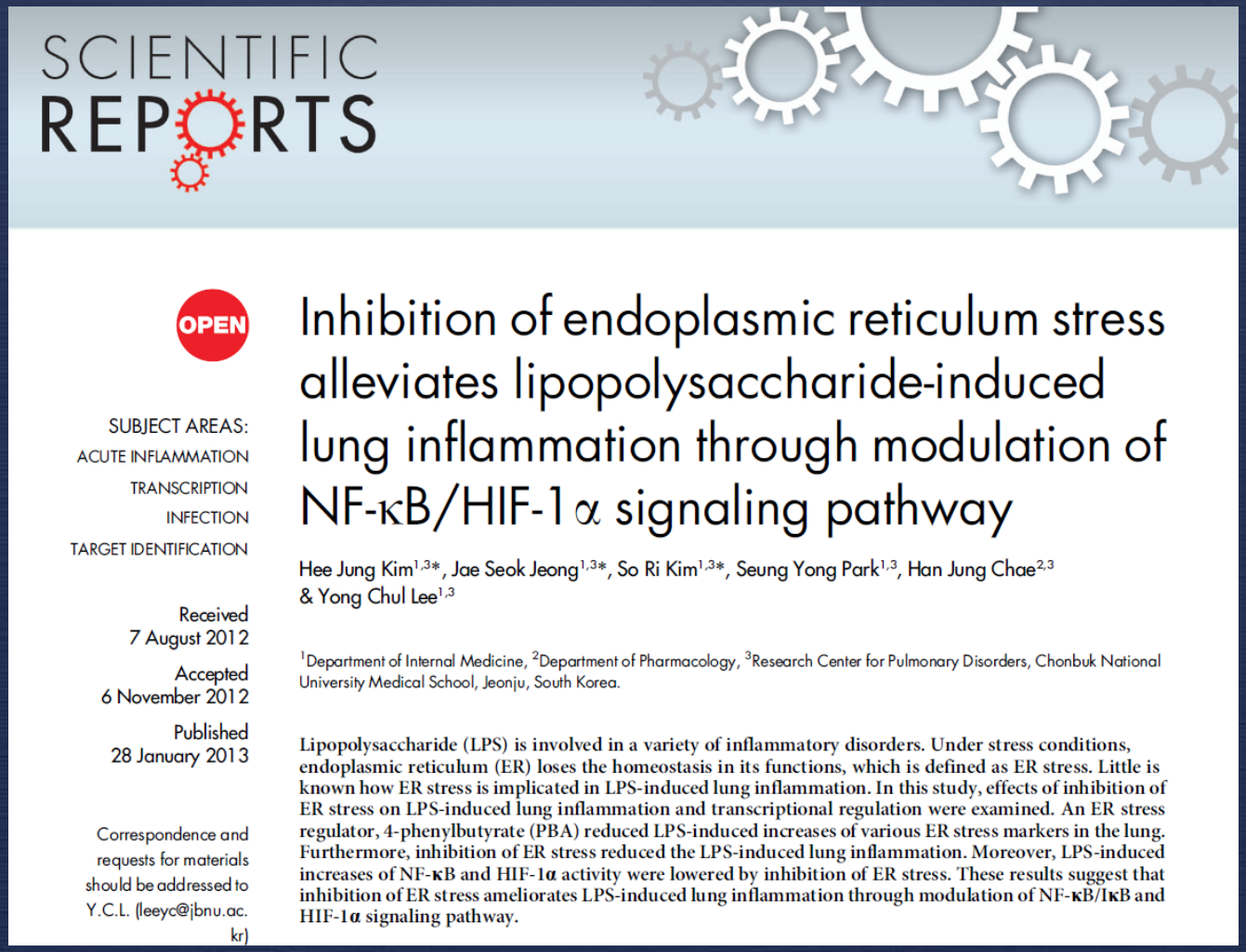

SCIENTIFIC REPORTS

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SUBJECT AREAS:
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Correspondence and requests for materials should be addressed to Y.C.L. (leeyc@jbnu.ac.kr)

Inhibition of endoplasmic reticulum stress alleviates lipopolysaccharide-induced lung inflammation through modulation of NF-κB/HIF-1α signaling pathway

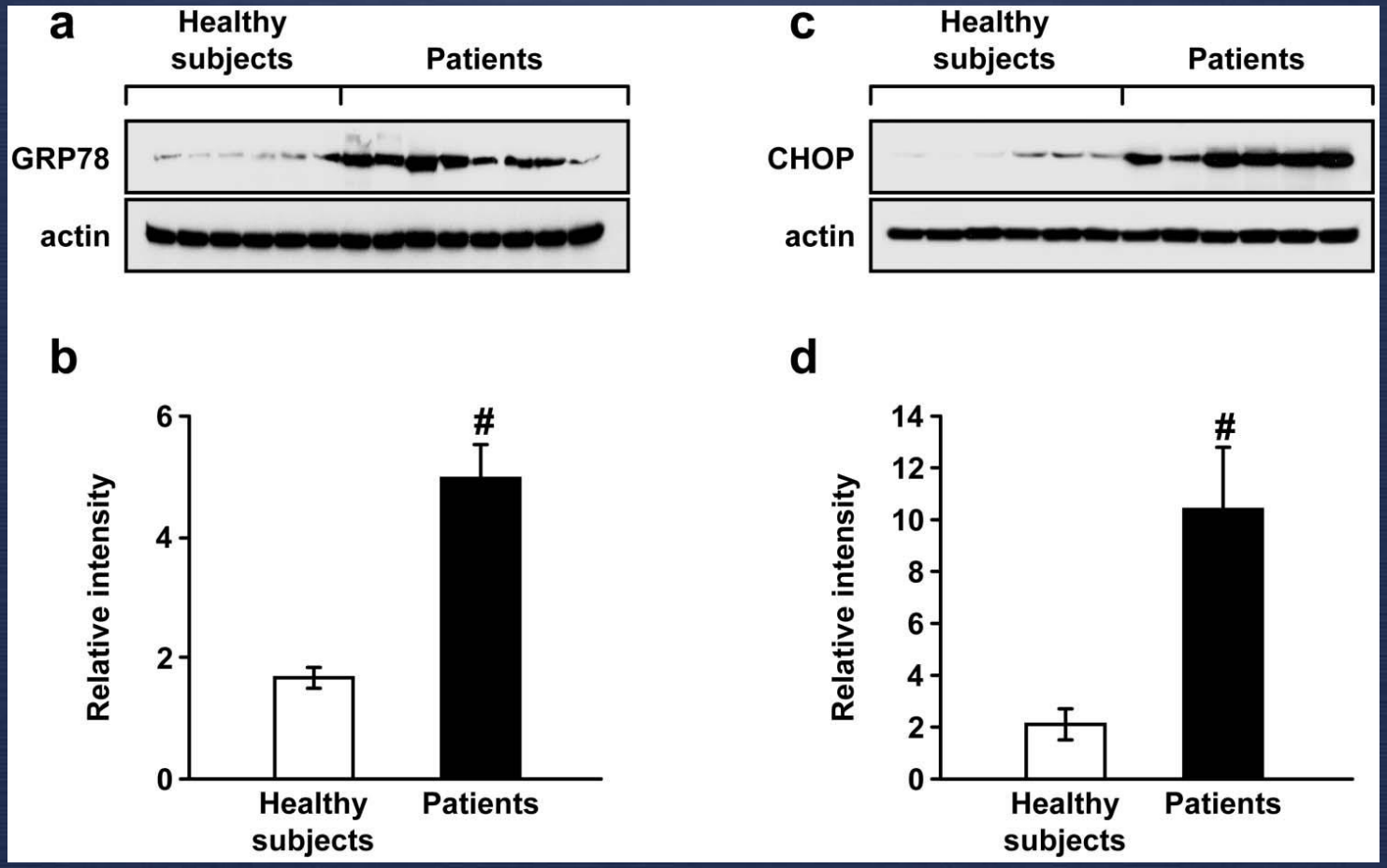
Hee Jung Kim[1,3]*, Jae Seok Jeong[1,3]*, So Ri Kim[1,3]*, Seung Yong Park[1,3], Han Jung Chae[2,3] & Yong Chul Lee[1,3]

[1]Department of Internal Medicine, [2]Department of Pharmacology, [3]Research Center for Pulmonary Disorders, Chonbuk National University Medical School, Jeonju, South Korea.

Lipopolysaccharide (LPS) is involved in a variety of inflammatory disorders. Under stress conditions, endoplasmic reticulum (ER) loses the homeostasis in its functions, which is defined as ER stress. Little is known how ER stress is implicated in LPS-induced lung inflammation. In this study, effects of inhibition of ER stress on LPS-induced lung inflammation and transcriptional regulation were examined. An ER stress regulator, 4-phenylbutyrate (PBA) reduced LPS-induced increases of various ER stress markers in the lung. Furthermore, inhibition of ER stress reduced the LPS-induced lung inflammation. Moreover, LPS-induced increases of NF-κB and HIF-1α activity were lowered by inhibition of ER stress. These results suggest that inhibition of ER stress ameliorates LPS-induced lung inflammation through modulation of NF-κB/IκB and HIF-1α signaling pathway.

Lee YC et al. Sci Rep 2013;3:1142

ER stress in LPS-induced lung inflammation



Lee YC et al. Sci Rep 2013;3:1142

ER stress in LPS-induced lung inflammation

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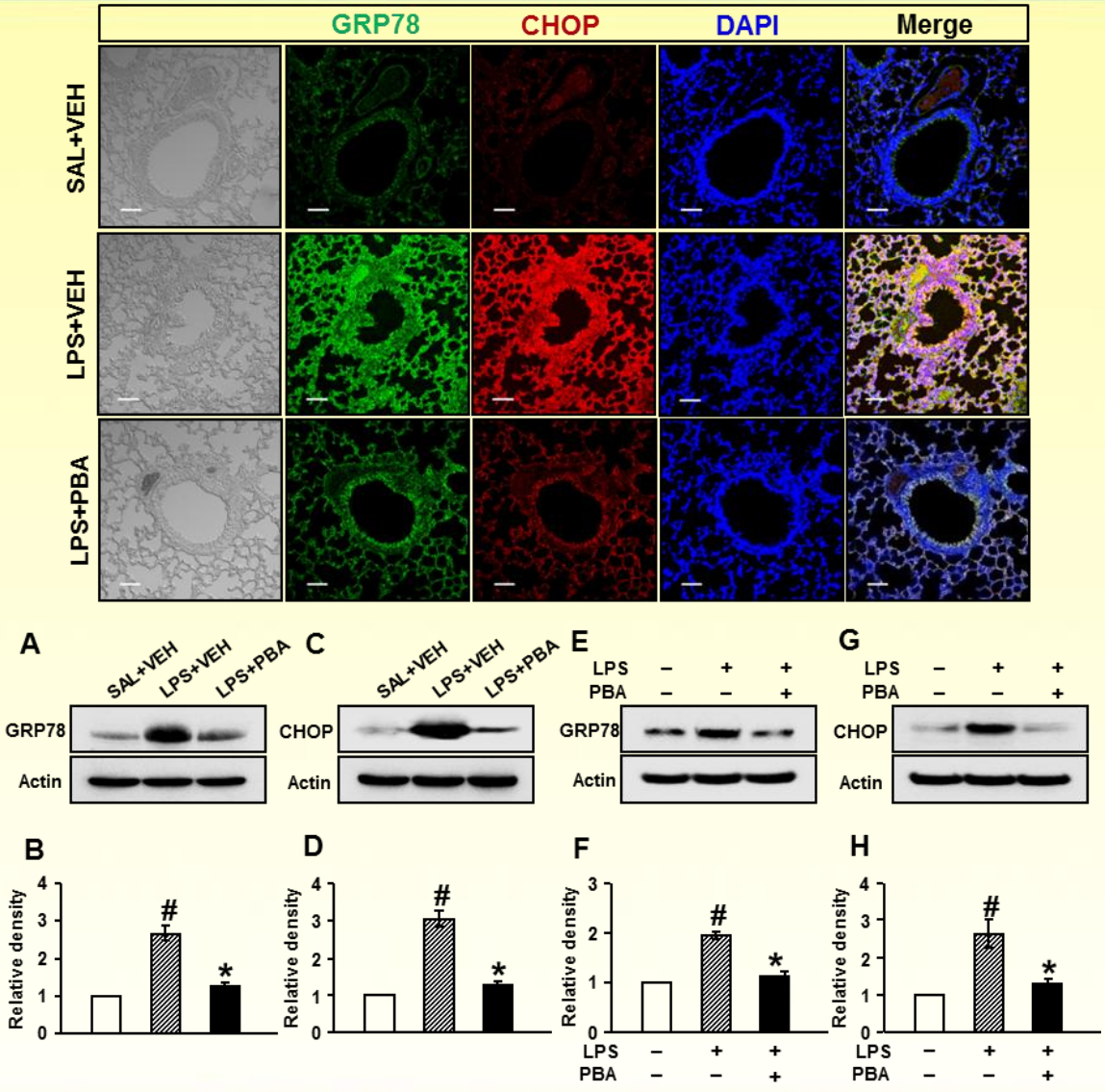

Lee YC et al. Sci Rep 2013;3:1142

ER stress in LPS-induced lung inflammation

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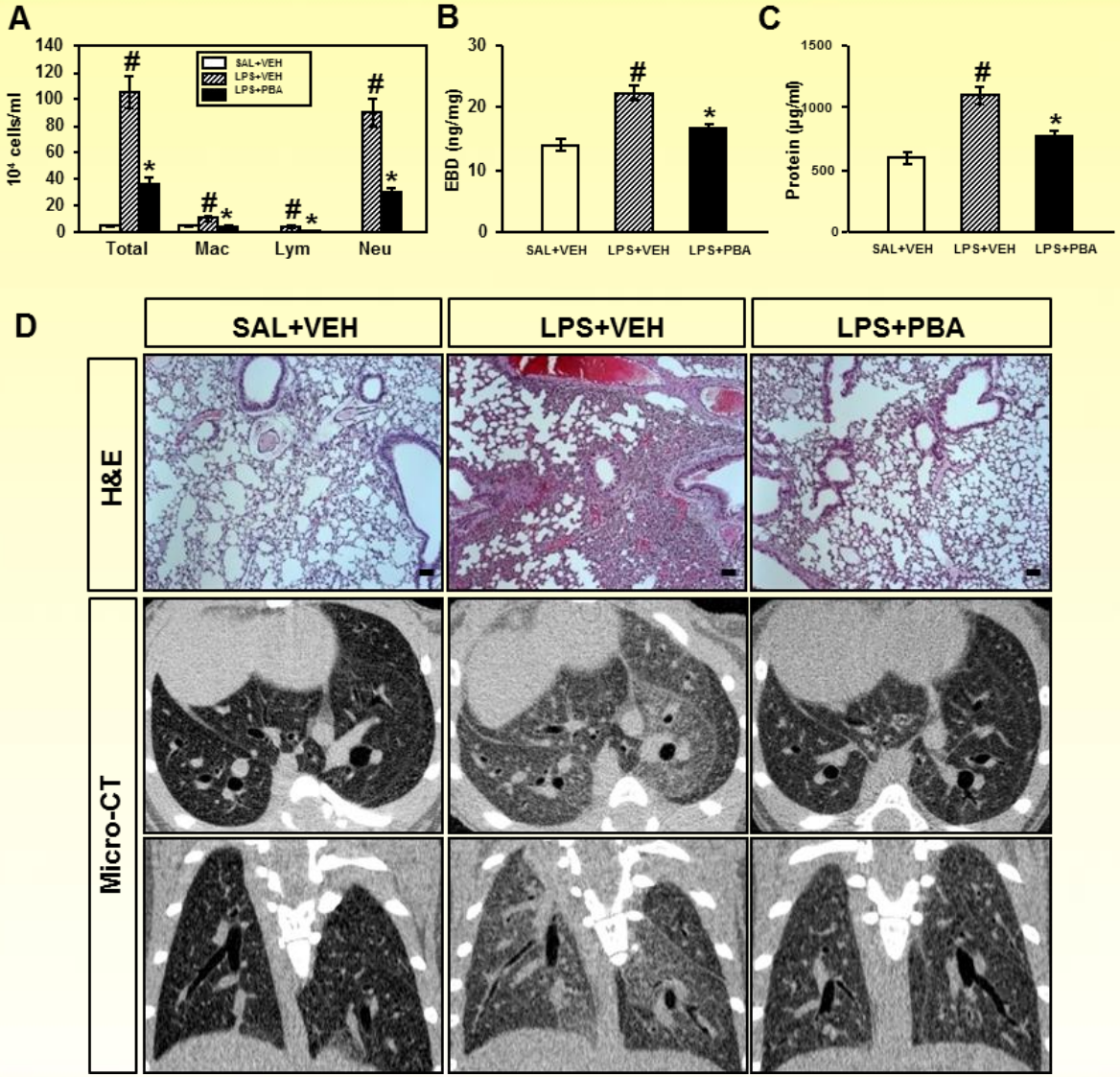

Lee YC et al. Sci Rep 2013;3:1142

INDUCTION OF INFLAMMATION THROUGH PATTERN RECOGNITION

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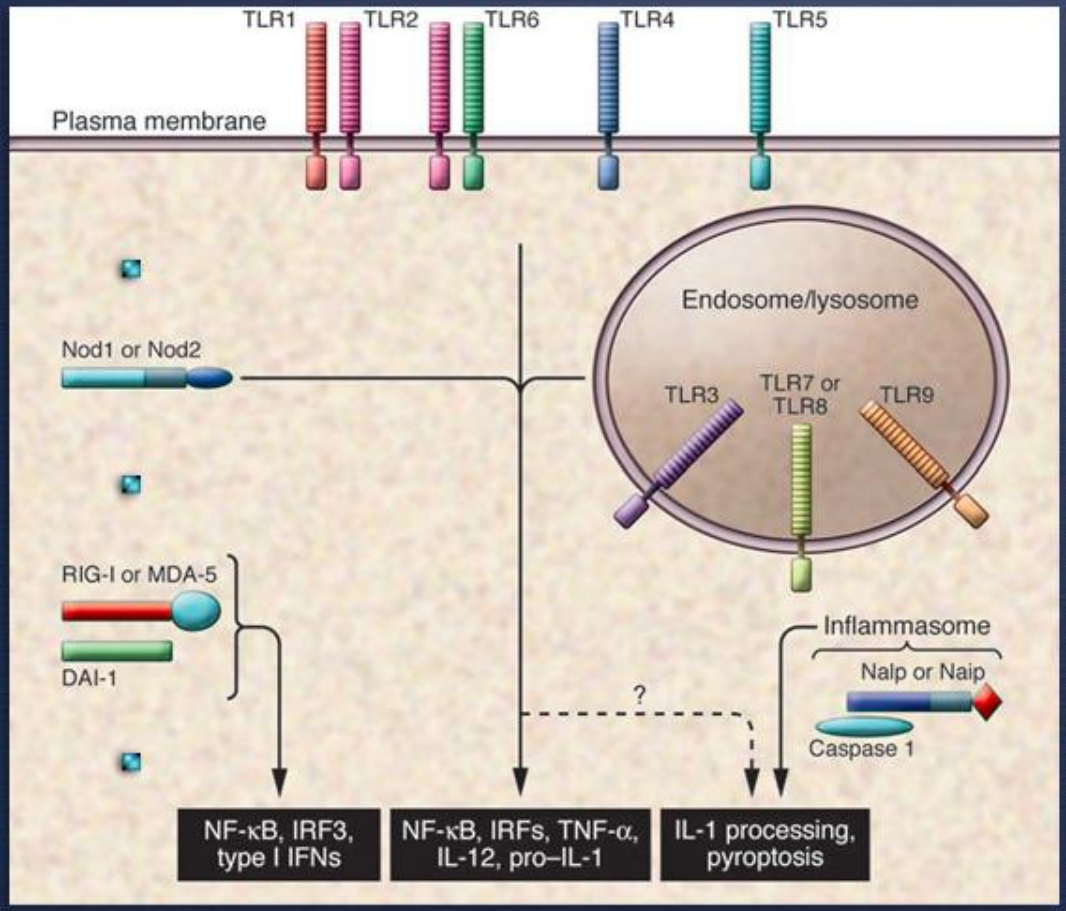

The PRRs of the innate immune system :

1. Toll-like receptors (TLRs)
2. C-type lectin receptors (CLRs)
3. Retinoic acid–inducible gene (RIG)-I-like receptors (RLRs)
4. The nucleotide-binding domain, leucine-rich repeat–containing (NBD-RR) proteins (NLRs).

Gregory M. Barton. J. Clin. Invest. 118:413–420 (2008)

The NLRs

- nod-like receptors (NLRs), - critical in the *initiation of inflammatory responses.*
- located in the cytoplasm.
- implicated in the sensing of invading microbes, tissue damage, or stress.

The NLRs

- comprises 22 family members in humans and 30 in mice.

- The NLR family can be further divided into 3 subfamilies based on modular structure:
  1. NODs (NOD1-5, CIITA)
  2. NLRPs (NLRP1-14)
  3. IPAF subfamily (IPAF/NLRC4,NAIP).

THE NLRs

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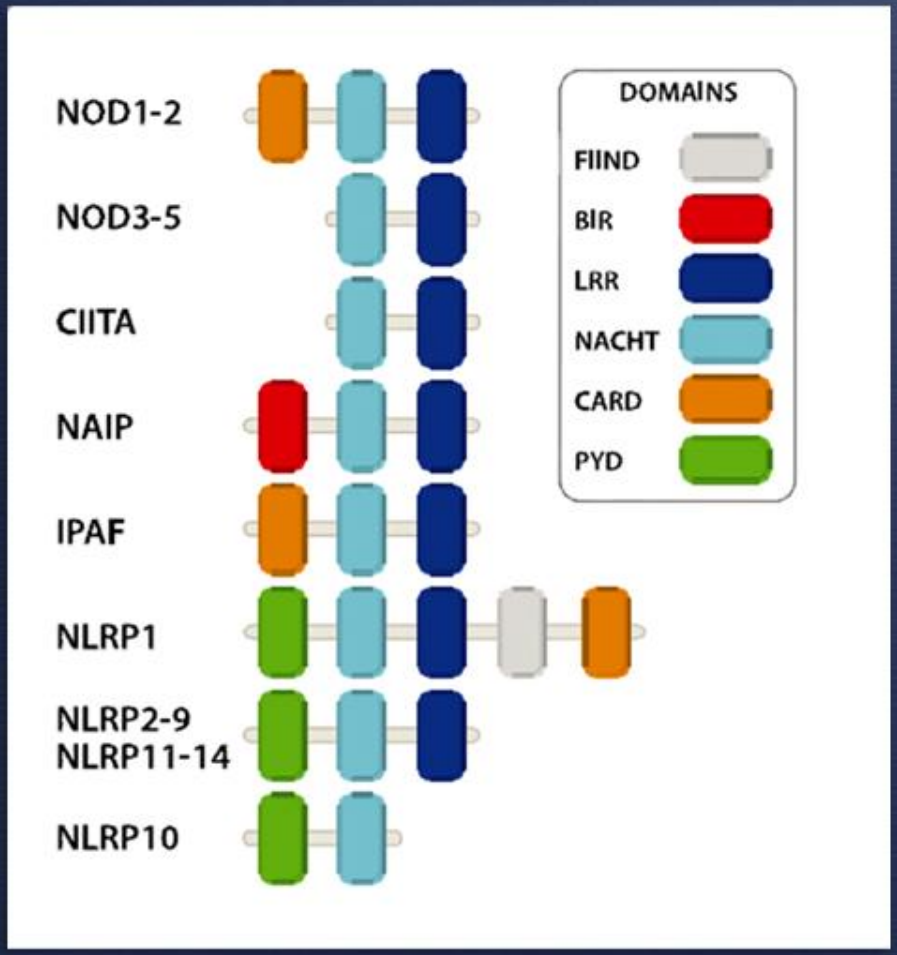


Each NLR contains three characteristic domains:

- An N-terminal effector domain responsible for signal transduction and activation of the inflammatory response. To date, four different N- terminal domains have been identified: acidic transactivation domain, caspase activation and recruitment domain (CARD), pyrin domain (PYD), and baculoviral inhibitor of apoptosis protein (IAP) repeat (BIR) domain.
- A central nucleotide binding and oligomerization (NACHT; NBD; NOD) domain that shares similarities with the NB-ARC motif of the apoptotic mediator APAF1.
- A C-terminal leucine-rich repeat (LRR) domain responsible for ligand sensing and autoinhibition

K. Schroder & J. Tschopp; Cell 140, 821 (2010)

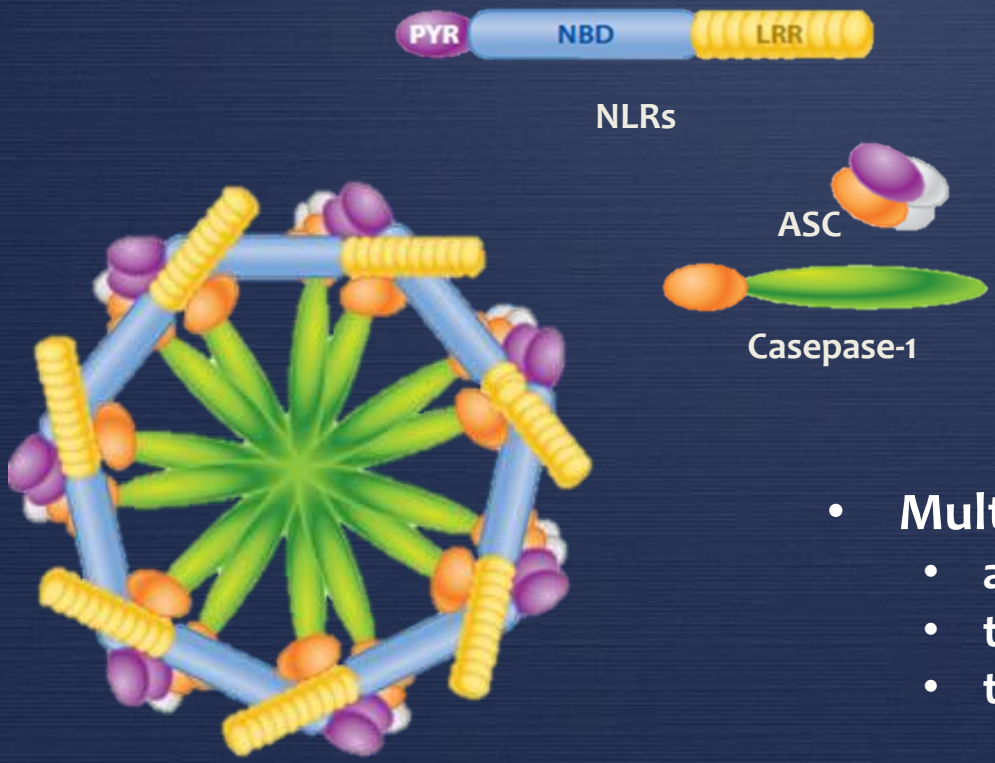
NLRP3

- NLRP3, also known as cryopirin or NALP3, - the one of the **best-characterized NLRP family members.**
- under resting conditions, expressed in ***myeloid immune cells***, particularly splenic conventional DCs, monocytes, macrophages, and neutrophils.

NLRP3 inflammasome

- forms a *large signal-induced multiprotein complex*, the inflammasome, resulting in pro-inflammatory caspase activation.
- a broad range of exogenous and endogenous stimuli activate the NLRP3 inflammasome.

Inflammasome



- Multiprotein complex
  - a sensor protein (NLRs)
  - the adapter protein ASC
  - the inflammatory protease caspase-1

Davis BK, et al. Annu. Rev. Immunol. 2011. 29:707–35

NLRP3

- Upon activation by diverse stimuli, NLRP3 proteins polymerize and bind to the *ASC adaptor*, - promotes the recruitment of *pro-caspase-1.*

- The NLRP3 inflammasome - promote caspase-1-mediated activation of *IL-1β and IL-18.*

NLRP3 inflammasome in allergic airway inflammation

CBNU

ORIGINAL ARTICLE EXPERIMENTAL ALLERGY AND IMMUNOLOGY

NLRP3 inflammasome is required in murine asthma in the absence of aluminum adjuvant

A.-G. Besnard[1], N. Guillou[1], J. Tschopp[2], F. Erard[1], I. Couillin[1], Y. Iwakura[3], V. Quesniaux[1], B. Ryffel[1] & D. Togbe[1,4]

[1]Université d'Orléans and CNRS, Molecular Immunology and Embryology (IEM), Orléans, France; [2]Department of Biochemistry, University of Lausanne, Chemin des Boveresses, Epalinges, Switzerland; [3]Center for Experimental Medicine, Institute of Medical Science, University of Tokyo, Shirokanedai, Minato-ku, Tokyo, Japan; [4]Artimmune SAS, Centre d'Innovation, 16 rue Léonard de Vinci, Orléans, France

To cite this article: Besnard A-G, Guillou N, Tschopp J, Erard F, Couillin I, Iwakura Y, Quesniaux V, Ryffel B, Togbe D. NLRP3 inflammasome is required in murine asthma in the absence of aluminum adjuvant. *Allergy* 2011; **66**: 1047–1057.

Keywords
allergic inflammation; asthma; IL-1R1; NLRP3 inflammasome.

Correspondence
Bernhard Ryffel, MD, UMR6218, Molecular Immunology and Embryology, 3B rue de la Férollerie, 45071 Orléans Cedex 2, France.
Tel.: + 33 238 25 5439
Fax: + 33 238 25 7979
E-mail: bryffel@cnrs-orleans.fr

Accepted for publication 2 March 2011

DOI:10.1111/j.1398-9995.2011.02586.x

Edited by: Angela Haczku

Abstract

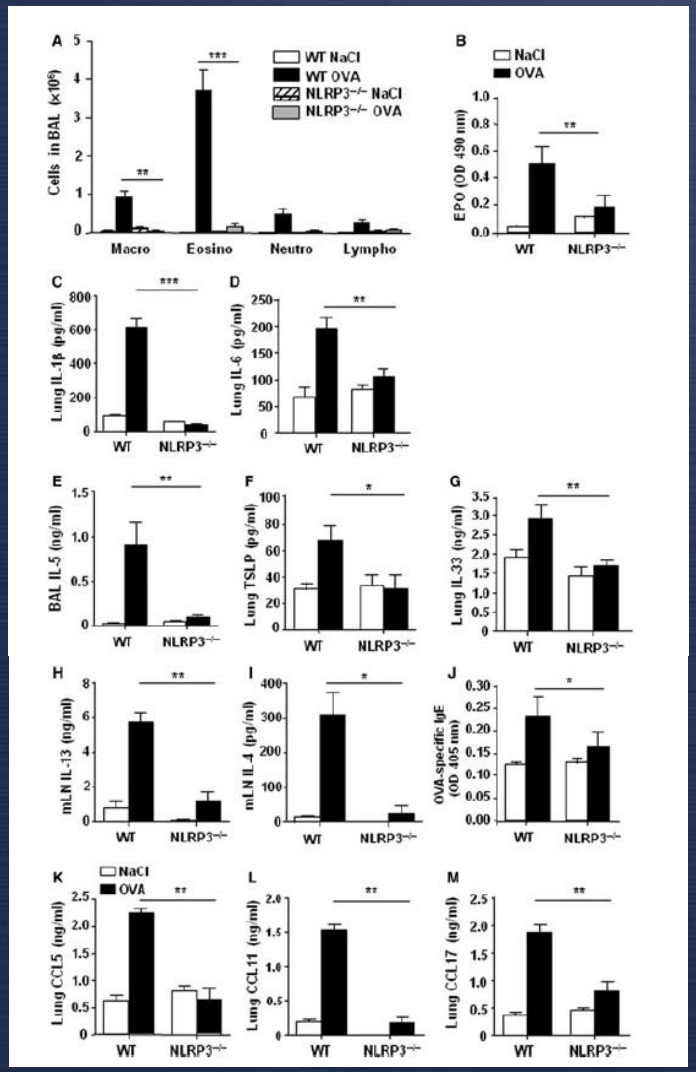
Background: Inflammasome activation with the production of IL-1β received substantial attention recently in inflammatory diseases. However, the role of inflammasome in the pathogenesis of asthma is not clear. Using an adjuvant-free model of allergic lung inflammation induced by ovalbumin (OVA), we investigated the role of NLRP3 inflammasome and related it to IL-1R1 signaling pathway.
Methods: Allergic lung inflammation induced by OVA was evaluated *in vivo* in mice deficient in NLRP3 inflammasome, IL-1R1, IL-1β or IL-1α. Eosinophil recruitment, Th2 cytokine, and chemokine levels were determined in bronchoalveolar lavage fluid, lung homogenates, and mediastinal lymph node cells *ex vivo*.
Results: Allergic airway inflammation depends on NLRP3 inflammasome activation. Dendritic cell recruitment into lymph nodes, Th2 lymphocyte activation in the lung and secretion of Th2 cytokines and chemokines are reduced in the absence of NLRP3. Absence of NLRP3 and IL-1β is associated with reduced expression of other proinflammatory cytokines such as IL-5, IL-13, IL-33, and thymic stromal lymphopoietin. Furthermore, the critical role of IL-1R1 signaling in allergic inflammation is confirmed in IL-1R1-, IL-1β-, and IL-1α-deficient mice.
Conclusion: NLRP3 inflammasome activation leading to IL-1 production is critical for the induction of a Th2 inflammatory allergic response.

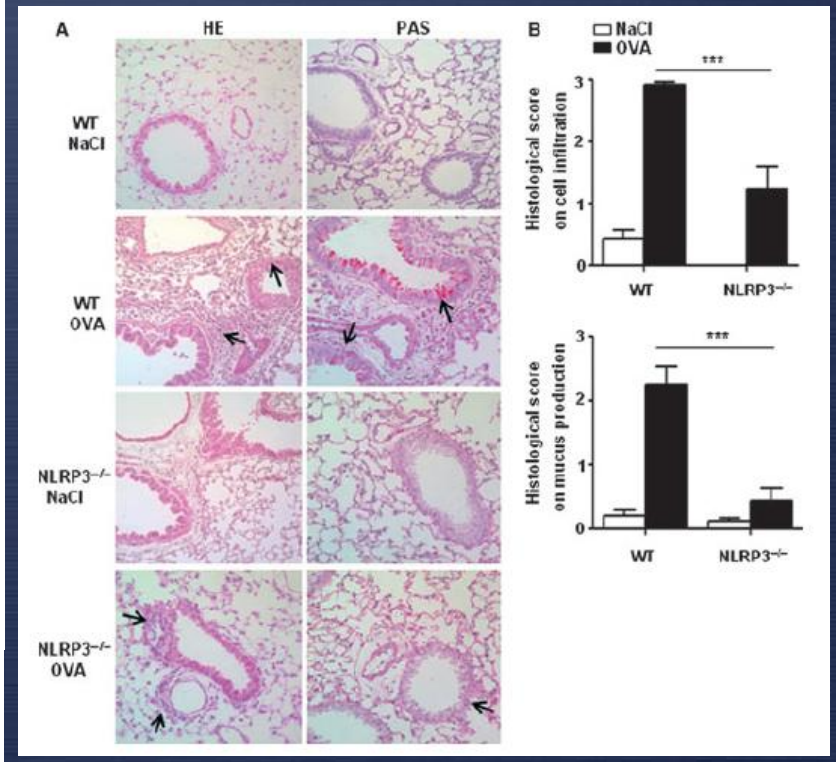
allergic lung inflammation was reduced in the absence of NLRP3

전북대학교병원 CHONBUK NATIONAL UNIVERSITY HOSPITAL 전북대학교

Besnard AG et al, Allergy 2011;66:1047-57

NLRP3 inflammasome in allergic airway inflammation

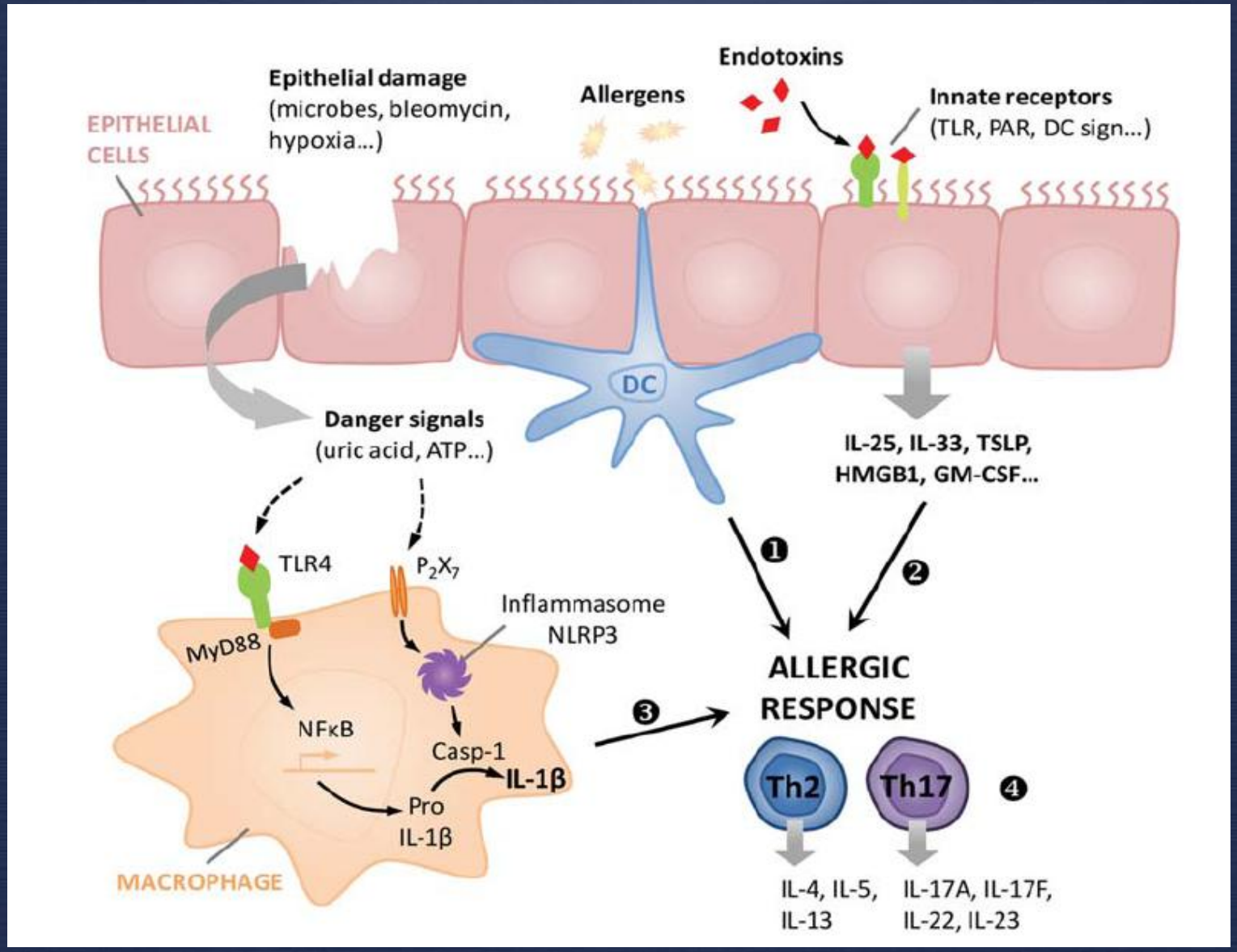




Eosinophil recruitment in the airways, mucus hypersecretion, and IL-1β were dramatically reduced in NLRP3-deficient mice, as well as Th2 cytokine and chemokine production.

전북대학교병원 CHONBUK NATIONAL UNIVERSITY HOSPITAL 전북대학교

Besnard AG et al, Allergy 2011;66:1047-57



Besnard AG et al, J Mol Cell Biol 2012;4:3-10

NLRP3 inflammasome

NLRP3 inflammasome activation - required **together with a TLR4 signal** to produce *mature IL-1β and allergic lung inflammation.*

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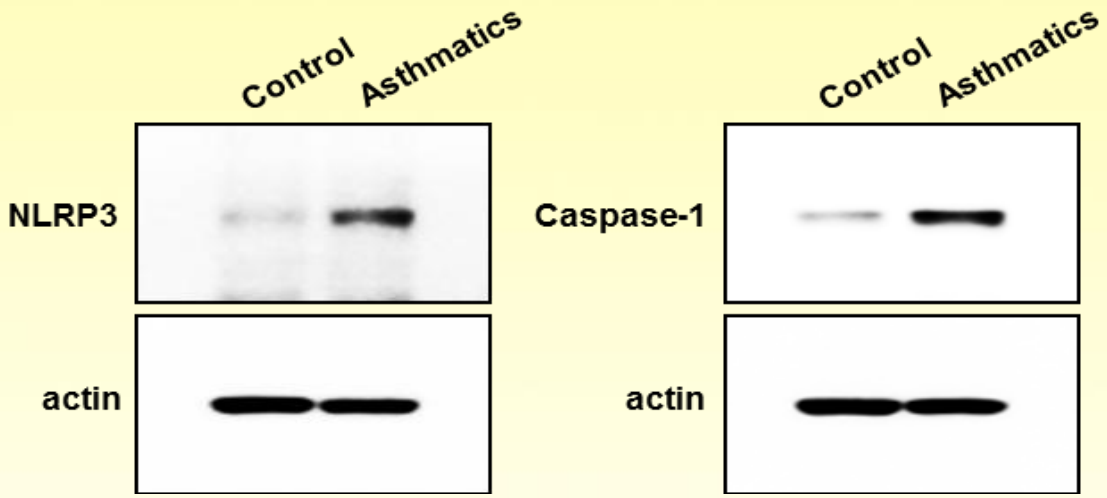
NLRP3 inflammasome activation in bronchial asthma : Human BAL sample



Unpublished data

ROS ACTIVATES NLRP3 INFLAMMASOME

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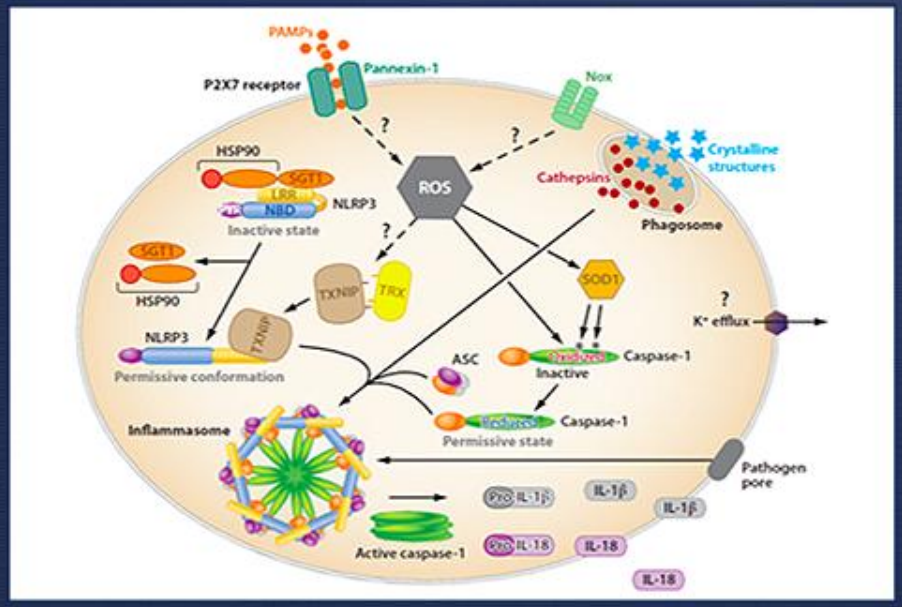
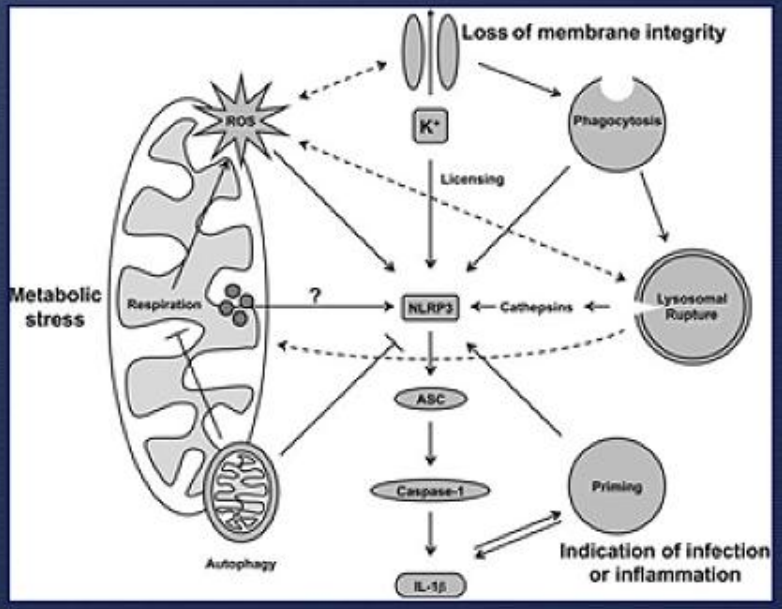
- There are three models to explain inflammasome activation:
  - Reactive oxygen species (ROS), esp. mitochondrial ROS?
  - Lysosomal destabilization
  - Pore formation and K^+ efflux and possibly PAMP influx





Davis BK, et al. Annu. Rev. Immunol. 2011. 29:707–35
Gross O, et al. Immunological Reviews 2011;243: 136–151

Mitochondrial ROS in allergic airway inflammation

Mitochondrial Dysfunction and Oxidative Stress in Asthma: Implications for Mitochondria-Targeted Antioxidant Therapeutics

P. Hemachandra Reddy[1,2]

[1]Neurogenetics Laboratory, Division of Neuroscience, Oregon National Primate Research Center, Oregon Health and Science University, 505 NW 185th Avenue, Beaverton, OR 97006, USA

[2]Department of Physiology and Pharmacology, Oregon Health and Science University, Portland, OR 97201, USA; reddyh@ohsu.edu; Tel.: 503-418-2625; Fax: 503-418-2701

Abstract

Asthma is a complex, inflammatory disorder characterized by airflow obstruction of variable degrees, bronchial hyper-responsiveness, and airway inflammation. Asthma is caused by environmental factors and a combination of genetic and environmental stimuli. Genetic studies have revealed that multiple loci are involved in the etiology of asthma. Recent cellular, molecular, and animal-model studies have revealed several cellular events that are involved in the progression of asthma, including: increased Th2 cytokines leading to the recruitment of inflammatory cells to the airway, and an increase in the production of reactive oxygen species and mitochondrial dysfunction in the activated inflammatory cells, leading to tissue injury in the bronchial epithelium. Further, aging and animal model studies have revealed that mitochondrial dysfunction and oxidative stress are involved and play a large role in asthma. Recent studies using experimental allergic asthmatic mouse models and peripheral cells and tissues from asthmatic humans have revealed antioxidants as promising treatments for people with asthma. This article summarizes the latest research findings on the involvement of inflammatory changes, and mitochondrial dysfunction/oxidative stress in the development and progression of asthma. This article also addresses the relationship between aging and age-related immunity in triggering asthma, the antioxidant therapeutic strategies in treating people with asthma.

Keywords

asthma; mitochondrial dysfunction; mitochondria-targeted antioxidants; reactive oxygen species

Mitochondrial dysfunction /oxidative stress in the development and progression of asthma

Pharmaceuticals (Basel). 2011 February 25; 4(3): 429–456.

MITOCHONDRIAL ROS DRIVE PROINFLAMMATORY CYTOKINE PRODUCTION

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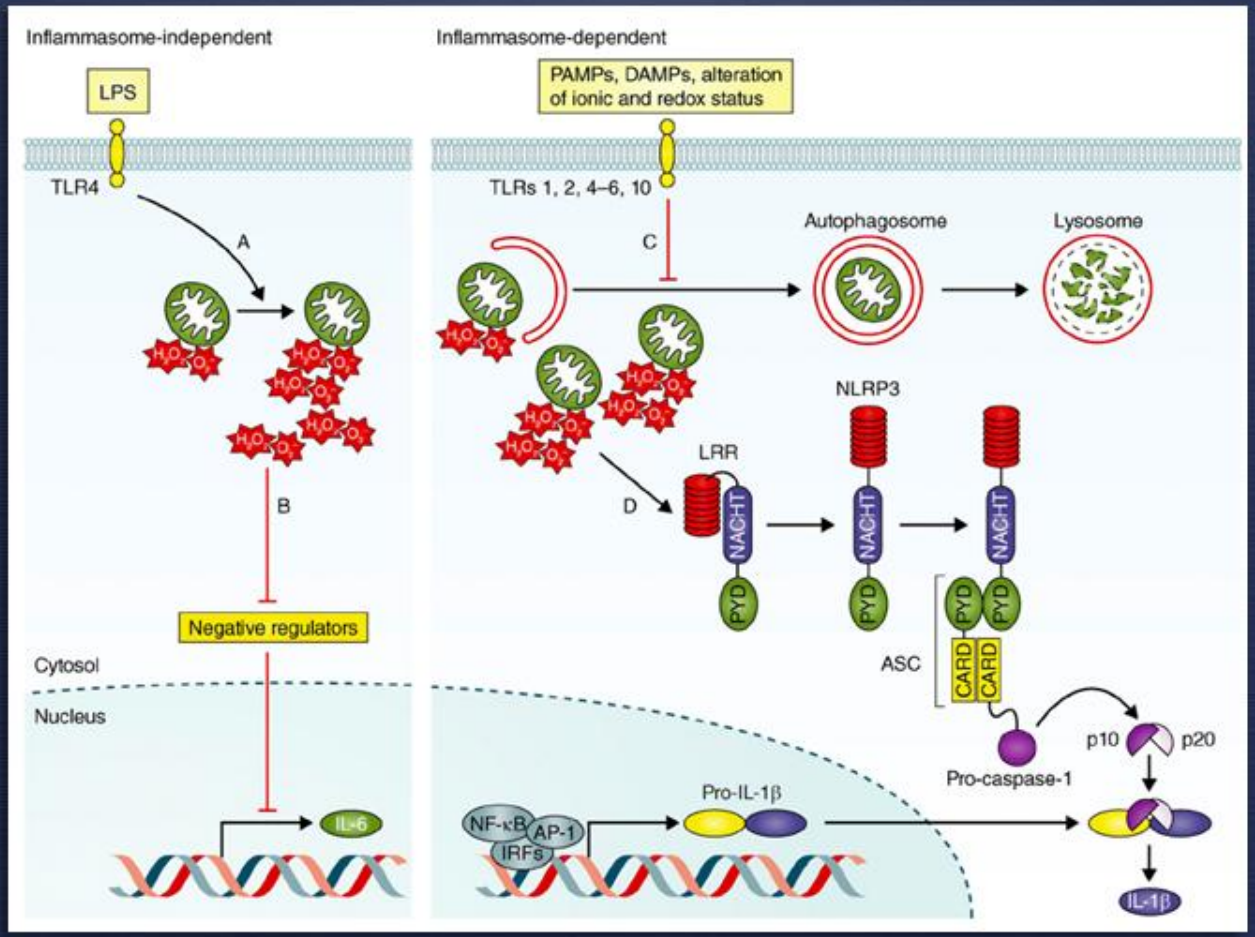
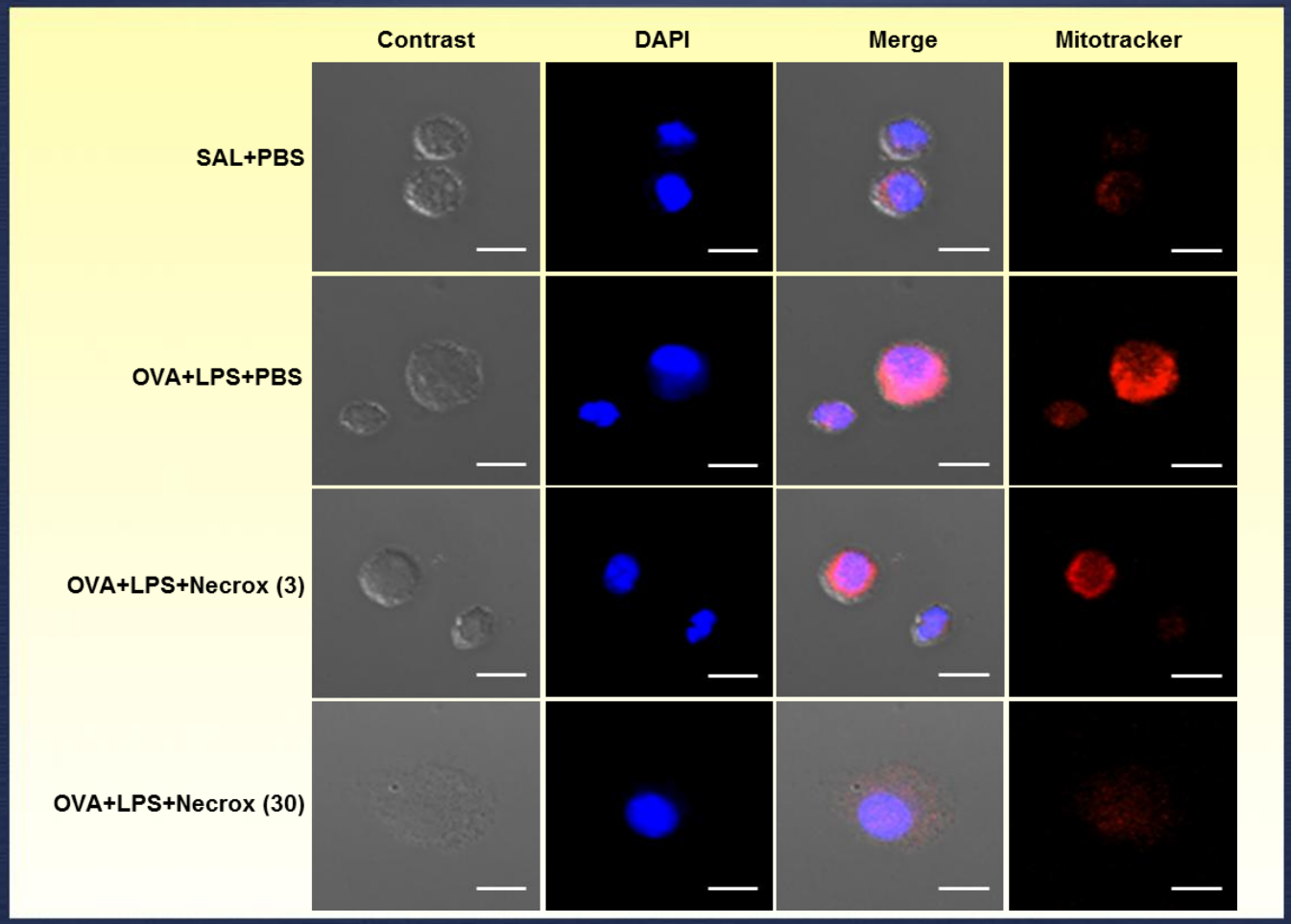
Mitochondria are the main source of ROS sensed by the NLRP3 inflammasome.



Naik E, Dixit VM. J Exp Med. 2011 Mar 14;208(3):417-20.

Mitochondrial ROS in allergic airway inflammation

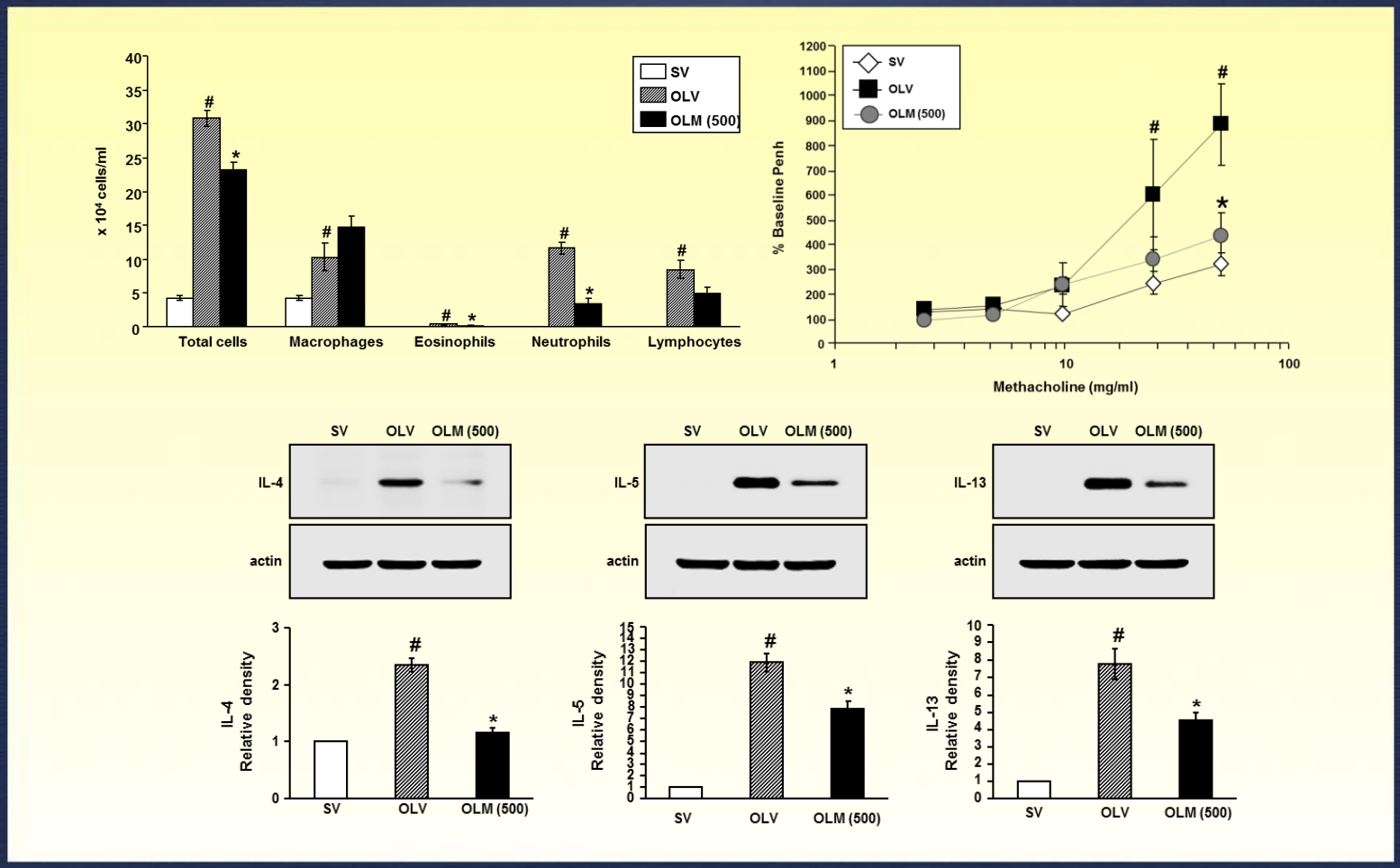
* Mitochondrial ROS (Mitotracker red CM-H2X ROS®) is enhanced in allergic airway inflammation



Unpublished data

Mitochondrial ROS in allergic airway inflammation

* Mitochondrial ROS inhibitor (Mito-tempo®) attenuates allergic airway inflammation



Unpublished data

Mitochondria and ER in innate immune response

EMBO reports

review

Mitochondria in innate immunity

Damien Arnoult[1], *Fraser Soares*[2], *Ivan Tattoli*[2] & *Stephen E. Girardin*[2*]

[1]INSERM U1014, Hopital Paul Brousse, Batiment Lavoisier, Villejuif, France, and [2]Department of Laboratory Medicine and Pathobiology, Medical Sciences Building, University of Toronto, Toronto, Ontario, Canada

Mitochondria are cellular organelles involved in host-cell metabolic processes and the control of programmed cell death. A direct link between mitochondria and innate immune signalling was first highlighted with the identification of MAVS—a crucial adaptor for RIG-I-like receptor signalling—as a mitochondria-anchored protein. Recently, other innate immune molecules, such as NLRX1, TRAF6, NLRP3 and IRGM have been functionally associated with mitochondria. Furthermore, mitochondrial alarmins—such as mitochondrial DNA and formyl peptides—can be released by damaged mitochondria and trigger inflammation. Therefore, mitochondria emerge as a fundamental hub for innate immune signalling.

Keywords: mitochondria; innate immunity; MAVS; pathogen; host metabolism

EMBO *reports* (2011) **12**, 901–910. doi:10.1038/embor.2011.157

See Glossary for abbreviations used in this article.

Introduction

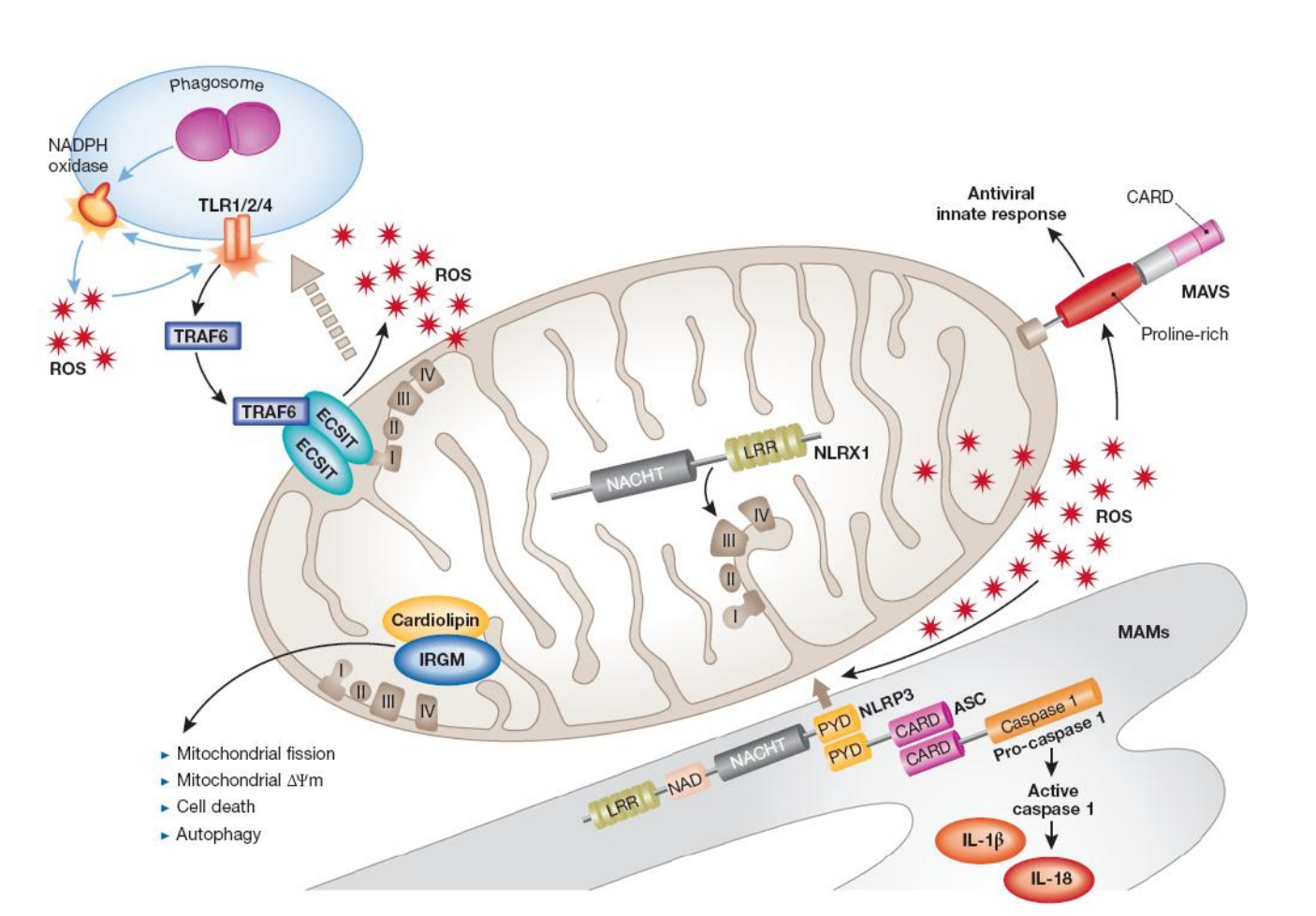
Innate immune responses in mammalian cells rely on the detection of trigger specific host defence signalling, the most commonly studied of which are pro-inflammatory pathways that activate nuclear factor κB (NF-κB), in response to most TLRs, the NLR family members NOD1 and NOD2, and RLRs; caspase 1 inflammasome, activated by the NLRP subfamily of NLR proteins and RLRs; and type I interferon (Elinav *et al*, 2011; Kawai & Akira, 2011; Loo & Gale, 2011). Finally, several new PRRs have been identified recently that detect intracellular foreign DNA, and these include AIM2, IFI16 and ZBP1/DLM1 (Barber, 2011).

With regards to the mechanisms that underlie PRR activation, a key remaining question is whether MAMP and DAMP detection is a direct ligand–receptor interaction or whether it requires multiple cofactors and complex modifications of host metabolic and homeostatic functions. In support of the latter hypothesis, numerous lines of evidence have recently highlighted the importance of mitochondria and mitochondrial functions in the regulation of host innate immune signalling. Importantly, although this organelle seems to be a signalling hub for innate immunity, there is strong compelling evidence to

Innate immune molecules such as NLRP3 have been associated with mitochondria.

Arnoult D, et al. EMBO reports (2011) 12, 901–910.

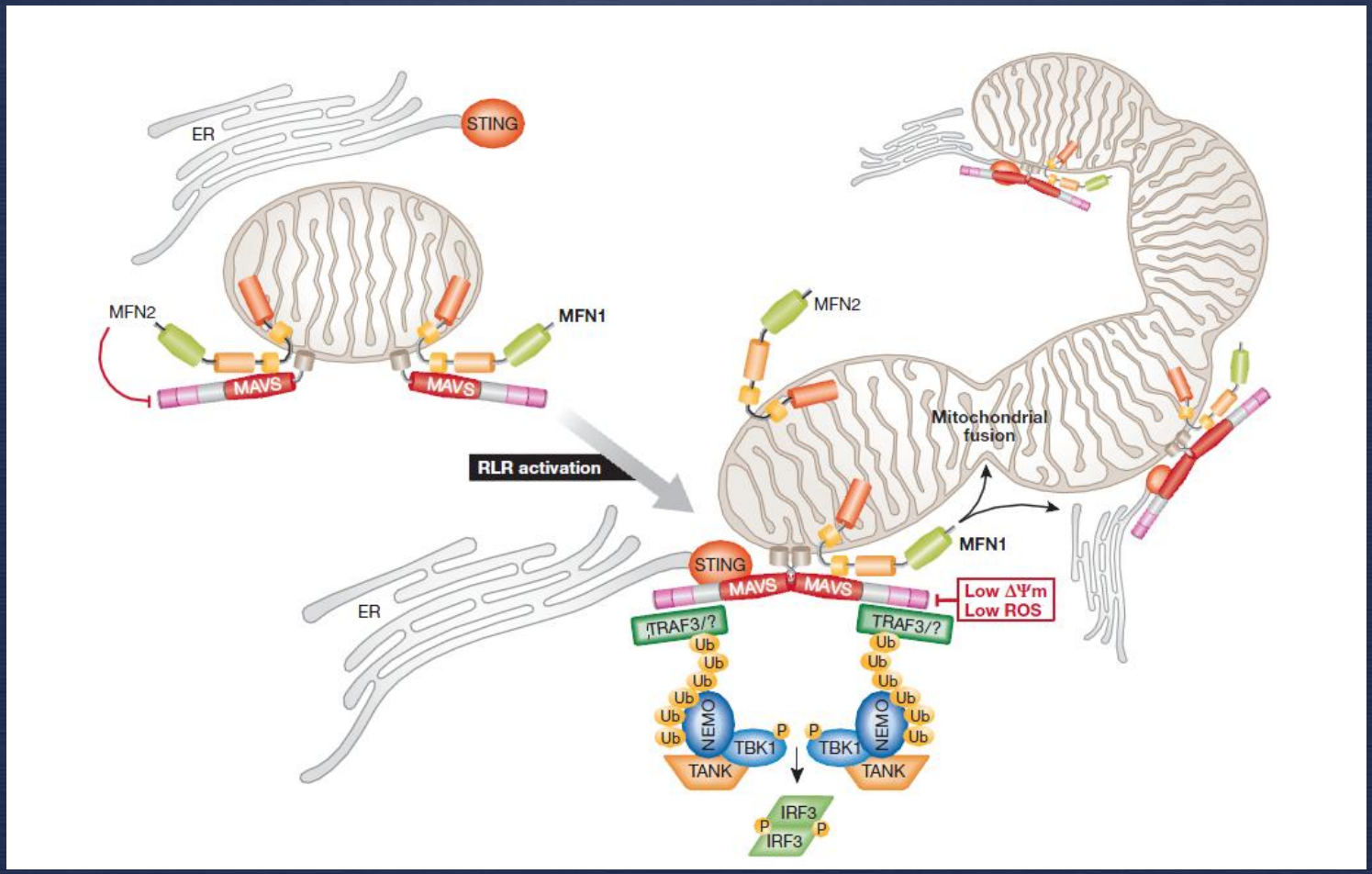
Mitochondria and ER in innate immune response



On induction of mitochondrial ROS, NLRP3 localizes to the mitochondrial-associated endoplasmic reticulum membrane (MAM) with ASC and pro-caspase 1, inducing caspase-1 activation and the production of IL-1β and IL-18.

Arnoult D, et al. EMBO reports (2011) 12, 901–910.

Mitochondria and ER in innate immune response



Both PAMPs and DAMPs trigger innate immunity following detection by PRRs. PRR activation leads to ER-inflammasome-mitochondrial fusion.

Arnoult D, et al. EMBO reports (2011) 12, 901–910.

ER stress and Inflammasome

CBNU

Citation: Cell Death and Disease (2012) 3, e261; doi:10.1038/cddis.2011.132

www.nature.com/cddis

ER stress activates the NLRP3 inflammasome via an UPR-independent pathway

P Menu*,[1,5], A Mayor[1,5], R Zhou[1,2], A Tardivel[1], H Ichijo[3], K Mori[4] and J Tschopp*,[1]

Uncontrolled endoplasmic reticulum (ER) stress responses are proposed to contribute to the pathology of chronic inflammatory diseases such as type 2 diabetes or atherosclerosis. However, the connection between ER stress and inflammation remains largely unexplored. Here, we show that ER stress causes activation of the NLRP3 inflammasome, with subsequent release of the pro-inflammatory cytokine interleukin-1β. This ER-triggered proinflammatory signal shares the same requirement for reactive oxygen species production and potassium efflux compared with other known NLRP3 inflammasome activators, but is independent of the classical unfolded protein response (UPR). We thus propose that the NLRP3 inflammasome senses and responds to ER stress downstream of a previously uncharacterized ER stress response signaling pathway distinct from the UPR, thus providing mechanistic insight to the link between ER stress and chronic inflammatory diseases.
Cell Death and Disease (2012) **3,** e261; doi:10.1038/cddis.2011.132; published online 26 January 2012
Subject Category: Immunity

Inflammation is the first response of the immune system to infection or tissue injury, and is meant to protect the body from these insults. Prolonged or chronic inflammation, however, can exacerbate tissue damage and is implicated in the development of diseases, such as arthritis, neurodegenerative diseases and type 2 diabetes.[1] The endoplasmic reticulum (ER) stress response is a potent, evolutionarily conserved response to misfolded proteins and cellular metabolic stress. The inflammatory response is frequently triggered as a consequence of ER stress, caused by metabolic problems or by the accumulation of misfolded proteins.[2] Under such conditions, the ER initiates the unfolded

Recent years have seen remarkable growth in our understanding of the cellular and molecular mechanisms that control the inflammatory response. In particular, proteins belonging to the NOD-like receptor (NLR) family have been identified as central players in innate immunity.[4,5] Some of these NLRs participate in multiprotein complexes termed inflammasomes, which mediate caspase-1-dependent maturation of the highly proinflammatory cytokine interleukin-1β (IL-1β). Of the thus far described inflammasomes, the NLRP3 inflammasome is most fully characterized.[6] In addition to microbial and viral danger signals (PAMPs), the NLRP3 inflammasome is unique

ER stress causes activation of the NLRP3 inflammasome, with subsequent release of the pro-inflammatory cytokine interleukin-1β.

전북대학교병원 CHONBUK NATIONAL UNIVERSITY HOSPITAL 전북대학교

Menu P, et al. Cell Death dis 2012; 3:e261.

ER-Mitochondrial ROS-NLRP3 inflammasome in allergic airway inflammation

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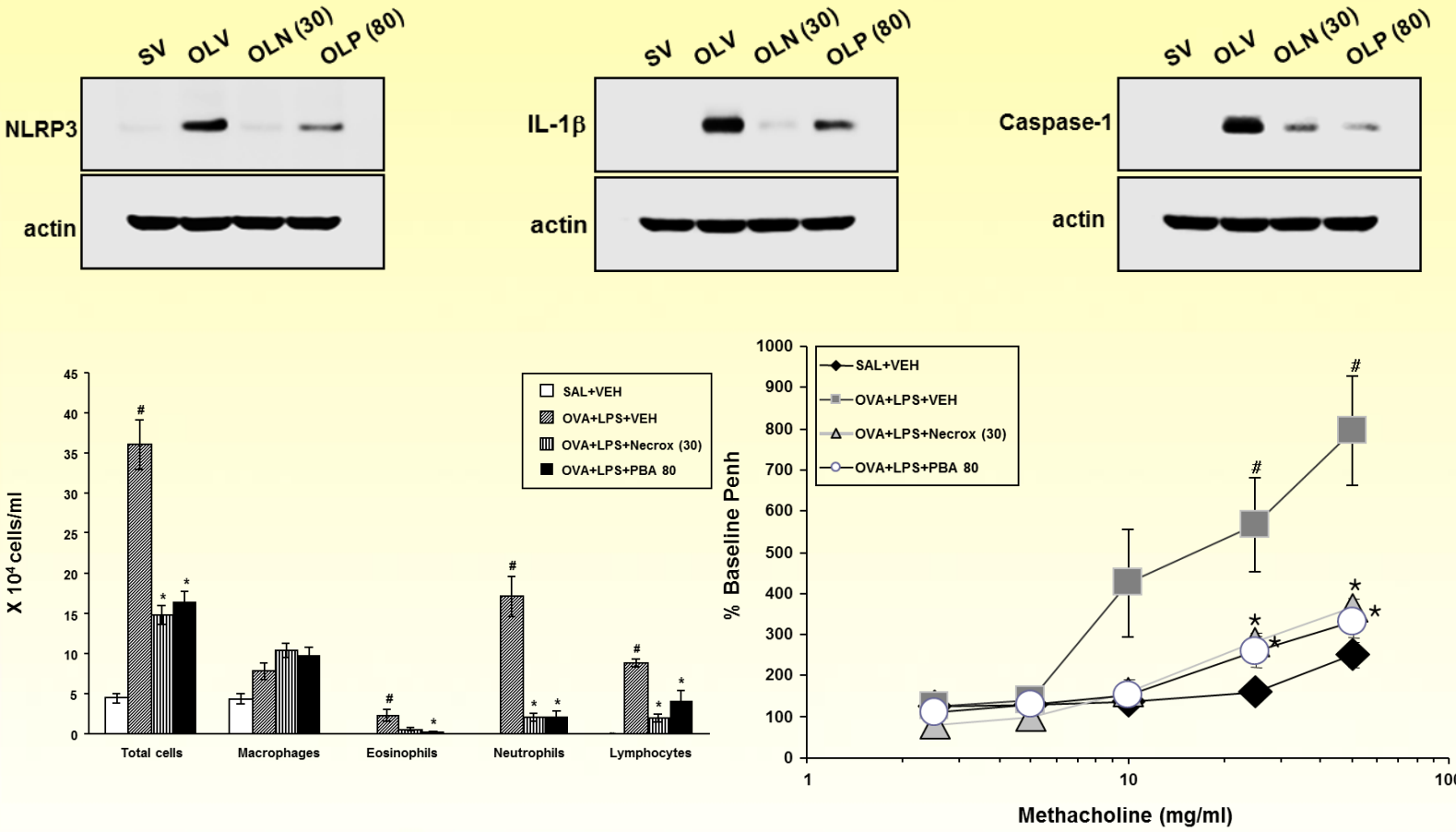
* Mitochondrial ROS inhibitor (Necrox-5®) or ER stress regulator (PBA) attenuates NLRP3 activation allergic airway inflammation



Unpublished data

ER-Mitochondrial ROS-NLRP3 inflammasome in allergic airway inflammation

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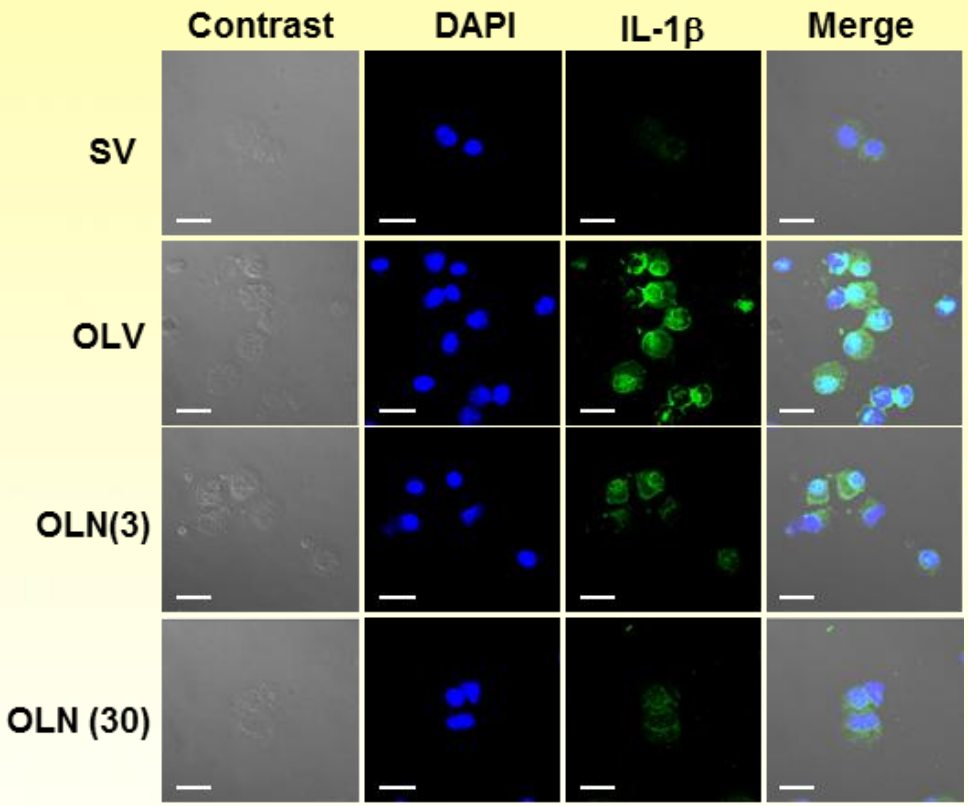
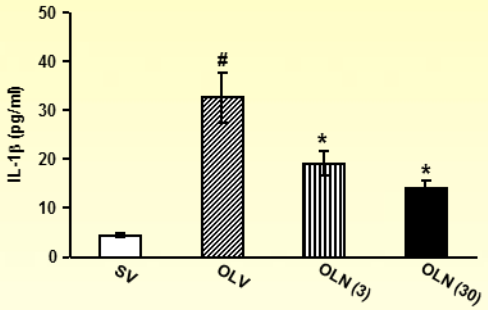
NLRP3 activation allergic airway inflammation





Unpublished data

ER-Mitochondrial ROS-NLRP3 inflammasome in severe asthma

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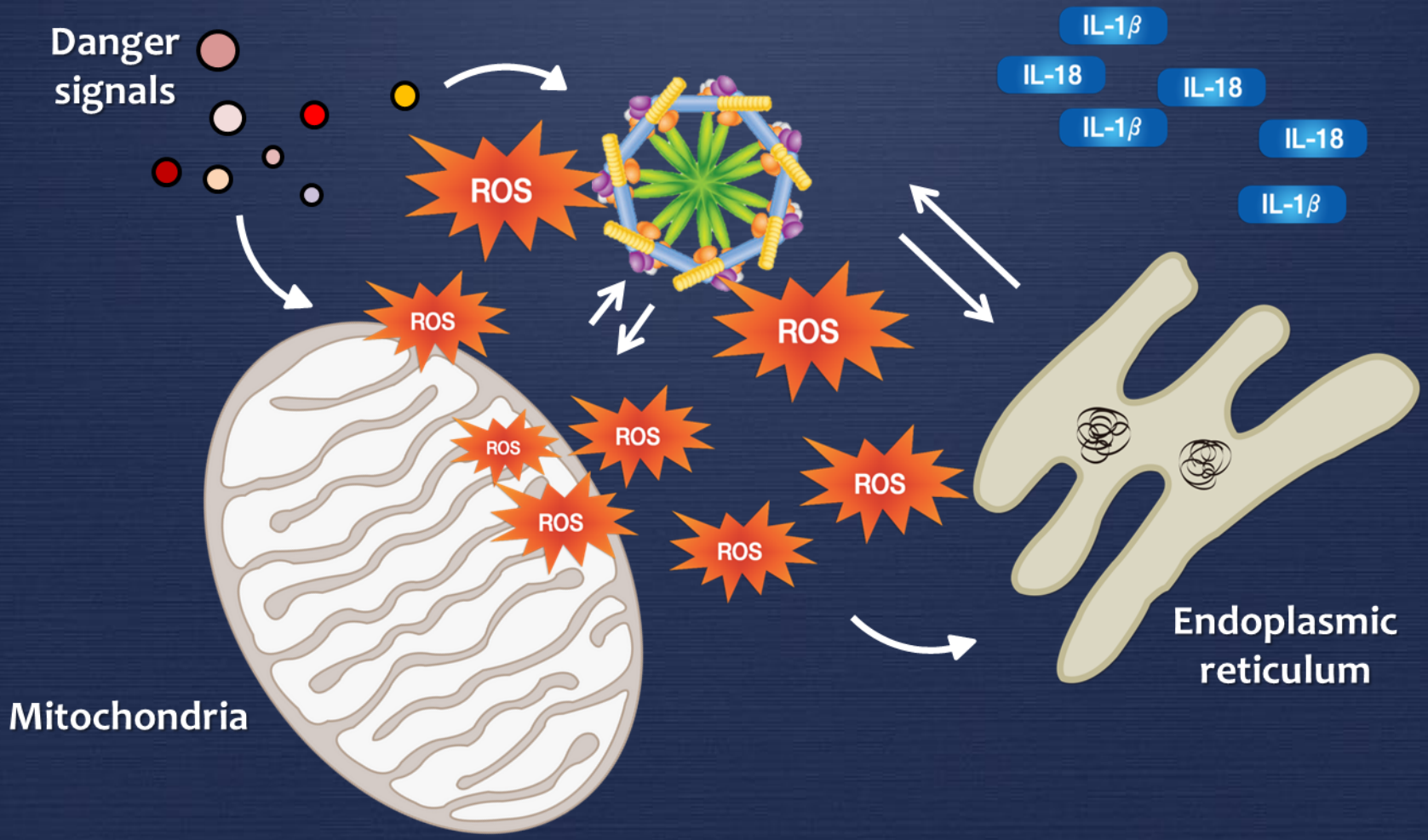

Take home message

- There is an extensive researches on biomarkers - limitation
- Severe asthma: *IL-17, PI3K- δ, ROS*
- organelle dysfunction and origin of inflammatory cascades in line with immune systems - especially severe phenotypes.

Take home message

- NLRP3 inflammasome activation is required together with a TLR4 signal to produce mature IL-1β and allergic lung inflammation.
- ER stress may be implicated in the pathogenesis of allergic lung inflammation.
- ER-Mitochondrial ROS- inflammasome complex is expected to be a novel concept in the pathogenesis of allergic inflammation. –GRP78, CHOP, Mitochondrial ROS, NLRP3

Take home message

- **several potential candidates of biomarkers** are expected to be applicable to clinical practice, specifically the estimation of therapeutic responses of orphan drugs such as 4-PBA or newly developing pharmacologic agents targeting severe asthma.

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- Ko Y H

CLOSING

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"I have a dream of taking our nation's science to a whole new level and I hope that these scientific developments return to the patients".

Thank you for your attention!!

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Pros and Cons of non-invasive techniques

Table 1
Pros and cons of non-invasive lower airways sampling techniques.

	Induced sputum	Exhaled NO	Exhaled breath condensate	Electronic nose
Pros	• Multiple biomarkers • Reproducible cell differentials on cytospins • Valid tool for diagnosis (e.g., 'refractory asthma') or assessment of anti-inflammatory therapy	• Non-invasive • Reproducible • Inexpensive measurements • Direct results • Allows serial measurements • Tool for diagnosis/assessment of anti-inflammatory therapy in (allergic) asthma	• Non-invasive • Multiple biomarkers • Allows serial measurements • Potential tool for diagnosis and assessment of anti-inflammatory therapy	• Non-invasive and portable • Almost real-time • Uses high-dimensional biomarker signal • Produces individual signature: 'breath'print • Allows serial measurements • Potential tool for diagnosis and monitoring of anti-inflammatory therapy
Contras	• Representative samples available in approx. 80–90% of subjects • Soluble markers subject to dilution • Non-repeatable over short time-period (<12–18 h) • Expertise & experience required (staff/lab) • Rescue medication needed • Contraindicated in severe persistent asthma/copd/active cardiovascular disorders	• Expensive equipment • Many perturbing factors • Longitudinal samplings within 1 patient are more informative than single measurements	• Detection assays not fully reproducible • Expensive & time-consuming procedure/assays • Soluble markers subject to dilution • Specialized lab needed	• Sensor technology still developing • Mapping between eNoses required • Offline SPSS- or R-analysis still required • External validation not completed yet
Overall assessment	• Validated tool for monitoring of the effects of (novel) anti-inflammatory drugs • Lengthy, expensive procedure requiring expertise/experience • Not suitable for patients with severe bronchoconstriction/comorbidities	• Validated tool for diagnosis/monitoring of anti-inflammatory drug-effects • Patient & researcher-friendly method	• Procedure awaits further validation • Patient & researcher-friendly method	• Patient & researcher-friendly method • Promising technique for both clinical and research applications
Refs[a]	[32,34,36,52,54]	[118,150]	[85,108]	[156,171]

Z. Diamant *et al. Pulmonary Pharmacology & Therapeutics* (2010)

Allergic reaction; acute to chronic

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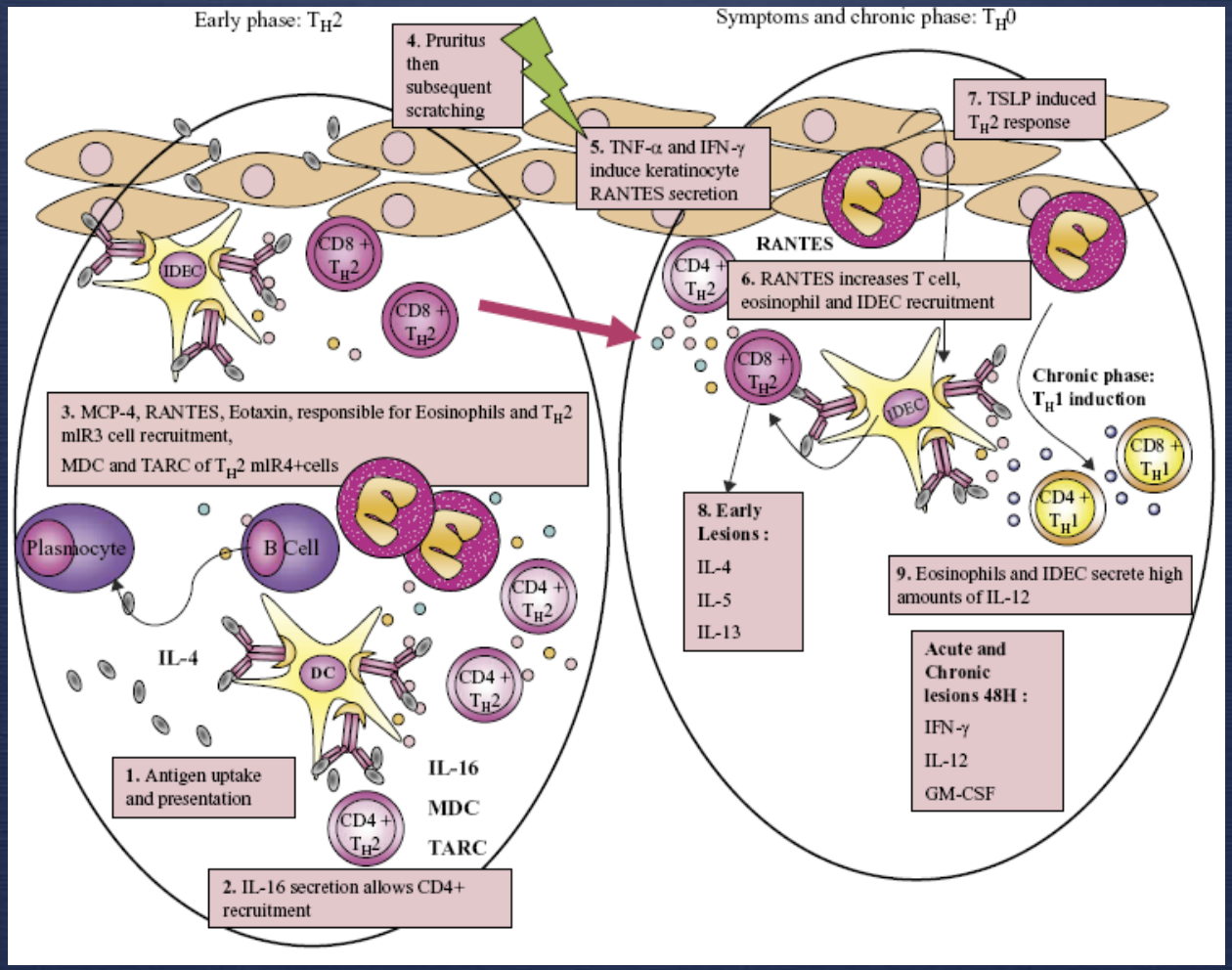
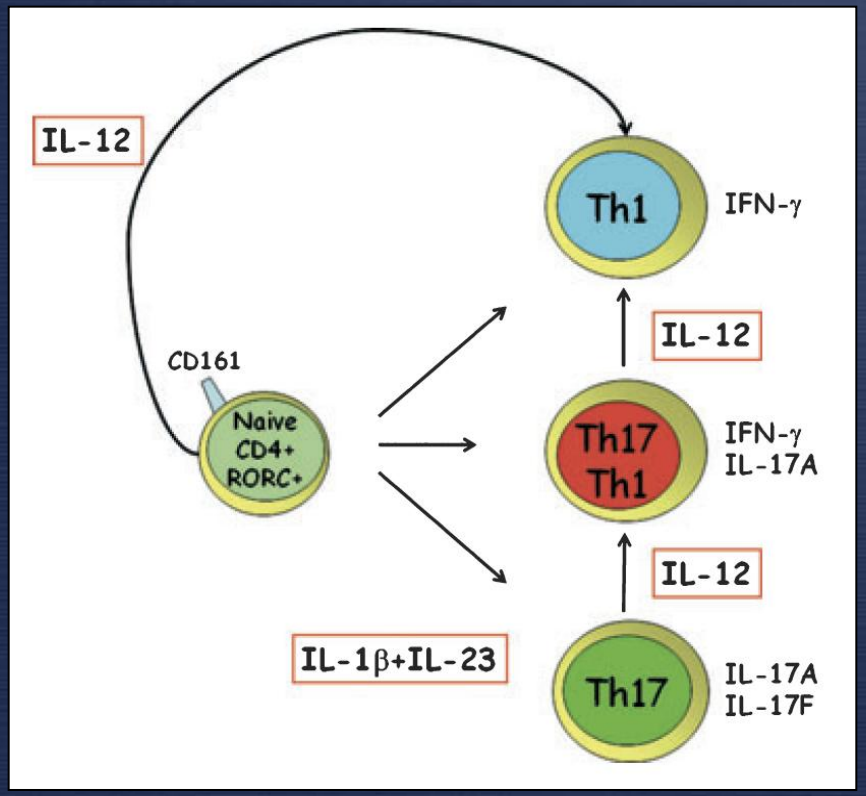
Th2 dominant ----------→ Th1 dominant



Mamessier E et al. Eur J Dermatol 2006; 16 (2): 103-13

Th1/Th17 cells in other inflammation



- **Th17 to Th1 plasticity** driven by the inflammatory environment in patients with autoimmune polyarthritis.

- The **respective role of Th17, Th17/Th1, and Th1** in the pathogenesis of chronic inflammatory disorders.

Cosmi L et el. Allergy 2011, In press
Nistala K et al. Proc Natl Acad Sci USA 2010;107:14751–14756
Annunziato F et al. Nat Rev Rheumatol 2009;5:325–331

Th2/Th17 cells in asthma

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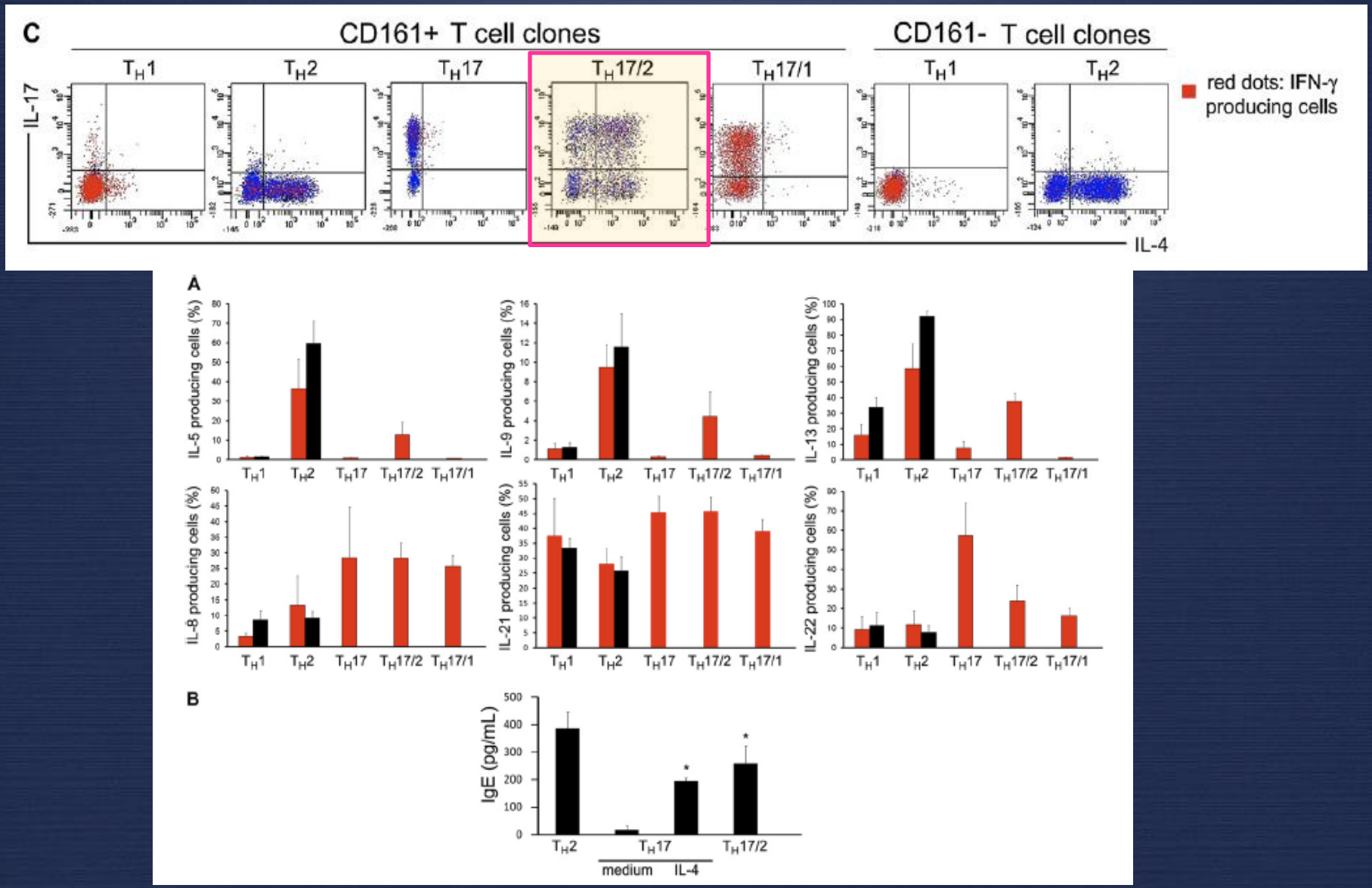

Cosmi L et el. J Allergy Clin Immunol 2010;125:222-230

Th2/Th17 cells in asthma

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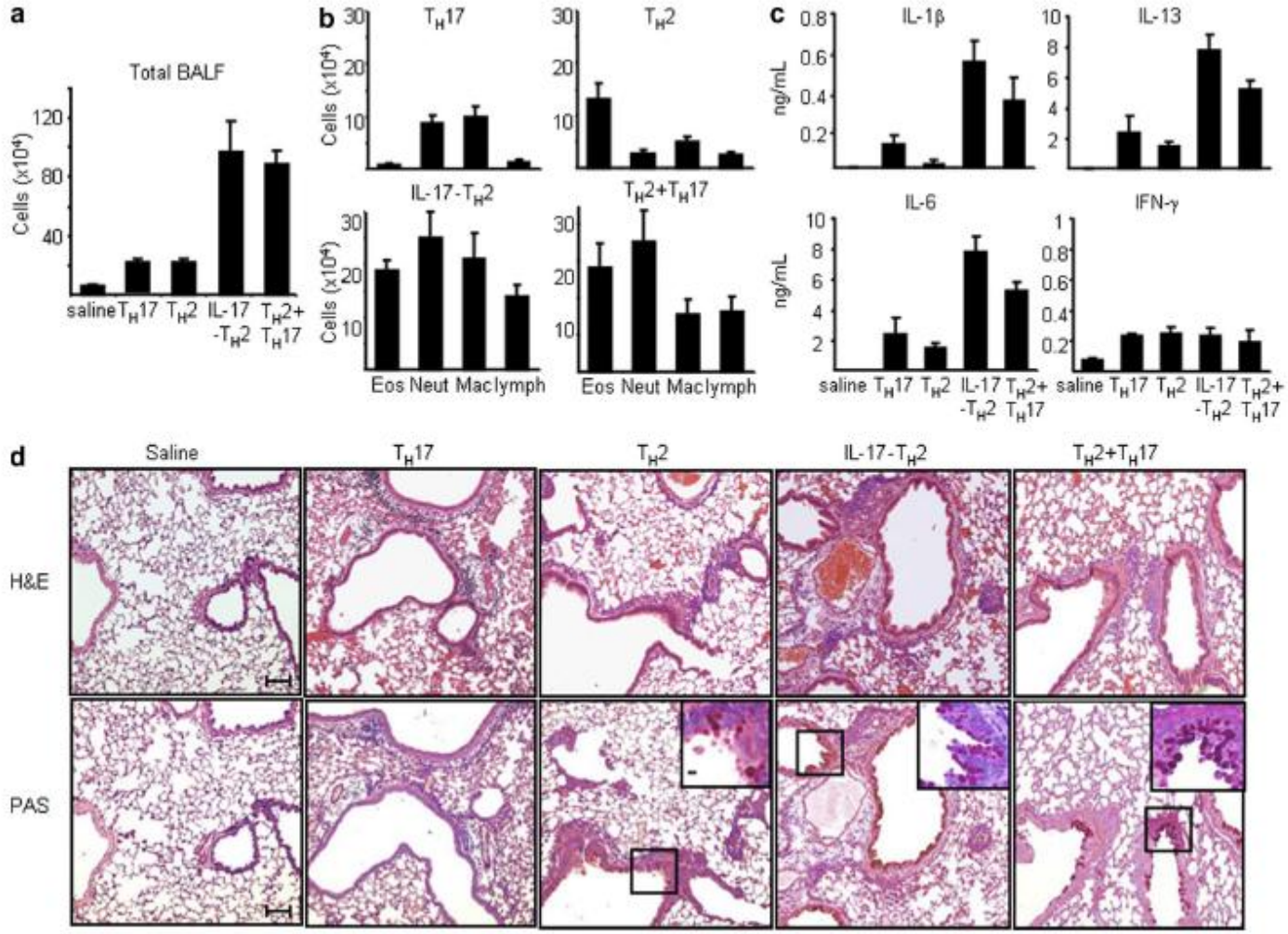

Wang YH et el.
J. Exp. Med. 2010;207 :2479-2491

Total and Allergen-Specific IgE

- The presence of allergen-specific IgE is a biomarker for atopic asthma
  - Atopic status is an important phenotype and should be documented in clinical research studies
- The *multiallergen screen* is a single semiquantitative serologic measure of IgE against major allergens
  - ***Core Biomarker***
  - It characterizes an individual as atopic but does not specify as to which allergen(s) a person is sensitized
- Quantitative serologic measures of individual *allergen-specific IgE antibodies*
  - Offer information on specific allergen sensitivities
  - Considered as ***supplemental biomarkers***

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Total and Allergen-Specific IgE

- Measurement of allergen-specific IgE by *skin prick test*, although widely used in the clinical setting,
  - is considered an ***emerging biomarker*** for research because of the variability of the test's performance

- *Total serum IgE* has been associated with asthma and is considered
  - a ***supplemental measure*** for study population characterization, as well as an outcome for intervention and observational studies, as deemed appropriate by study design

Exhaled nitric oxide

- *FENO* measured at a constant flow rate is
  - a simple, safe, and reproducible biomarker for use in asthma clinical trials

- Although FENO values overlap among healthy, atopic, and asthmatic cohorts,
  - Changes in FENO values over time in individuals who have asthma are relevant to clinical research studies that seek to
    - **Measure effects of interventions on airway inflammation**, in particular the effects of antiinflammatory (eg, corticosteroid) therapies

Exhaled nitric oxide

- FENO levels of *less than 25 ppb*
  - Generally indicate lower likelihood for eosinophilic inflammation and responsiveness to corticosteroids

- However, FENO cannot be used interchangeably with sputum eosinophilia as an outcome measure,
  - Given that eosinophilic inflammation and FENO levels ***do not always respond identically*** to treatment

Exhaled nitric oxide

- FENO is recommended
  - As a ***supplemental outcome*** in clinical trials that seek to evaluate effects of interventions on airway disease and/or to characterize corticosteroid-responsive phenotypes of asthma

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Sputum eosinophils

- Analysis of *eosinophil counts in induced sputum*
  - Identifies patients who have eosinophilic and non-eosinophilic phenotypes of asthma
  - These inflammatory phenotypes can *predict response to treatment*
  - ***Supplemental outcome***

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CBC/Blood eosinophils

- Analysis of *blood eosinophils* by automated CBC provides useful information to
  - Characterize study populations for prospective clinical trials and observational studies in asthma

- Blood eosinophils can be used as a biomarker to monitor
  - Systemic biological ***effects of*** pharmacologic and immunologic ***interventions*** in patients with asthma

Urinary leukotriene E4

- Urinary leukotriene E4 (*LTE4*) is a
  - Validated marker of cysteinyl leukotriene activity
  - Should be considered for incorporation in clinical trials of molecules that may directly or indirectly affect this pathway

NLRP3

- mediate secretion of IL-1β and IL-18 to initiate the inflammatory process.