Inflammasome in asthma

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Contents

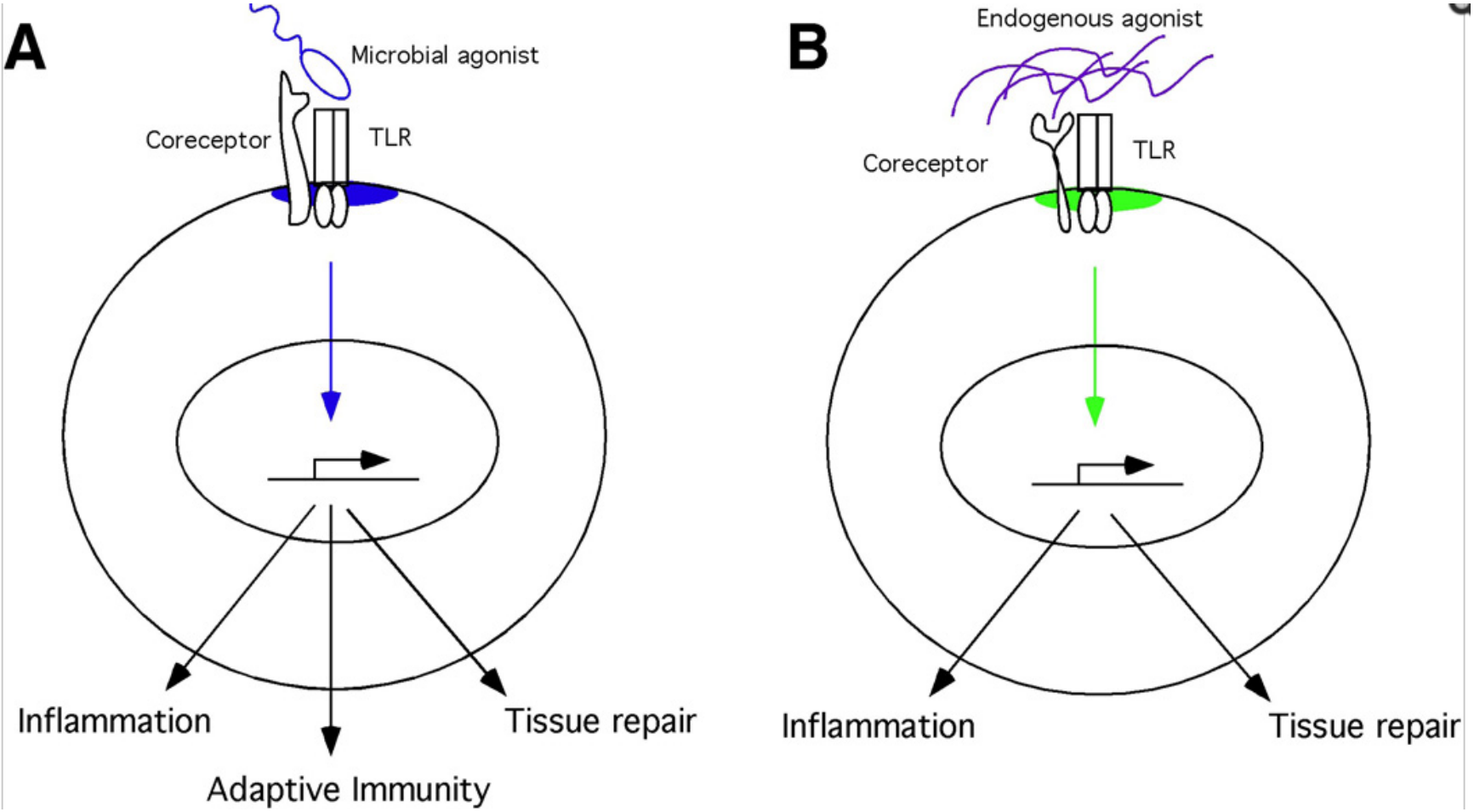
Inflammasome

- large macromolecular signaling complexes that control the proteolytic activation of two highly proinflammatory IL-1 family cytokines, IL-1β and IL-18.
- an intracellularmultimeric protein complex that regulates the maturation and release of proinflammatory cytokines of the IL-1 family in response to pathogens and endogenous danger signals.
- plays a role in the chronic inflammation of the airways of patients with asthma and chronic obstructive pulmonary disease, as well as in the initiation and progression of the inflammatory process in pulmonary fibrosis.

Historical Perspective

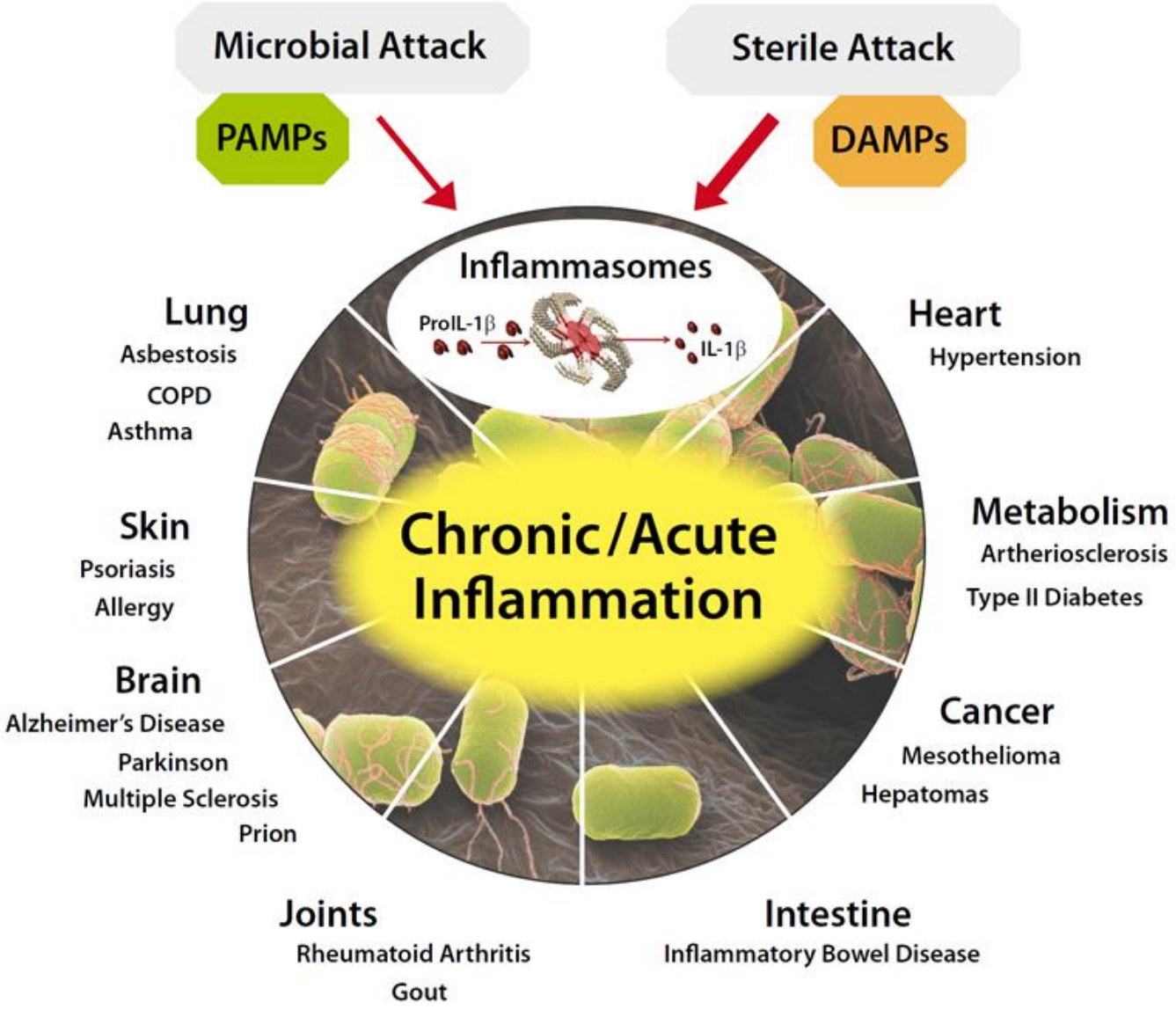
- The link between mutations in NLR genes and inflammatory diseases was established by Hoffmann and colleagues in 2001, when they described mutations in NLRP3 in individuals affected by Muckle-Wells syndrome.
- In 2002, Martinon et al. described, for the first time, an inducible high-molecular-weight complex containing NLRP3, an adaptor protein [apoptosis-associated speck-like protein containing a CARD domain (ASC)], and proinflammatory caspases, which they called the inflammasome.

Regulation of adaptive immunity by the innate immune system



Proposed consequences of Toll like receptor (TLR) recognition of exogenous versus endogenous ligands

Science 2010;327(5963):291-5



http://www.adipogen.com/inflammasomes/ (Fig : Overview on inflammasome-associated diseases.)

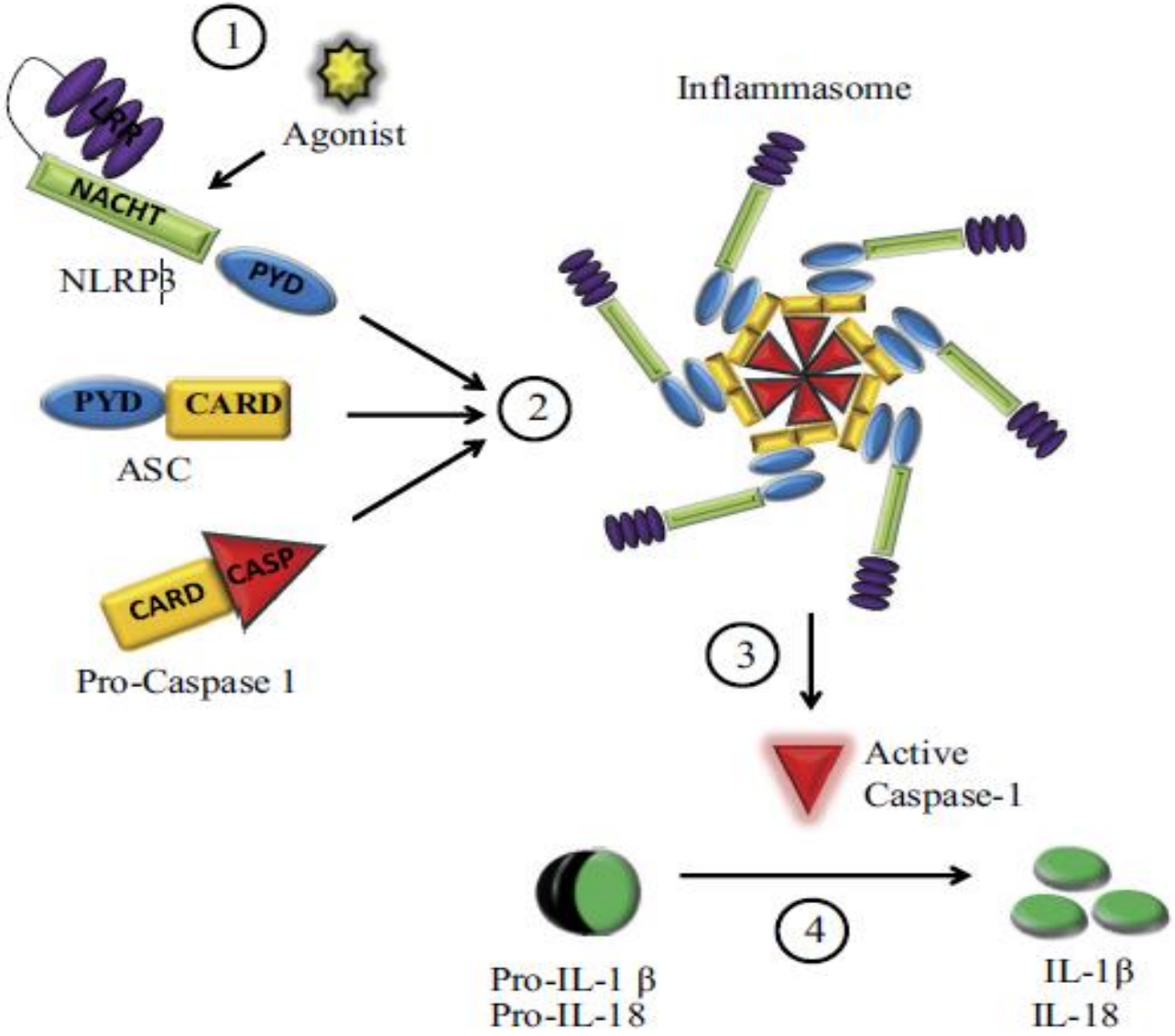


Fig. 1. Nucleotide oligomerization domain (NOD)-like receptor containing a pyrin domain (NLRP3) inflammasome.

Am J Physiol Lung Cell Mol Physiol 2012;303: L627–L633

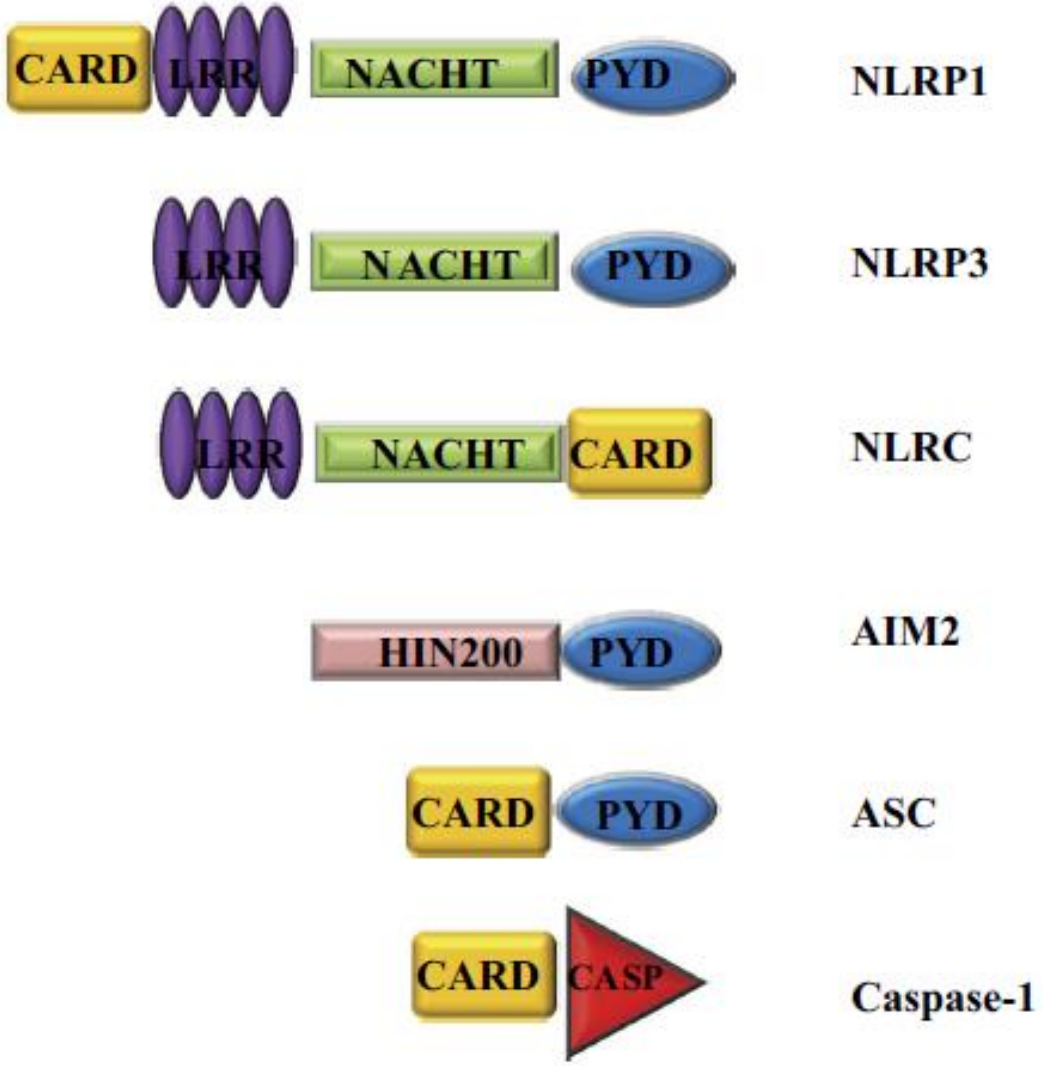


Fig. 3. Human inflammasome-related proteins. NLRPs contain an NH2-terminal PYD, a conserved NACHT domain, and a COOH-terminal LRR domain.

Am J Physiol Lung Cell Mol Physiol 2012;303: L627–L633

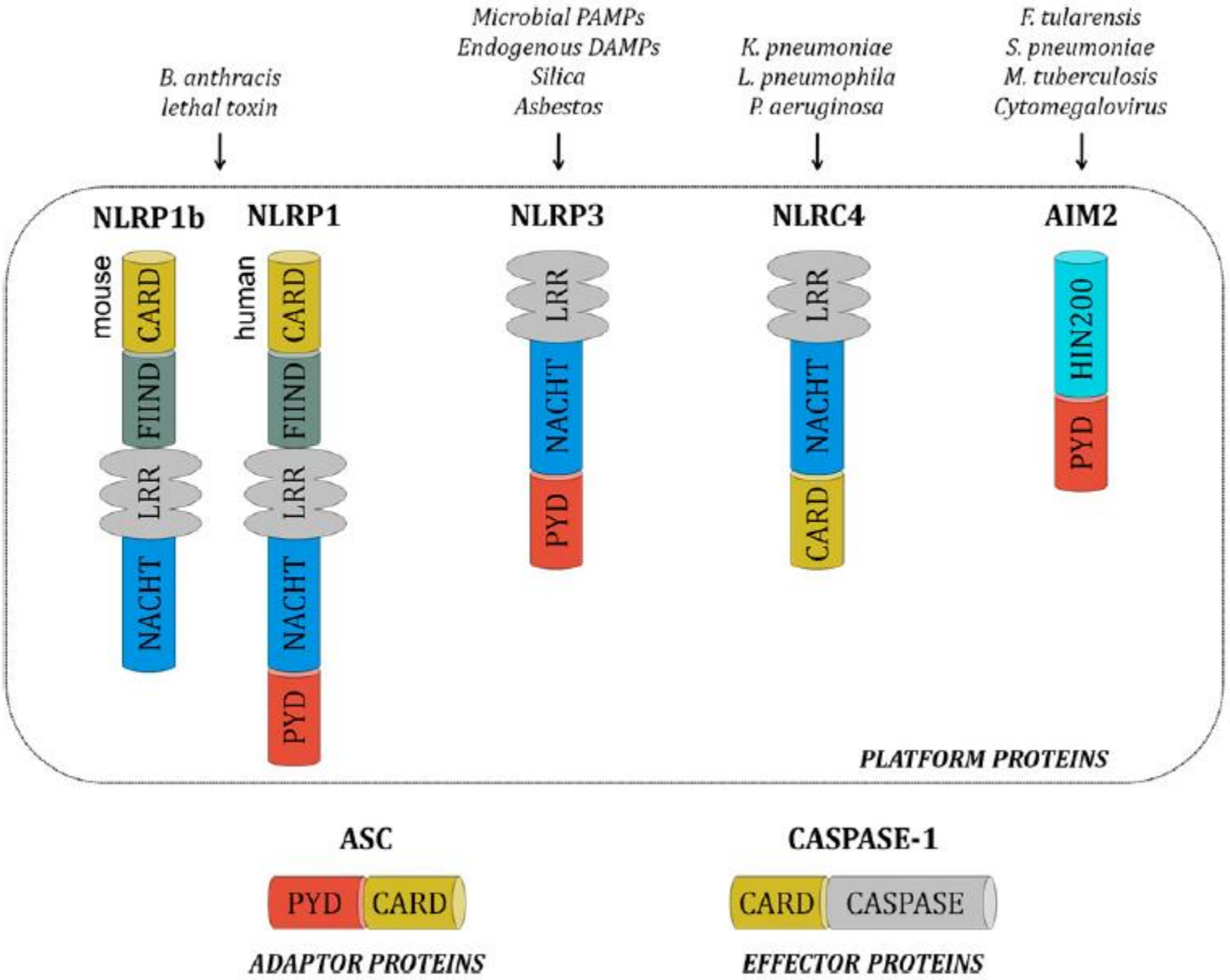


Figure 1. Overview of inflammasome composition and activating stimuli.

Chest 2014;145:1121 – 1133

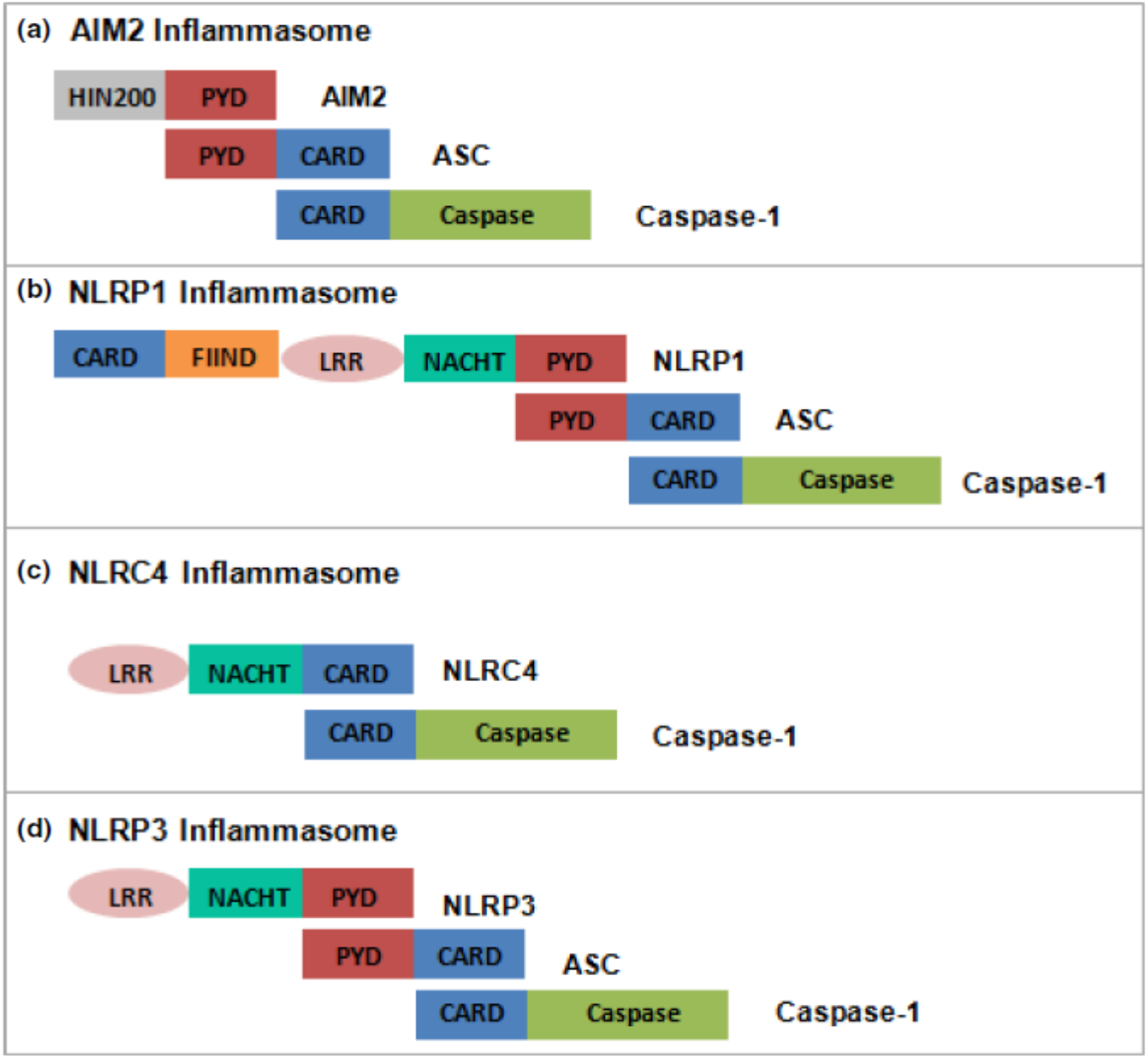


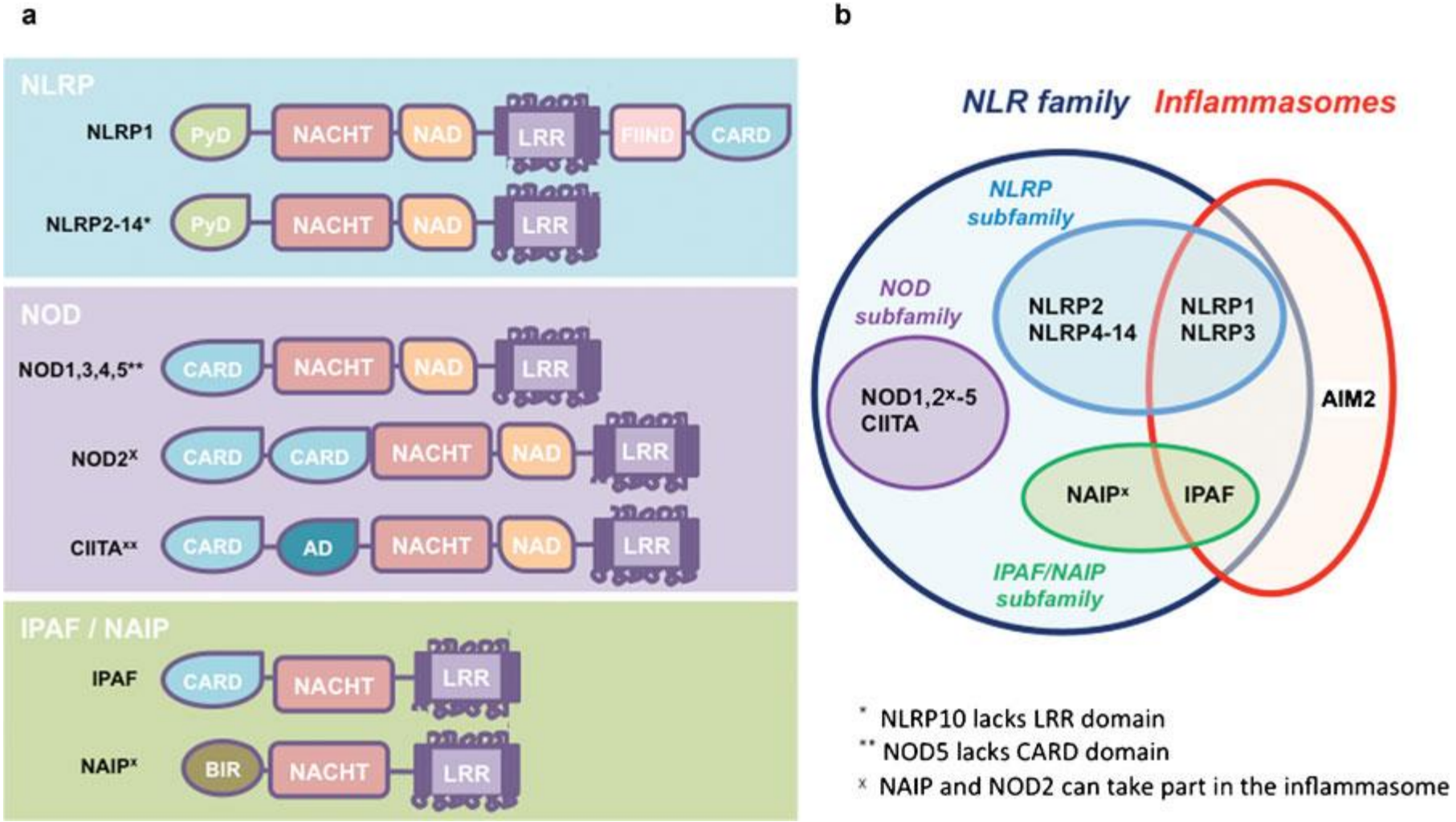
Fig. 1. AIM2, NLRP1, NLRC4 and NLRP3 inflammasomes (nucleotide-binding domain, leucine-rich repeats containing family, pyrin domain-containing-3)

Clin Exp Allergy 2014;44:160–172

Name	Domains	Activator	Adapter/ binder	Function	Expression (lung)
NOD1	CARD NOD LRR	mDAP-Tri-[h]/Tetrapeptide [m]	Rip2, ATGL16	NF-κB activation, autophagy	yes
NOD2	CARD CARD NOD LRR	MDP and other PG motifs	Rip2, ATGL16	NF-κB activation, autophagy	Myeloid cells, monocytes, bronchial epithelial cells
NLRC3	NOD LRR	?	TRAF6	Negative TLR regulator?	?
NLRC4	CARD NOD LRR	flagellin, T3SS components	ASC, caspase-1	Inflammasome formation	macrophages
NLRC5	NOD LRR	virus	?	Transcription MHC class I related genes	high
NLRX1	MT NOD LRR	RNA?	UQCRC2, MAVS, TUFM	ROS production, autophagy, negative regulator of TLR and MAVS-dep. signaling	yes
NLRP1	human PYD NOD LRR CARD mouse ? NOD LRR CARD	LF [m], MDP [h]	ASC, caspase-1	Inflammasome formation	leukocytes and epithelial cells
NLRP2	PYD NOD LRR	?	ASC?	Inflammasome formation?	yes
NLRP3	PYD NOD LRR	pore-forming toxins, nucleic acids, ATP, uric acid, hyaluronan…	ASC, caspase-1	Inflammasome formation	Myeloid cells monocytes, DC
NLRP4	PYD NOD LRR	?	Beclin-1	Autophagy and negative reg. of NF-κB	?
NLRP6	PYD NOD LRR	?	ASC	Inflammasome formation, negative TLR regulator	yes [h]
NLRP7	PYD NOD	microbial acylated lipopeptide	ASC	Inflammasome formation	?
NLRP10	PYD NOD LRR	?	?	Negative NF-κB regulator, DC migration	?
NRLP12	PYD NOD LRR	Lipopeptide (*Yersinia pestis*)	ASC	Negative TLR regulator	myeloid cells
NAIP [h]	BIR BIR BIR NOD LRR	TTSS needle protein (CprI) flagellin?	NLRC4	Inflammasome formation	yes
NAIP2 [m]	BIR BIR BIR NOD LRR	T3SS components (PrgJ)	NLRC4	Inflammasome formation	yes
NAIP5 [m]	BIR BIR BIR NOD LRR	flagellin	NLRC4	Inflammasome formation	yes
CIITA	CARD NOD LRR	?	?	MHCII regulation	lymphocytes, endothelial cells

Figure . Summary of the main characteristics of the NLRs

Frontiers in Immunology 2013;4:393



Cell & Mol Immunol 2011: 8;146–156

Activation of the inflammasome

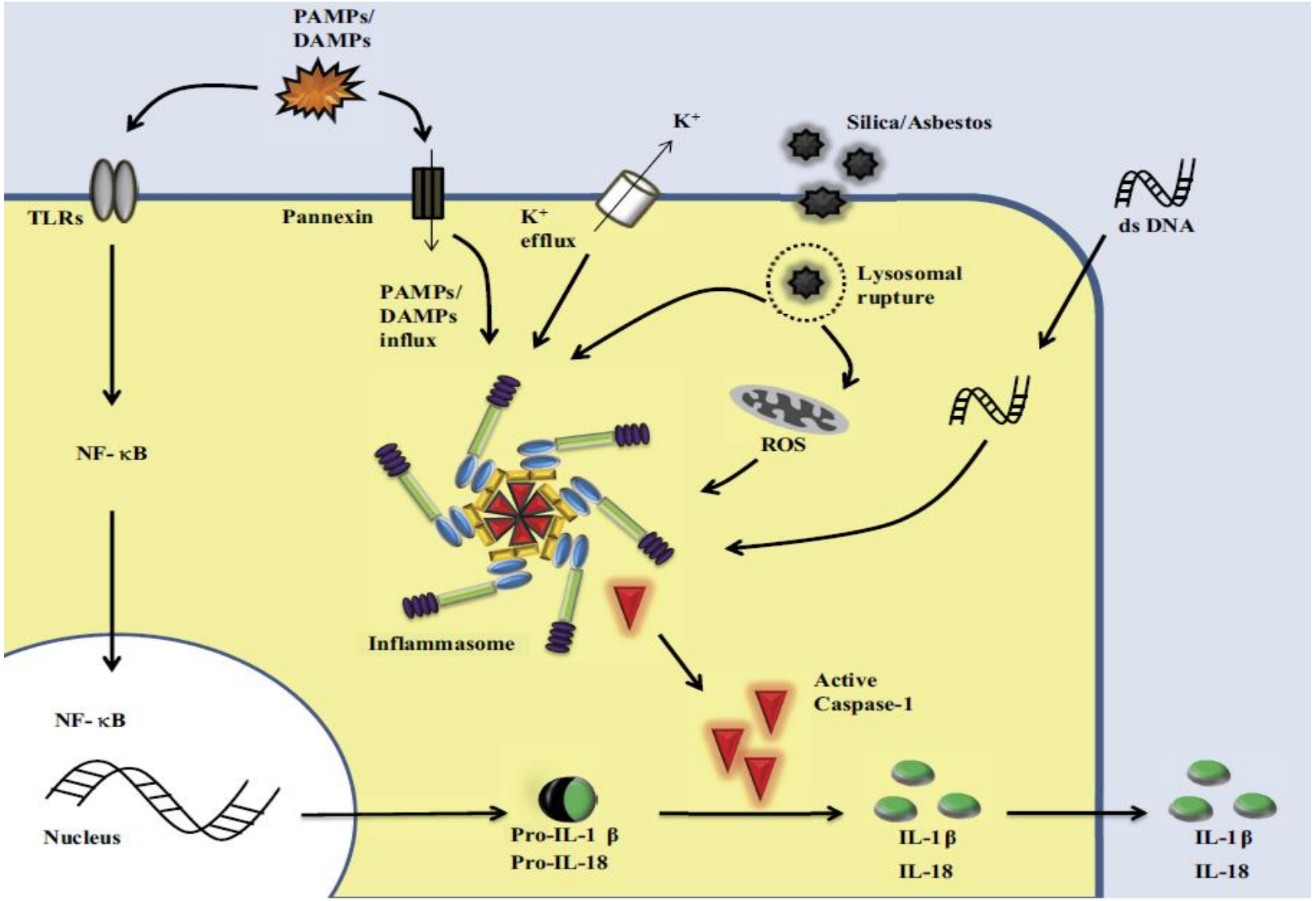


Fig. 2. Activation of the inflammasome.

Am J Physiol Lung Cell Mol Physiol 2012;303: L627–L633

Table 1. *Selected triggers for inflammasome activation*

Inflammasome	Activated by	Source	Reference
NLRP1	*Bacillus anthracis* lethal toxin	*Bacillus anthracis*	Boyden et al. (7)
NLRP3	ATP	Injured cells	Mariathasan et al. (37)
	MSU	Injured cells	Martinon et al. (38a)
	ROS	Cellular stress	Zhou et al. (66)
	Whole pathogens	*Candida albicans Saccharomyces cerevisiae*	Schroder and Tschopp (53)
	Virus	Sendai virus	Kanneganti et al. (27)
		Adenovirus	
		Influenza virus	
	Bacterial toxins	*Streptococcus pneumoniae*	Mariathasan et al. (37)
		Listeria monocytogenes	
		Staphylococcus aureus	
	Environmental irritants	Silica	Hornung et al. (25)
		Asbestos	Dostert et al. (18)
NLRC4	Flagellin	*Salmonella typhimurium*	Franchi et al. (20a)
	Type II/IV secretion systems	*Shigella flexneri*	Suzuki et al. (56)
		Legionella pneumophila	Case et al. (9a)
		Pseudomonas aeruginosa	Sutterwala et al. (55)
AIM2	dsDNA	*Fransicella tularensis*	Rathinam et al. (49)
		Cytomegalovirus	
		Listeria monocytogenes	

NLR, nucleotide oligomerization domain (NOD)-like receptor; NLRP, NLR subfamily containing a pyrin domain; NLRC, NLR subfamily containing a caspase recruitment domain; MSU, monosodium urate; ROS, reactive oxygen species; dsDNA, double-stranded DNA.

Am J Physiol Lung Cell Mol Physiol 2012;303: L627–L633

Table 1 — *Activation of Infl ammasomes by Respiratory Pathogens*

Pathogen	Microbial Activator	Inflammasome
Bacterial		
Bacillus anthracis	Anthrax lethal toxin	NLRP1b
Bordetella pertussis	CyaA	NLRP3
Chlamydia pneumoniae	Unknown	NLRP3
Francisella tularensis	DNA	AIM2
Haemophilus influenzae	Unknown	NLRP3
Klebsiella pneumoniae	Unknown	NLRP3, NLRC4
Legionella pneumophila	Flagellin	NLRC4
Mycoplasma pneumoniae	Acylated lipopeptides	NLRP7
Pseudomonas aeruginosa	Flagellin	NLRC4
Staphylococcus aureus	Hemolysins	NLRP3
Streptococcus pneumoniae	Pneumolysin, DNA	NLRP3, AIM2
Fungal		
Aspergillus fumigatus	Unknown	NLRP3
Candida albicans	Unknown	NLRP3
Mycobacterial		
Mycobacterium tuberculosis	ESAT-6, DNA	NLRP3, AIM2
Viral		
Cytomegalovirus	Viral double-stranded DNA	AIM2
Influenza A	Viral M2, viral RNA?	NLRP3
Respiratory syncytial virus	Unknown	NLRP3

CHEST 2014;145: 1121 – 1133

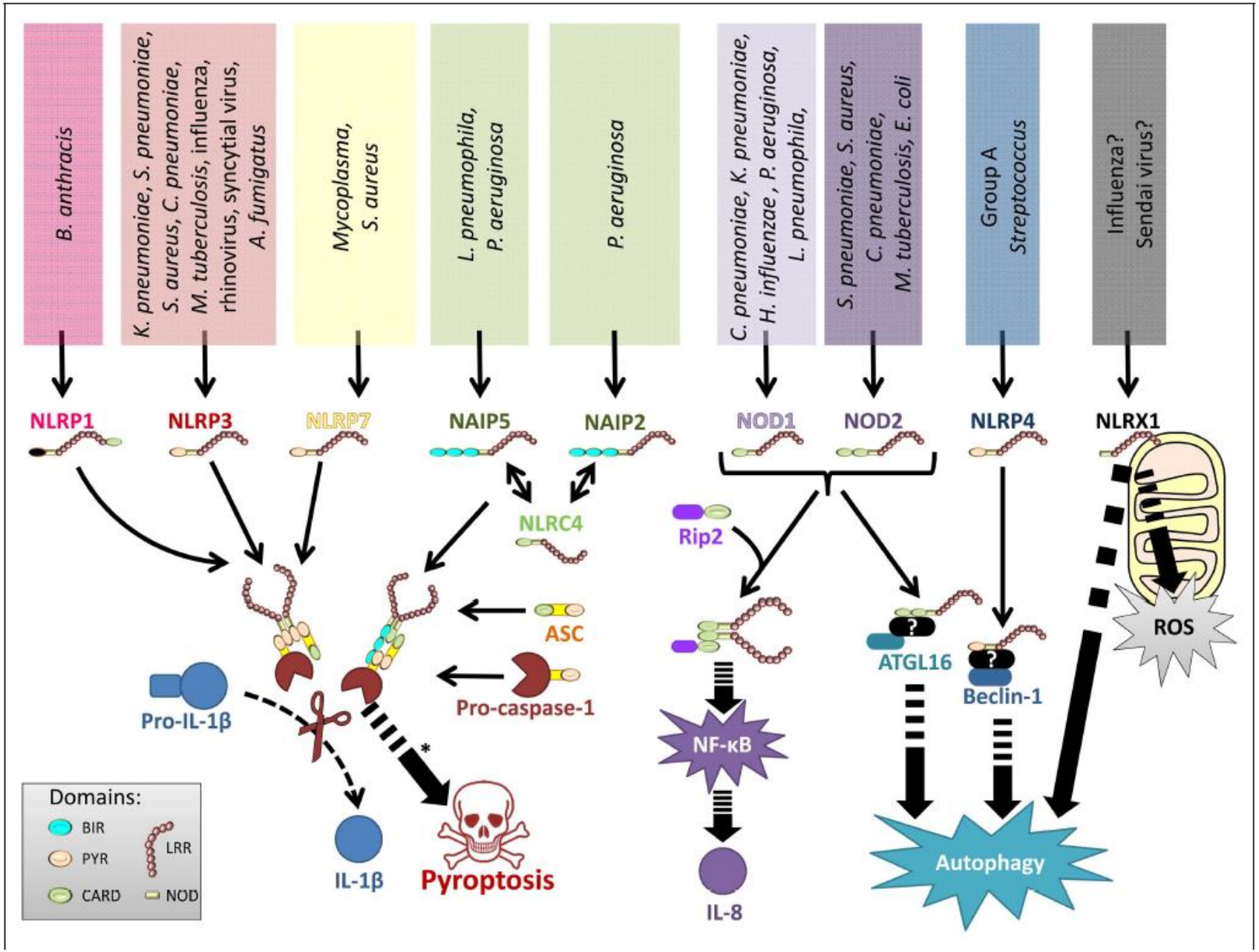


Figure 2. Representation of NLRs involved in lung infections

Frontiers in Immunology 2013;4:393

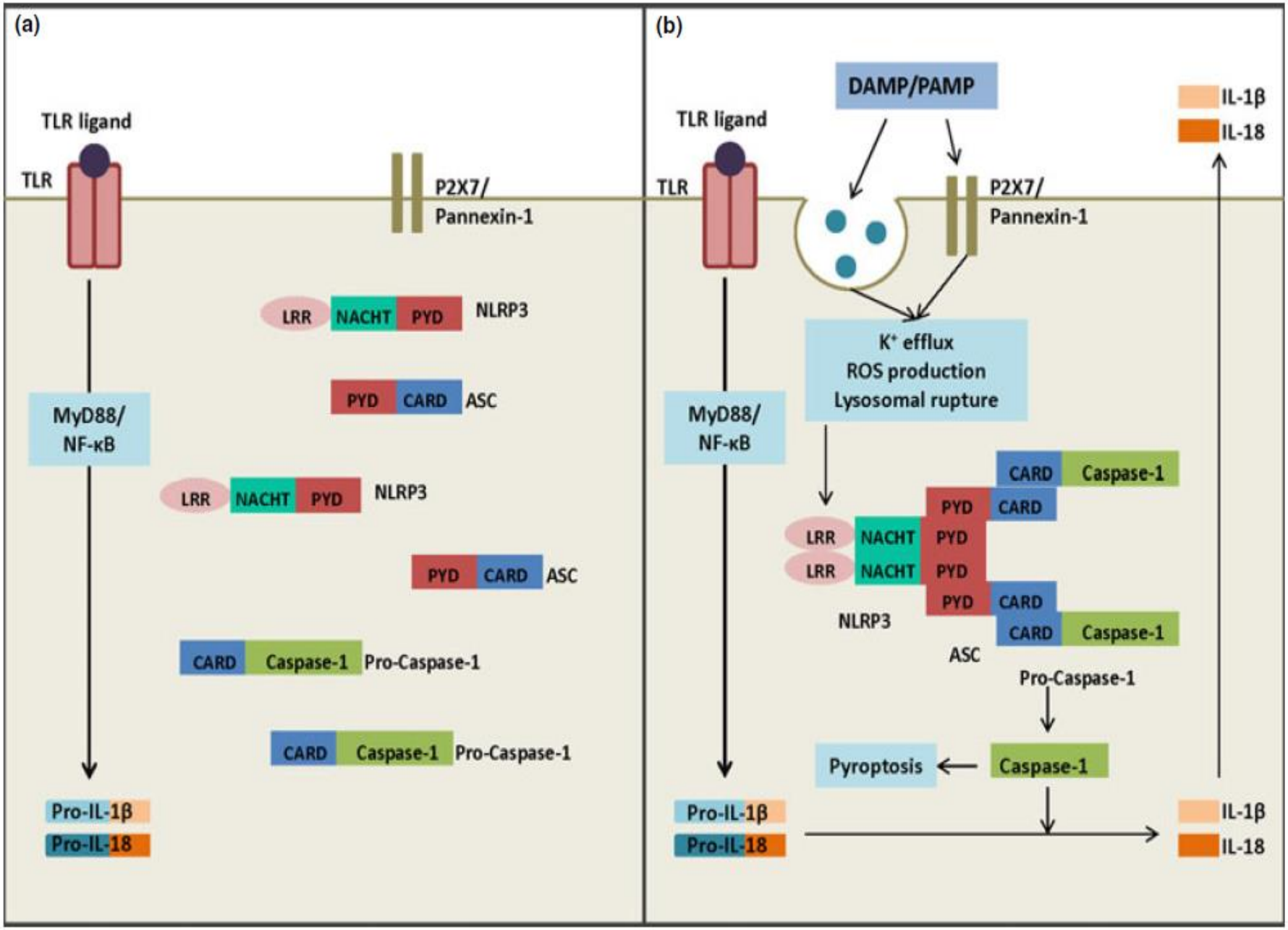


Fig. 2. NLRP3 inflammasome activation

Clin Exp Allergy 2014;44:160–172

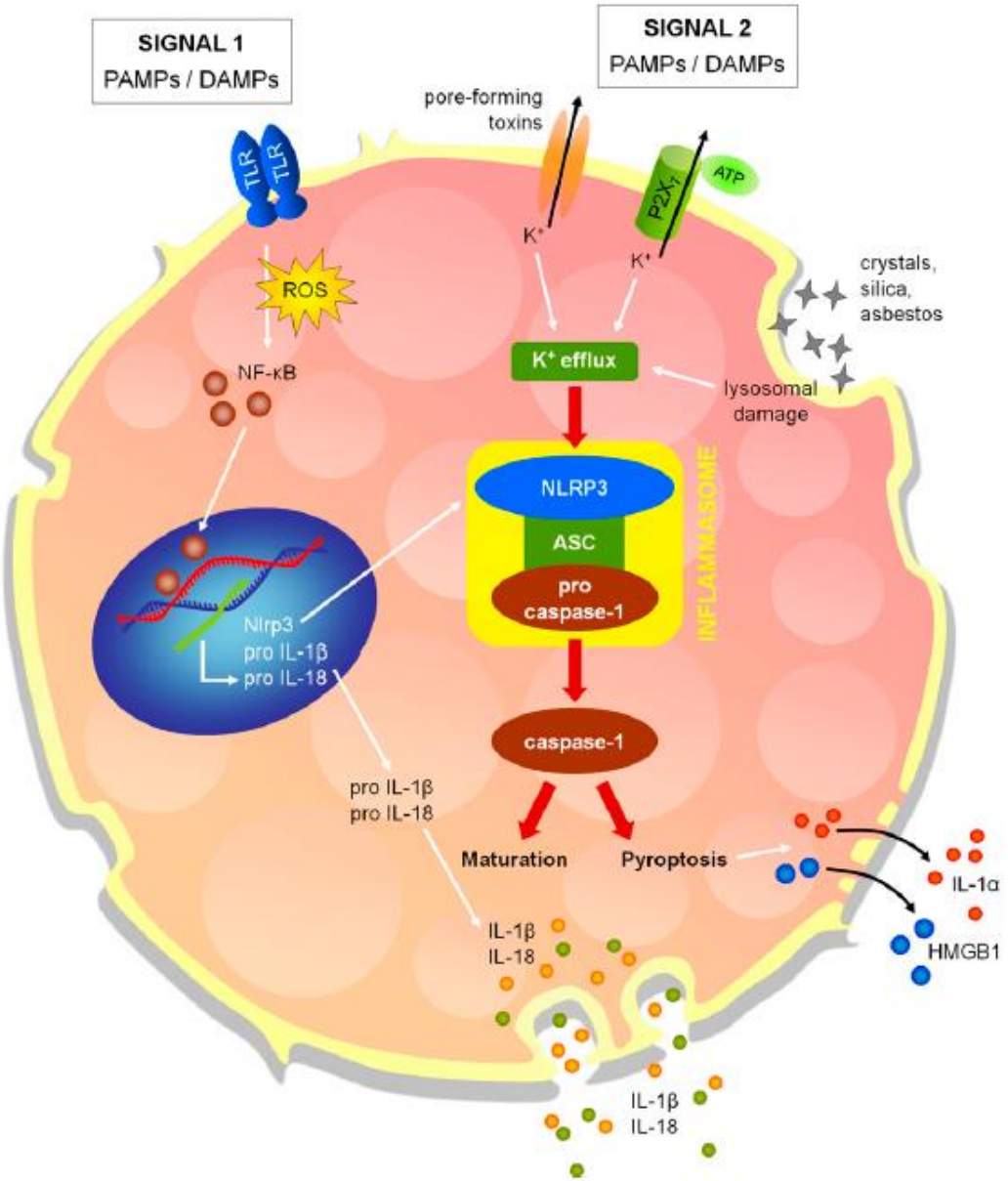


Figure 2. Two signals are needed to activate the NLRP3 inflammasome.

Chest 2014; 145 : 1121 – 1133

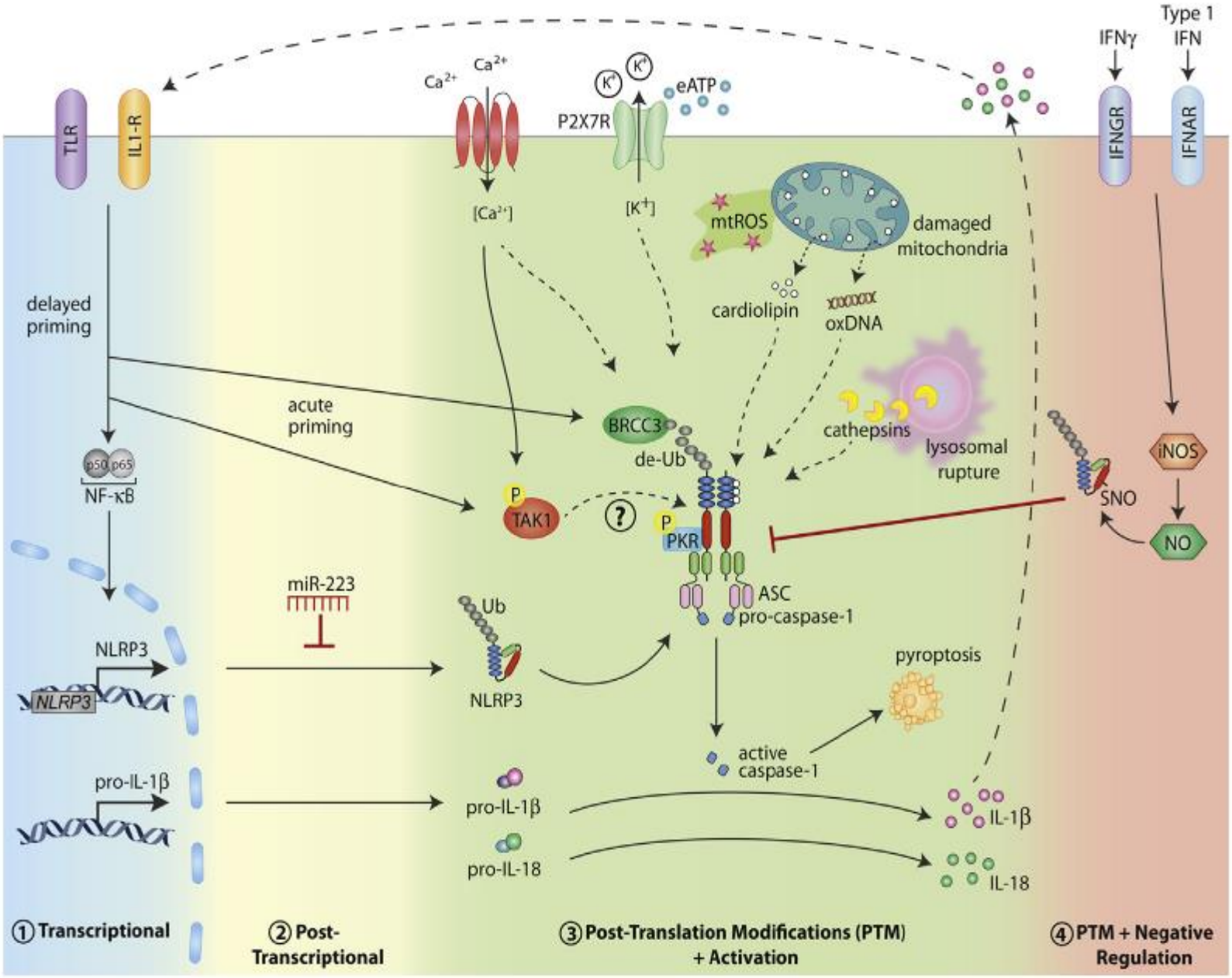


Figure 1 Multiple levels of NLRP3 inflammasome regulation.

Am J Pathol 2014; 184: 42-54

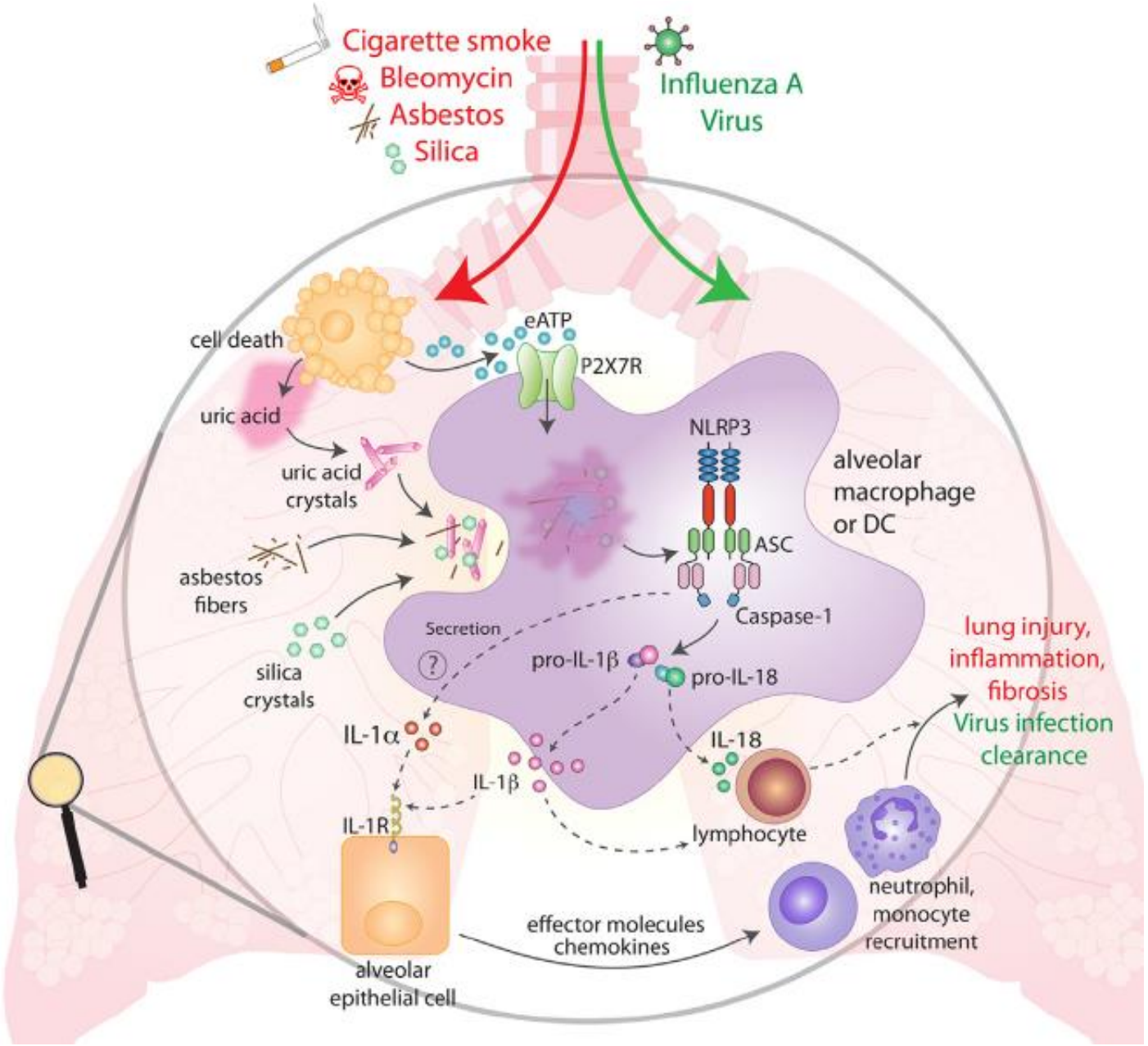


Figure 2 The NLR 3 inflammasome in lung inflammation and injury.

Am J Pathol 2014; 184: 42-54

천식에서 inflammasome

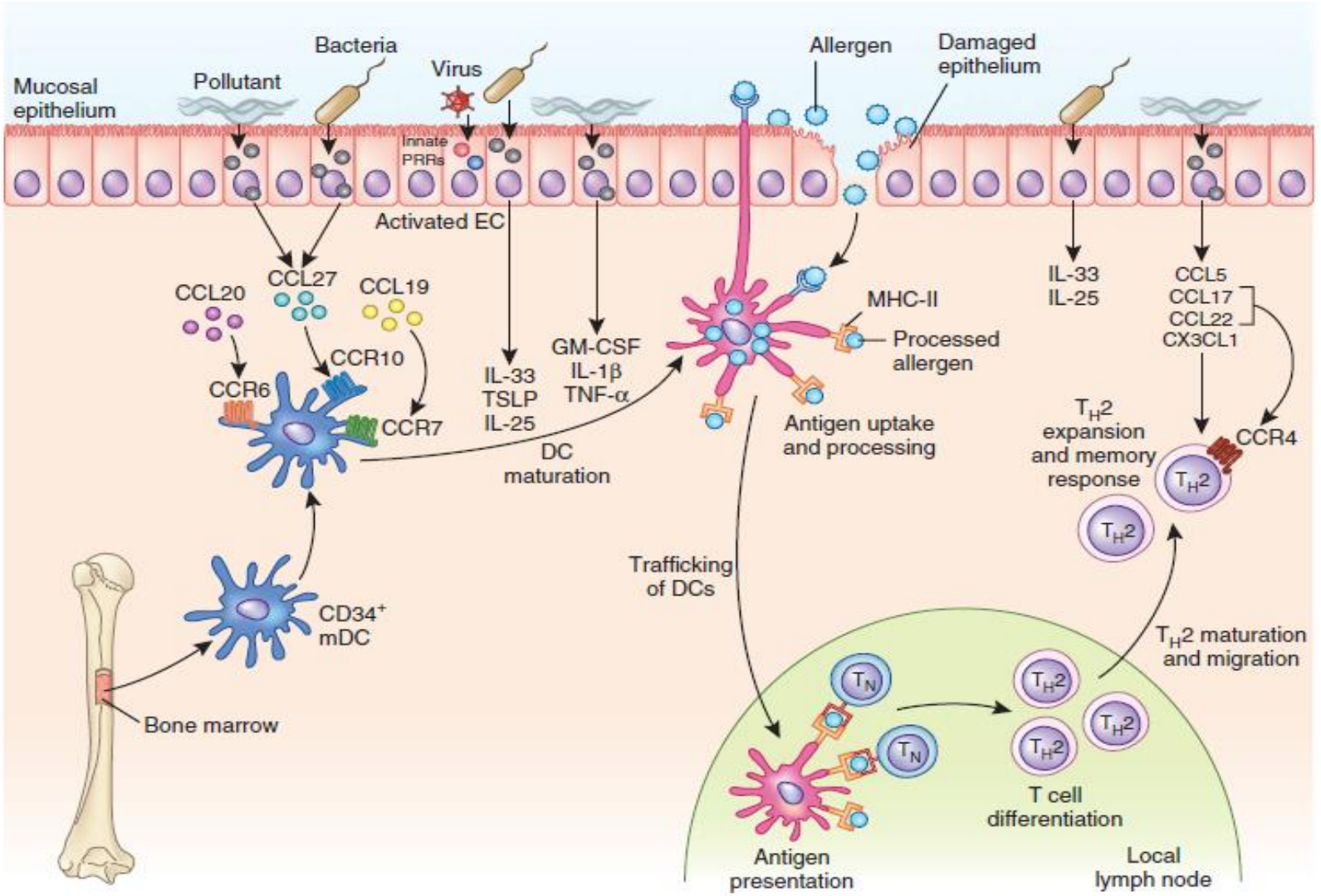


Figure 1 Primary sensitization of the airways in the induction of allergic-type asthma.

Nat Med 2012 18;672-683

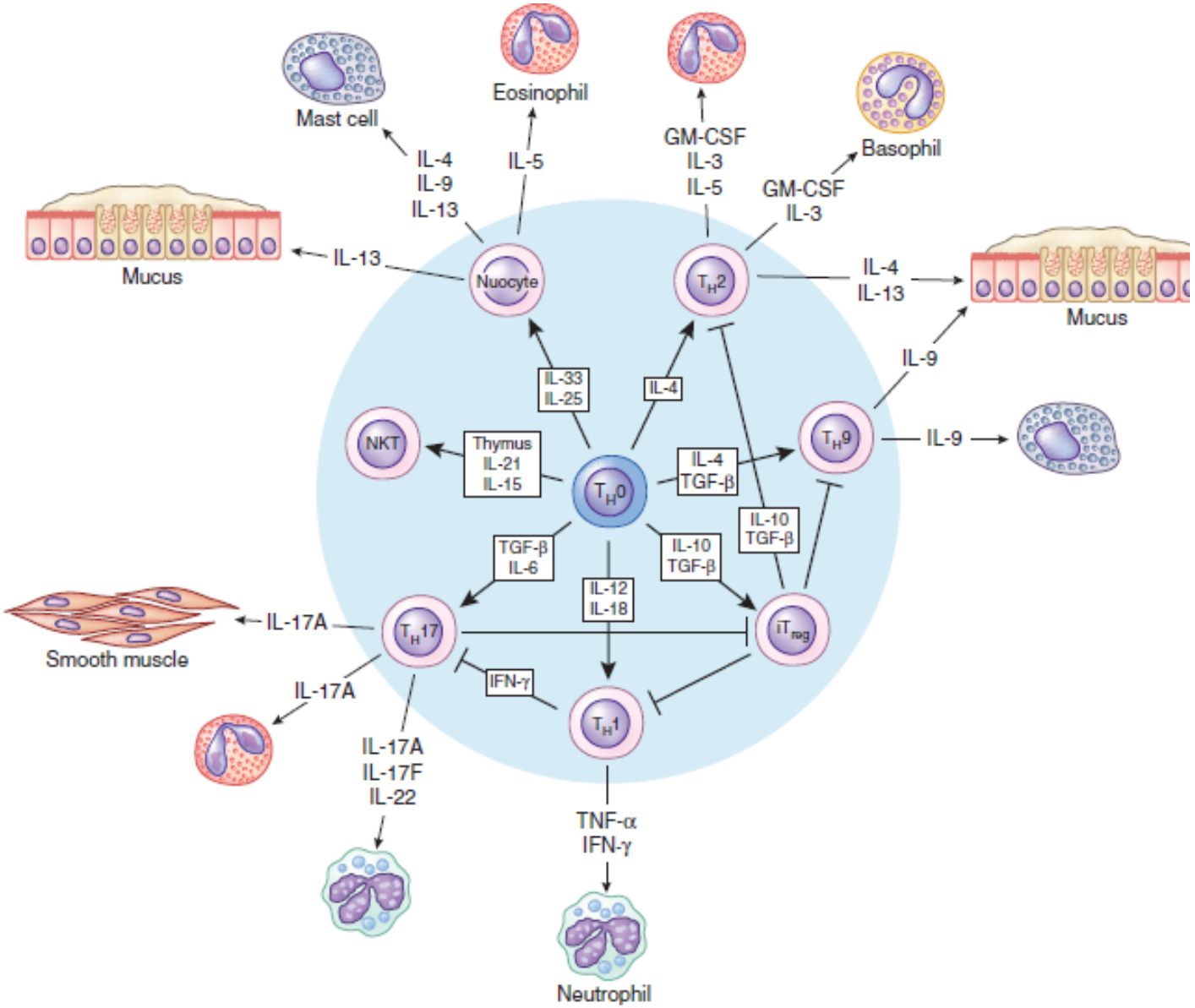
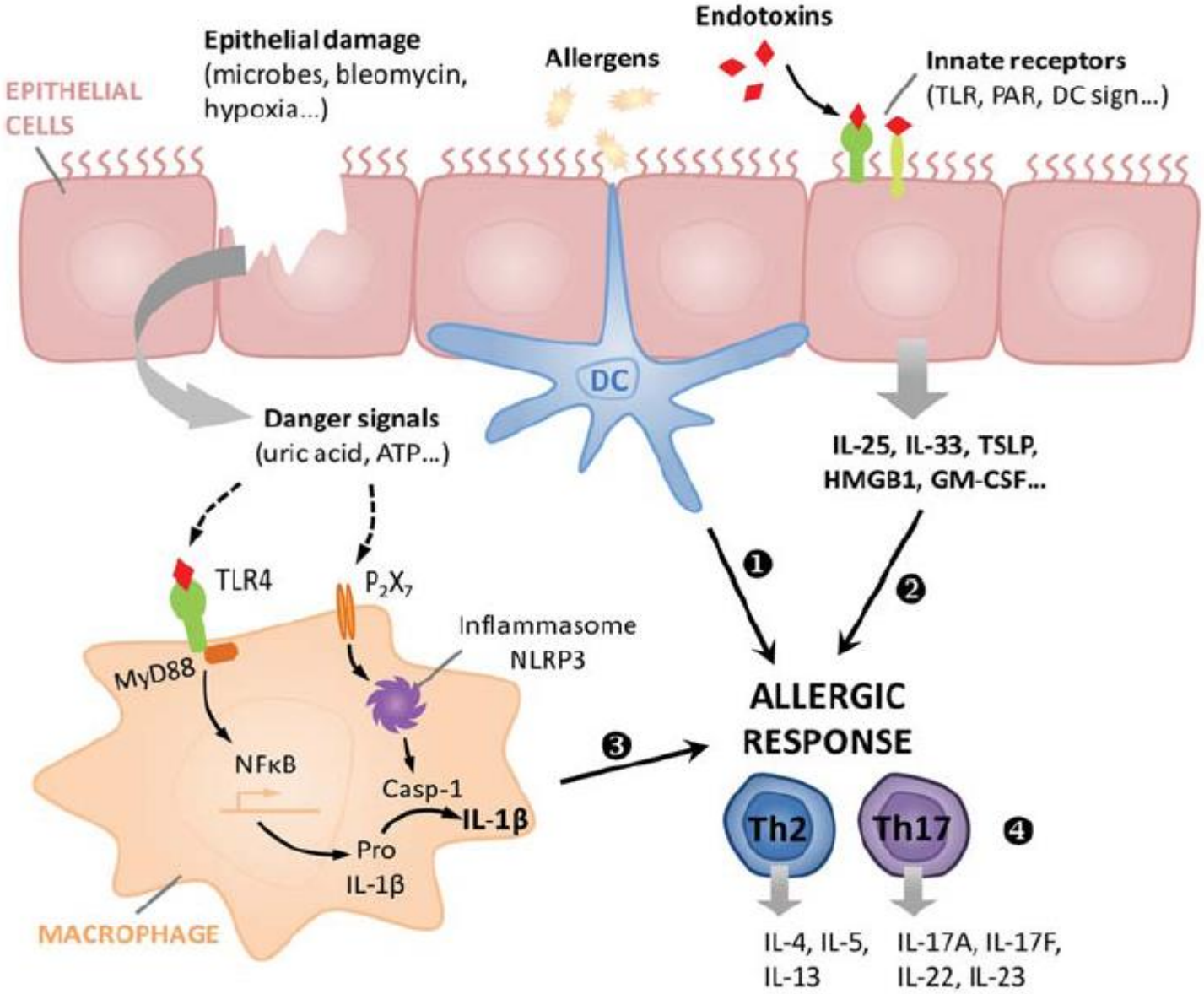


Figure 3. Different T cell subtypes involved in the pathogenesis of asthma and its differing endotypes.

Nat Med 2012 18;672-683



J Mol Cell Biol 2012; 4:3–10

Table 1. Innate immune system pattern recognition receptors (PRRs) in allergic diseases

PRRs
Toll-like receptors (TLR)
Nucleotide binding domain/lucine rich repeat receptors (NLR)
C-type lectin receptors (CLR)
Scavenger receptors
Adenosine receptors
Secreted PRRs
Complement system proteins
Collectins
Mucins
Pentraxins
Ficolins
Resolvins

Table 2. Innate immune cells in allergic diseases

Lung
Dendritic cells
Resident alveolar macrophages
Epithelial cells
Granulocytes
Mast cells
Basophils
Eosinophils
Polymorphonuclear neutrophils
NK, NKT, iNKT Cells
γδT cells
GI tract
Dendritic cells
Oral cavity dendritic cells
Epithelial cells
M cells
Eosinophils
Skin
Dendritic cells
Langerhans cells (skin)
Keratinocytes
Inflammatory mononuclear phagocytes

Immunol Rev 2011;242:106-27

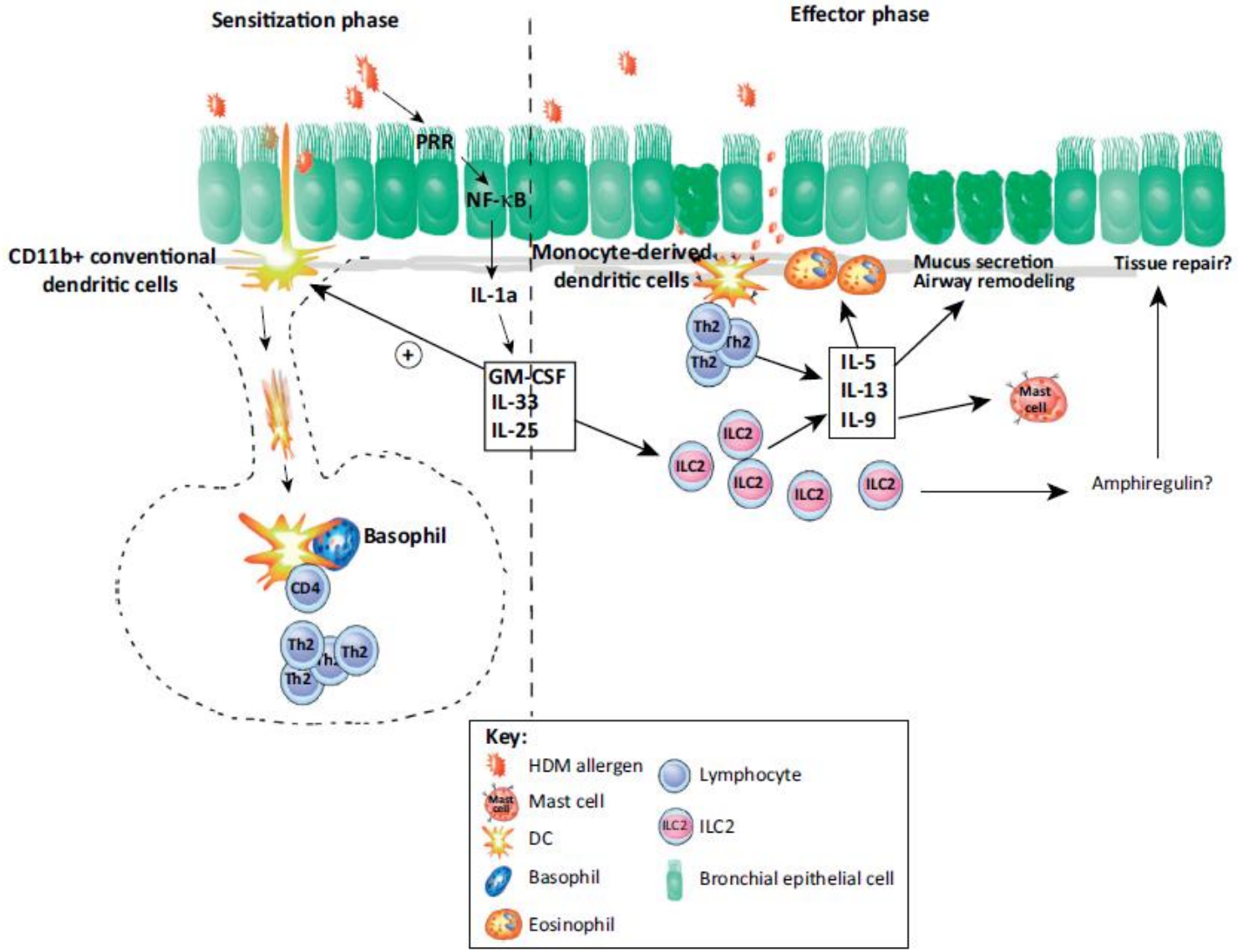


Figure 1. Current model for T helper (Th)2 sensitization to inhaled allergens.

Trends Immunol 2013;34:540-7.

Influenza enhances caspase-1 in bronchial epithelial cells from asthmatics and is associated with pathogenesis

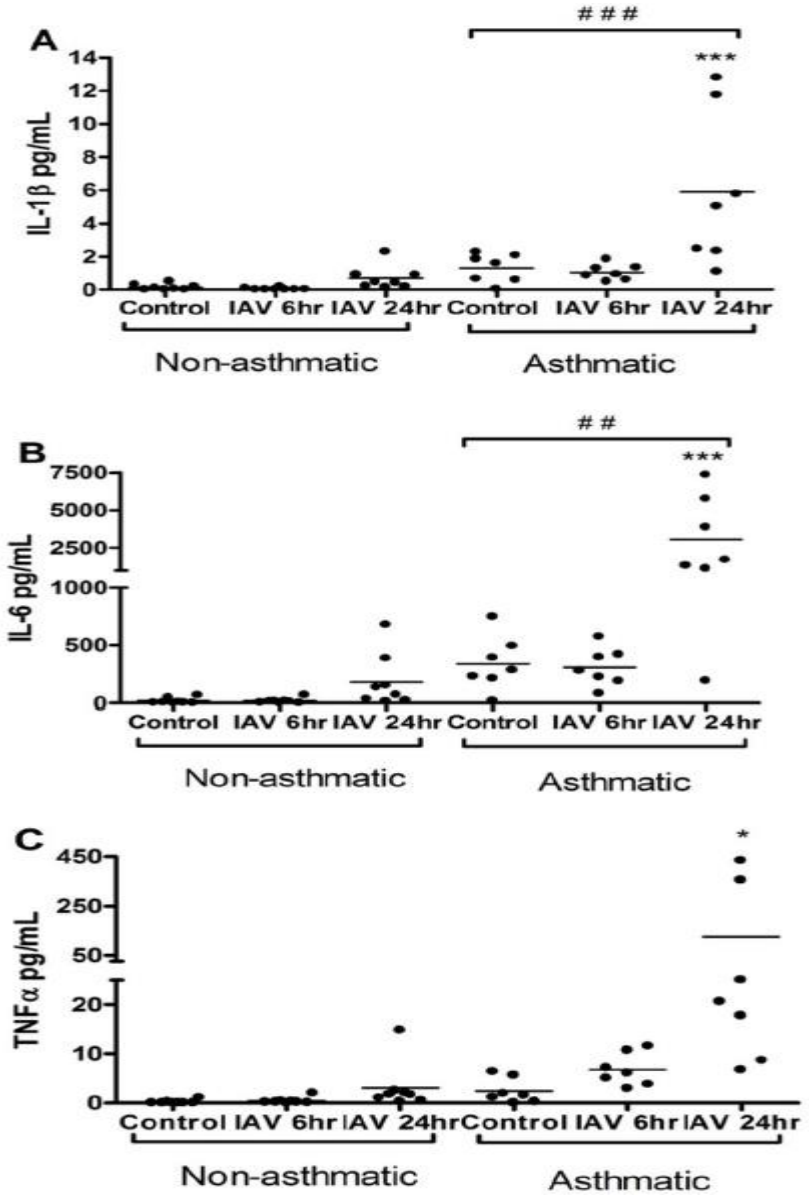


Figure 1.
HBEC from asthmatics have enhanced production of pro-inflammatory cytokines in response to IAV infection. Apical washes from HBEC from asthmatics (n=7) and nonasthmatics (n=8) were collected 6 or 24 hours post-IAV infection or 24 hours HBSS control treatment and analyzed for (A) IL-1β; (B) IL-6; and (C) TNFα concentrations by ELISA.

J Allergy Clin Immunol. 2012; 130: 958–967.

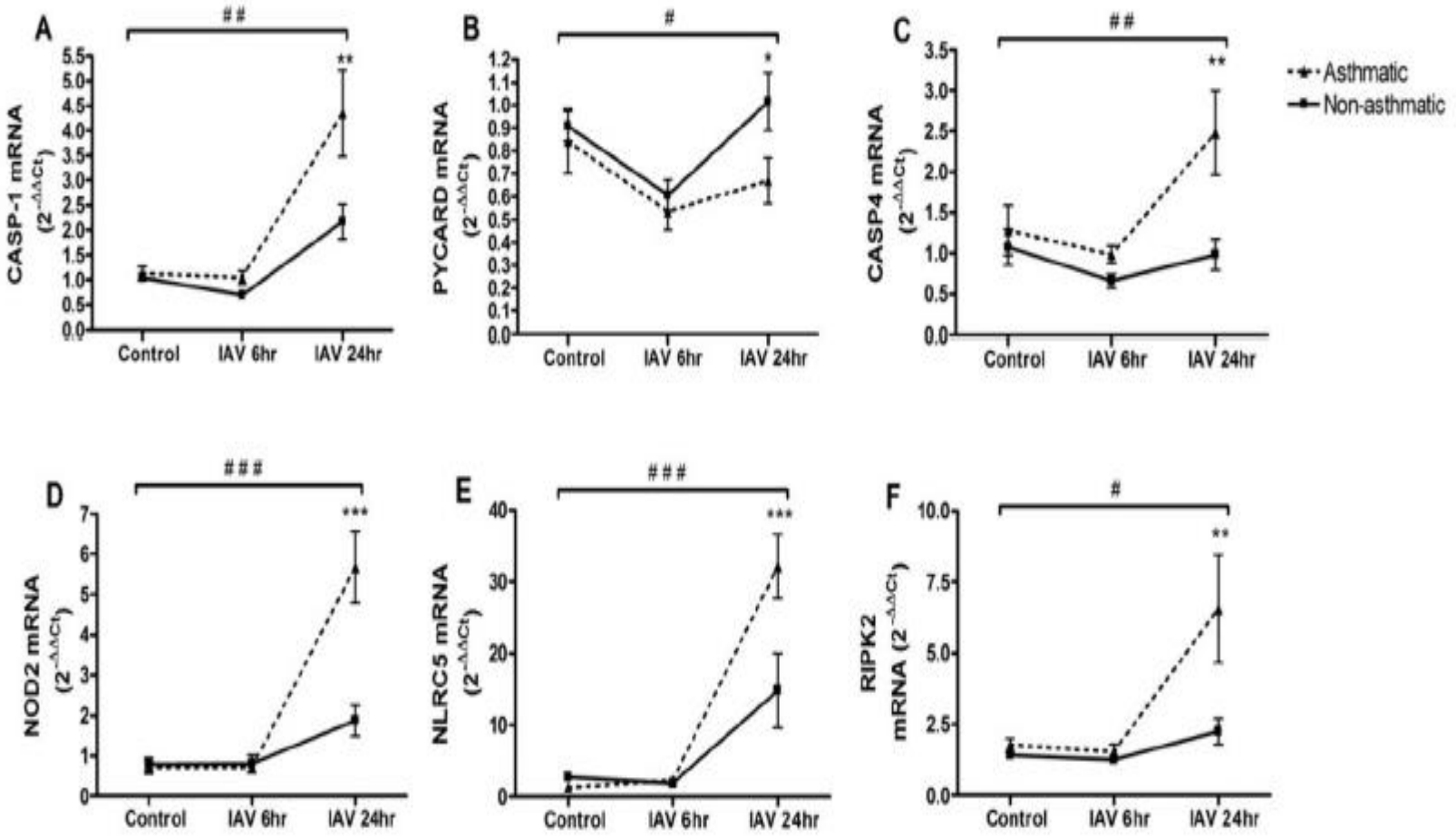


Figure 2. Gene- specific qRT-PCR confirmation of inflammasome-related gene expression. Total RNA from HBEC from asthmatics (n=7) and non-asthmatics (n=8) at 6 or 24 hours post-IAV infection or 24 hours HBSS control treatment was analyzed for expression of (A) CASP1; (B) PYCARD; (C) CASP4; (D) NOD2; (E) NLRC5; and (F) RIPK2 by qRT-PCR. Ct values were normalized to β-actin.

J Allergy Clin Immunol. 2012; 130: 958–967.

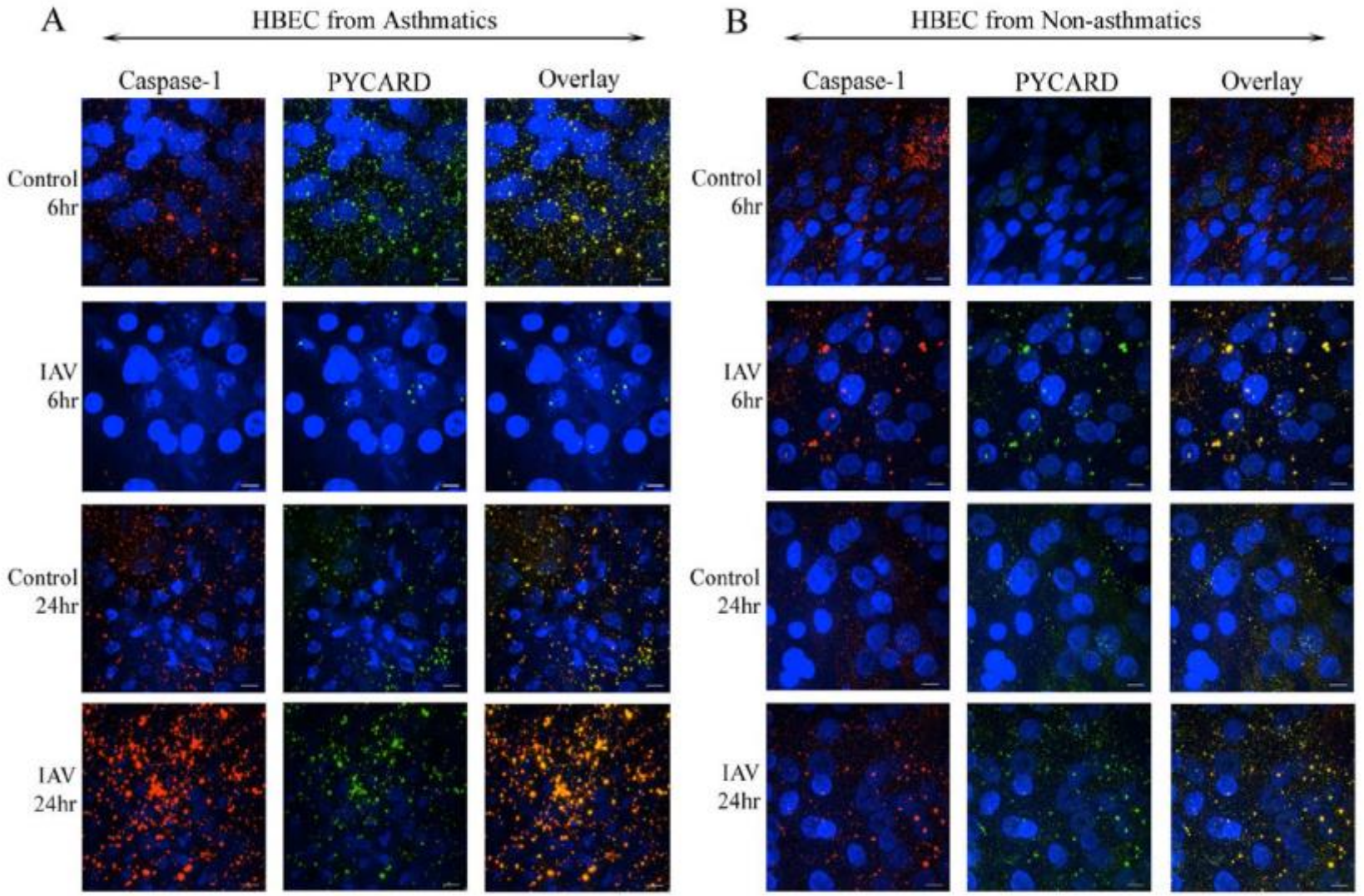


Figure 3. Caspase-1 and PYCARD co-localize with IAV-infection. HBEC from (A) asthmatics and (B) non-asthmatics 6 or 24 hours post-IAV infection or HBSS control treatment were probed for Caspase-1 (red) and PYCARD (green). DAPI stain identified nucleic acid (blue). Images are representative of 3 asthmatic and 3 non-asthmatic isolates. White bars=10μm.

J Allergy Clin Immunol. 2012; 130: 958–967.

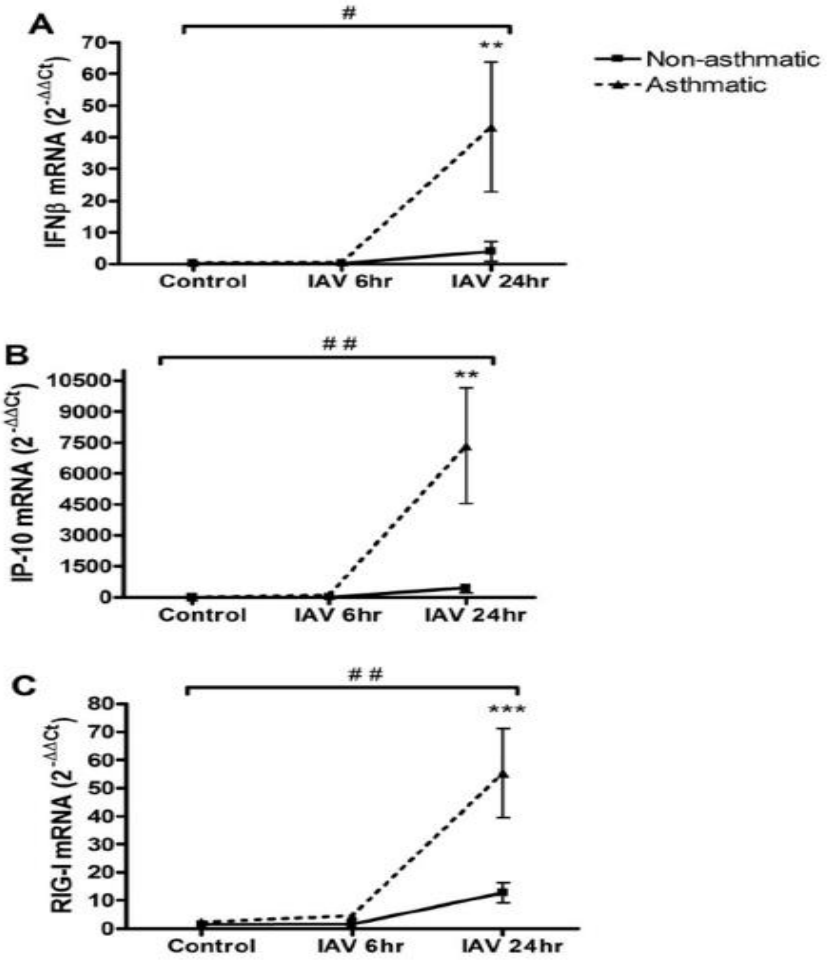


Figure 4. HBEC from asthmatics have enhanced expression of antiviral genes. Total RNA from HBEC from asthmatics (n=7) and non-asthmatics (n=8) at 6 or 24 hours post-IAV infection or 24 hours HBSS control treatment was analyzed for expression of (A) IFNβ; (B) IP-10; and (C) RIG-I by qRT-PCR. Ct values were normalized to β-actin.

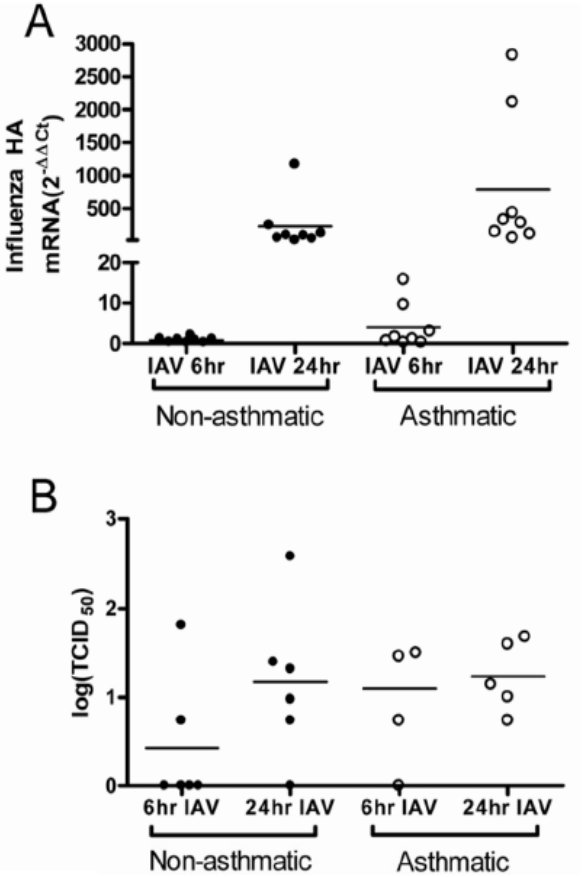


Figure 5. Viral replication is not significantly increased in HBEC from asthmatics. (A) Total RNA from IAV-infected HBEC from asthmatics (n=7) and non-asthmatics (n=8) were analyzed for IAV hemagglutinin (HA) RNA by qRT-PCR. Ct values were normalized to β-actin. (B) Vial titer of apical washes from HBEC from asthmatics (n=4–5) and non-asthmatics (n=6) at 6 and 24 hours post-IAV infection.

J Allergy Clin Immunol. 2012; 130: 958–967.

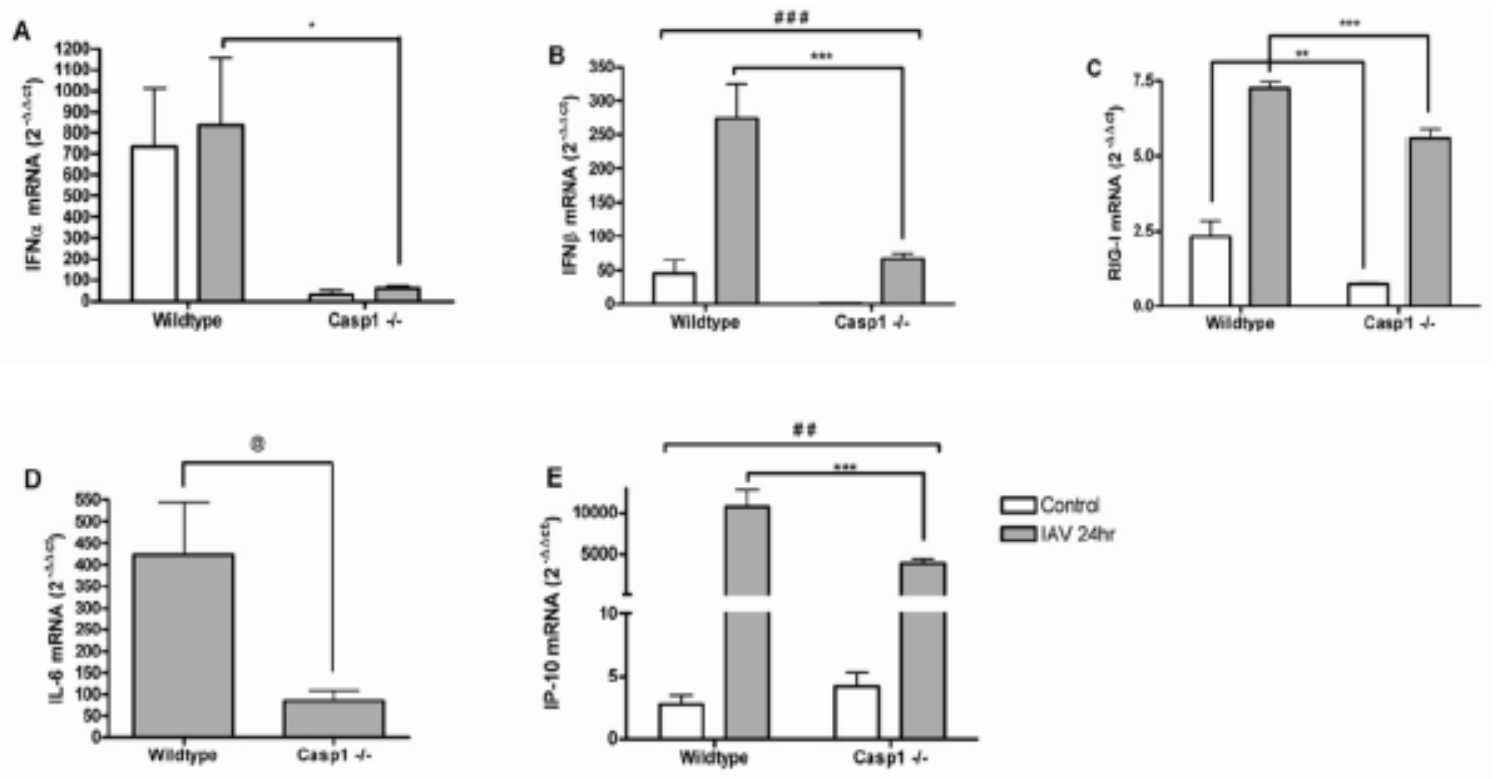


Figure 6. Casp1 −/− MTEC have diminished antiviral gene expression in response to IAV infection.RNA from wildtype (n=8) and Casp1 −/− (n=9) MTEC 24 hours post-IAV infection or control were analyzed for expression of (A) IFNα; (B) IFNβ; (C) RIG-I; (D) IL-6; (E) IP-10 by qRT-PCR. Ct values were normalized to β-actin. @ $p<0.05$ Student's T-test Casp1 −/−vs. wildtype, 24hrs post-IAV infection only; * $p<0.05$, ** $p<0.01$; *** $p<0.001$ ANOVA and Tukey post-hoc test, Casp1 −/− vs. wildtype 24hrs post-IAV and control infection; ## $p<0.01$, ### $p<0.001$ Factorial ANOVA, interaction between genotype and infection.

J Allergy Clin Immunol. 2012; 130: 958–967.

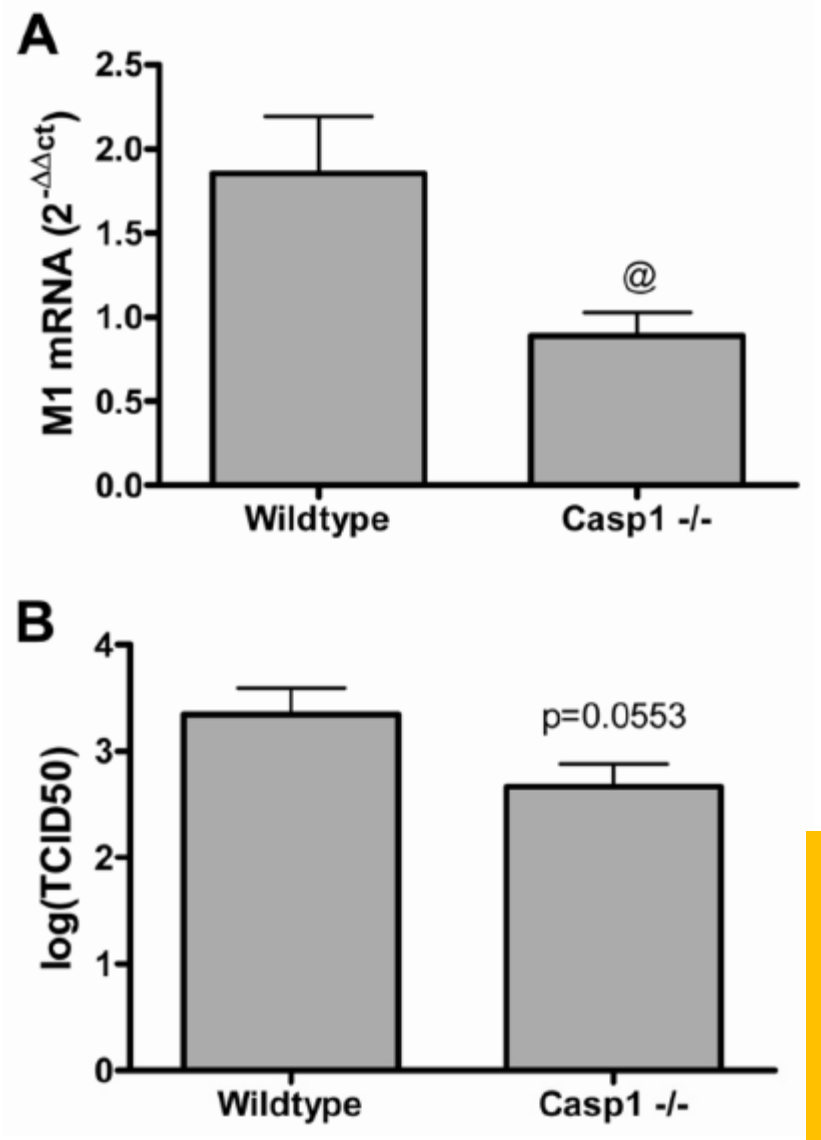


Figure 7. CASP1 −/− MTEC have diminished viral replication. (A) Total RNA from Casp1 −/− (n=9) and wildtype (n=8) MTEC 24 hours post-IAV infection or media control treatment were analyzed for IAV matrix 1 (M1) RNA by qRT-PCR. Ct values were normalized to β-actin. (B) Vial titer was assessed using media supernatents from Casp1 −/− (n=9) and wildtype (n=8) MTEC 24 hours post-IAV infection.

Conclusion:Caspase-1 plays an important role in the airway epithelial cell response to influenza infection, which is enhanced in asthmatics and may contribute to the enhanced influenza related pathogenesis observed in vivo.

J Allergy Clin Immunol. 2012; 130: 958–967.

Elevated expression of the NLRP3 inflammasome in neutrophilic asthma

TABLE 1 Participant demographics and sputum inflammatory cell counts for participants with eosinophilic, neutrophilic or paucigranulocytic asthma and healthy controls

	Eosinophilic asthma	Neutrophilic asthma	Paucigranulocytic asthma	Healthy controls	p-value[#]
Subjects n	30	20	35	27	
Age mean (range)	53 (23–82)[¶]	64 (43–76)[¶]	60 (31–80)[¶]	41 (20–69)	<0.001
Sex male/female n	17/13	10/10	21/14	12/15	0.647
Ex-smoker n (%)	10 (33)	8 (40)	14 (40)	3 (11)	0.052
Smoking history pack-years	0.5 (0.3–1.0)	5.0 (2.7–14.6)[+]	7.0 (2.0–10.5)[+]	0.1 (1.1–2.9)	0.004
FEV1 % pred	75±20[¶]	62±17[¶]	74±20[¶]	104±12	<0.001
FEV1/FVC %	68±11[¶]	59±11[¶,+,§]	68±11[¶]	82±7	<0.001
ICS dose	1000 (1000–2000)	2000 (800–2000)	2000 (1000–2000)		0.531
Total cells $\times 10^6$ per mL	2.97 (1.53–5.31)	9.63 (4.73–14.63) [¶,§,+]	2.88 (1.89–4.59)	2.30 (1.53–4.46)	<0.001
Viability %	74 (65–84)	91 (83–94) [¶,§,+]	71 (55–81)	67 (58–81)	<0.001
Neutrophils %	27.1 (18.3–36.3)	81.6 (68.8–90.1)[¶,+,§]	25.5 (15.3–39.0)	25.6 (13.3–41.8)	<0.001
Neutrophils $\times 10^4$ per mL	89.9 (38.9–130)	815 (385–1218)[¶,+,§]	63.8 (34.7–176)	53.4 (22.4–131)	<0.001
Eosinophils %	7.9 (4.3–17)[¶,§]	0.4 (0.1–1.0)[+]	0.5 (0.0–1.0)	0.3 (0–0.5)	<0.001
Eosinophils $\times 10^4$ per mL	20.4 (8.4–68.7)[¶,§]	2.6 (0.3–5.7)[+]	0.8 (0.0–3.8)	0.6 (0.0–1.8)	<0.001
Macrophages %	52.0 (41.3–69.5)	15.3 (8.6–22.2)[¶,+,§]	66.7 (48.0–76.3)	64.8 (47.8–75.5)	<0.001
Macrophages $\times 10^4$ per mL	172 (68.0–281)	108 (67.5–228)	164 (119–296)	131 (81.9–268)	0.137
Lymphocytes %	0.5 (0.3–1.3)	0.5 (0.0–1.0)[¶]	0.9 (0.0–1.5)	1.8 (0.5–3.3)	0.035
Lymphocytes $\times 10^4$ per mL	1.6 (0.7–4.3)	2.7 (0.0–7.4)	2.4 (0.0–5.5)	3.2 (1.3–8.4)	0.465
Columnar epithelial cells %	3.1 (0.8–5.8)	1.4 (0.0–3.4)	4.8 (1.0–6.5)	2.8 (1.0–12.8)	0.093
Columnar epithelial cells $\times 10^4$ per mL	6.7 (3.9–17.3)	8.2 (0.0–21.9)	2.7 (0.0–8.3)	4.5 (2.4–11.3)	0.533
Squamous %	3.9 (1.5–7.8)	1.4 (0.6–2.9)[#,+]	2.9 (1.0–11.9)	5.7 (2.0–8.5)	0. 041

Data are presented as median (interquartile range) or mean±SD, unless otherwise stated. FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; ICS: inhaled corticosteroid. [#]: for four-group comparison; [¶]: $p<0.008$ *versus* healthy controls; [+]: $p<0.008$ *versus* eosinophilic asthma; [§]: $p<0.008$ *versus* paucigranulocytic asthma.

Eur Respir J 2014; 43: 1067–1076

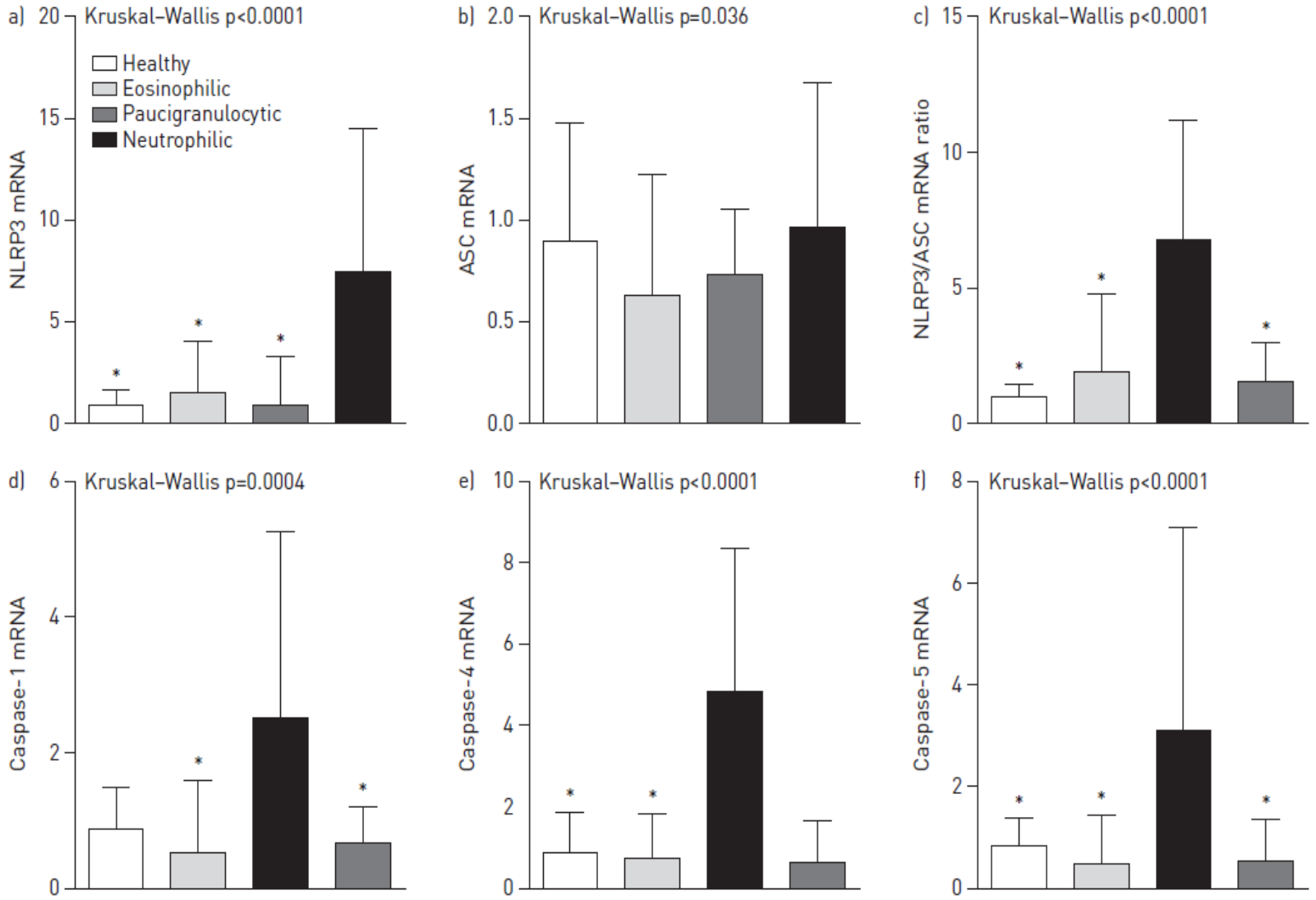


FIGURE 1 a) Nucleotide-binding domain, leucine-rich repeat-containing family protein (NLRP)3, b) apoptosis-associated speck-like protein containing a caspase-recruitment domain (ASC), c) NLRP3/ASC ratio, d) caspase-1, e) caspase-4 and f) caspase-5 gene expression for healthy controls, eosinophilic asthma, neutrophilic asthma and paucigranulocytic asthma. Bars represent median and error bars show the third quartile. *: $p<0.05$ *versus* neutrophilic.

Eur Respir J 2014; 43: 1067–1076

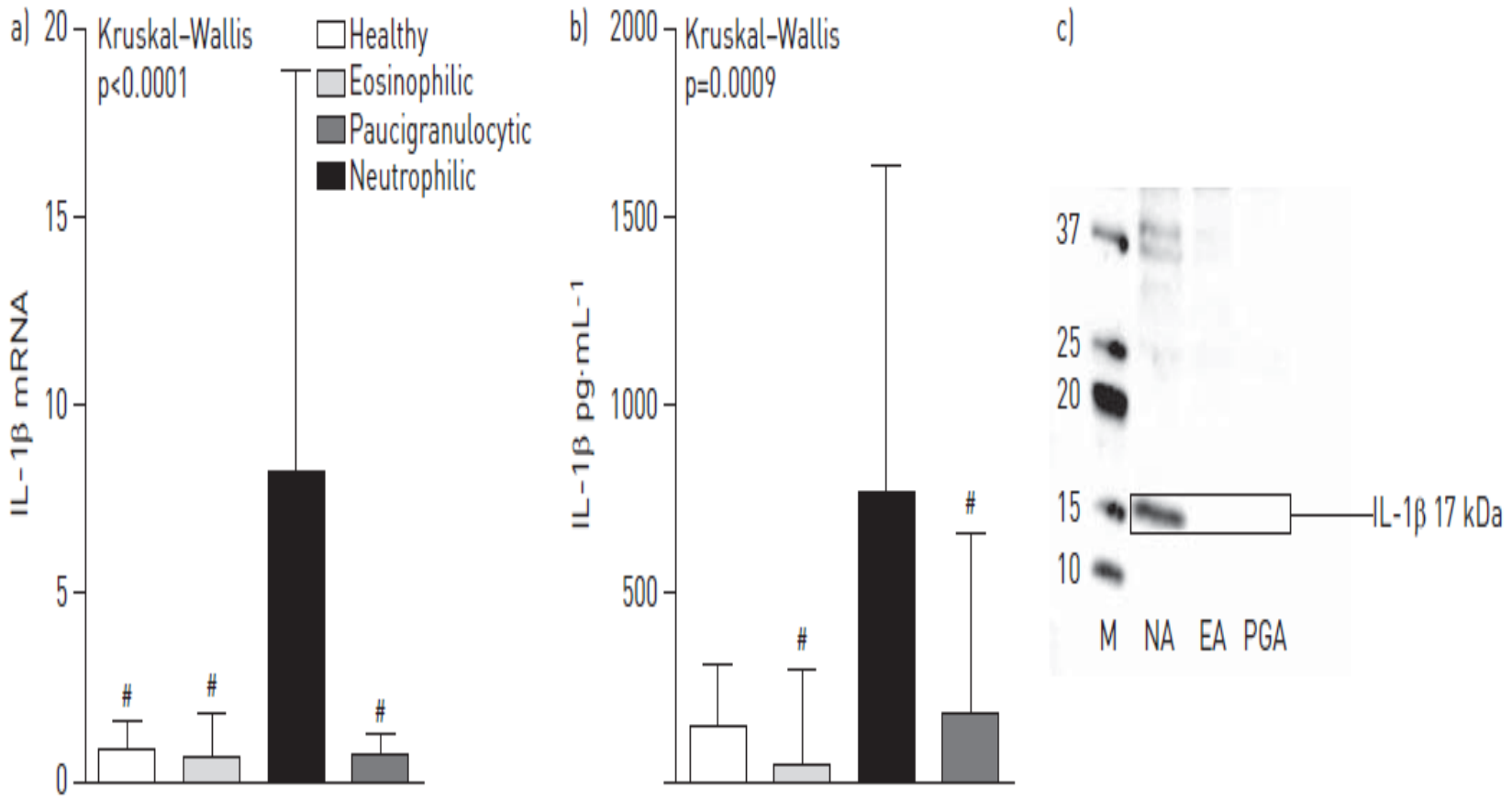


FIGURE 2 a) Sputum interleukin (IL)-1β gene expression and b) sputum IL-1β protein levels for healthy controls, eosinophilic asthma, neutrophilic asthma and paucigranulocytic asthma. Bars represent median and error bars show the third quartile. c) Representative Western blot image showing 17-kDa mature IL-1β observed in neutrophilic asthma (NA). M: molecular weight marker; EA: eosinophilic asthma; PGA: paucigranulocytic asthma. #: $p<0.008$ *versus* neutrophilic.

Eur Respir J 2014; 43: 1067–1076

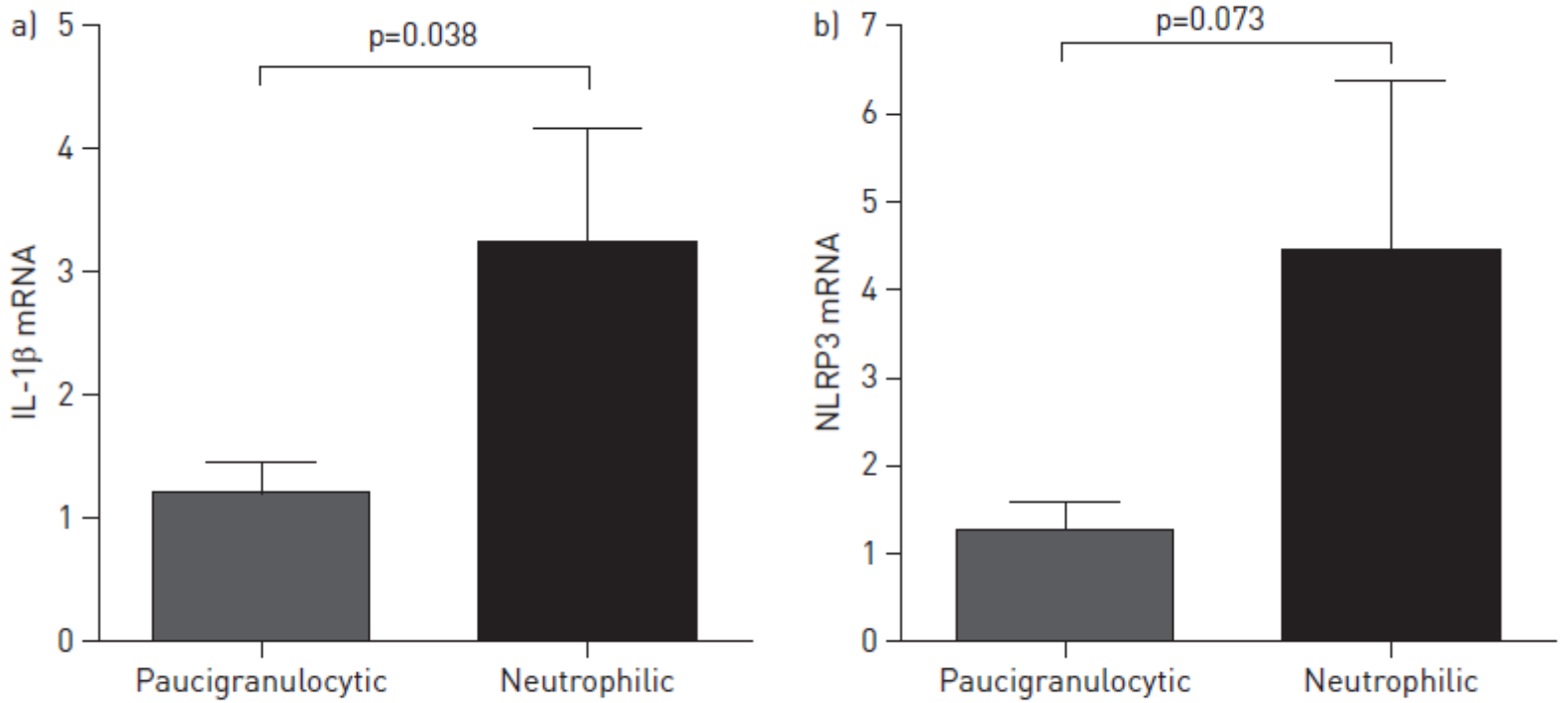


FIGURE 4 Gene expression of a) interleukin (IL)-1β and b) nucleotide-binding domain, leucine-rich repeat-containing family protein (NLRP)3 is increased in isolated sputum macrophages in neutrophilic asthma compared with paucigranulocytic asthma.

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TABLE 2 NOD2 and TLR gene expression and inflammatory protein expression for participants with eosinophilic, neutrophilic and paucigranulocytic asthma and healthy controls

	Eosinophilic asthma	Neutrophilic asthma	Paucigranulocytic asthma	Healthy controls	p-value[#]
Gene expression					
NOD2 mRNA	0.68 (0.37–1.52)	2.71 (0.92–6.87)[¶,+]	0.54 (0.35–1.56)	0.95 (0.53–1.28)	0.001
TLR2 mRNA	1.29 (0.55–3.52)	8.59 (4.46–17.61) [¶,+,§]	2.74 (0.67–7.57)	0.86 (0.39–2.40)	<0.001
TLR4 mRNA	0.78 (0.26–2.45)	2.48 (1.01–6.81)	1.19 (0.35–5.02)	0.71 (0.51–2.57)	0.070
Protein level					
IL-8 ng·mL^{-1}	5.0 (2.9–11.1)	17.2 (4.8–59.3)[+,§]	7.1 (2.7–16.9)	3.5 (1.8–6.0)	0.002
IL-8 samples n	30	18	35	24	
NE ng·mL^{-1}	414.6 (240–1086)	2995 (529–9188)[¶,+,§]	521.7 (189–845)	493 (110–678)	0.001
NE samples n	28	16	29	13	

Data are presented as median (interquartile range), unless otherwise stated. NOD: nucleotide-binding oligomerisation domain-containing protein; TLR: Toll-like receptor; IL: interleukin; NE: neutrophil elastase. [#]: for four-group comparison; [¶]: p<0.008 *versus* paucigranulocytic asthma; [+]: p<0.008 *versus* eosinophilic asthma; [§]: p<0.008 *versus* healthy controls.

TABLE 3 Multivariate linear regression outcomes with dependent variable sputum NLRP3 gene expression

Variable	Coefficient	SE	p-value	95% CI
Age	0.003	0.004	0.436	-0.005–0.012
Sex	0.031	0.102	0.761	-0.173–0.234
NOD2 mRNA	**0.147**	**0.0215**	**<0.001**	**0.104–0.190**
Neutrophils $\times 10^4$ per mL	**2.65×10^{-5}**	**1.32×10^{-5}**	**0.049**	**9.80×10^{-7}–5.30×10^{-5}**
IL-1β pg·mL^{-1}	3.28×10^{-6}	1.74×10^{-6}	0.064	-2.01×10^{-6}–6.75×10^{-6}
IL-8 ng·mL^{-1}	**-0.007**	**0.003**	**0.039**	**-0.013– $-0.3.5 \times 10^{-4}$**
Constant	-0.222	0.299	0.459	-0.819–0.375

NLRP: nucleotide-binding domain, leucine-rich repeat-containing family protein; NOD: nucleotide-binding oligomerisation domain-containing protein; IL: interleukin. Values in bold are statistically significant.

TABLE 4 Multivariate linear regression outcomes with dependent variable sputum supernatant interleukin (IL)-1β levels

Variable	Coefficient	SE	p-value	95% CI
Age	9.19×10^{-6}	0.006	0.988	-0.012–0.012
Sex	-0.0590	0.150	0.695	-0.358–0.240
TLR2 mRNA	0.016	0.009	0.075	-0.002–0.034
Caspase-1 mRNA	**-0.121**	**0.049**	**0.017**	**-0.220– -0.023**
IL-1β mRNA	**0.018**	**0.006**	**0.006**	**0.005–0.030**
IL-8 ng·mL^{-1}	**0.016**	**0.002**	**<0.001**	**0.011–0.021**
Constant	0.323	0.452	0.478	-0.851–1.228

TLR: Toll-like receptor. Values in bold are statistically significant.

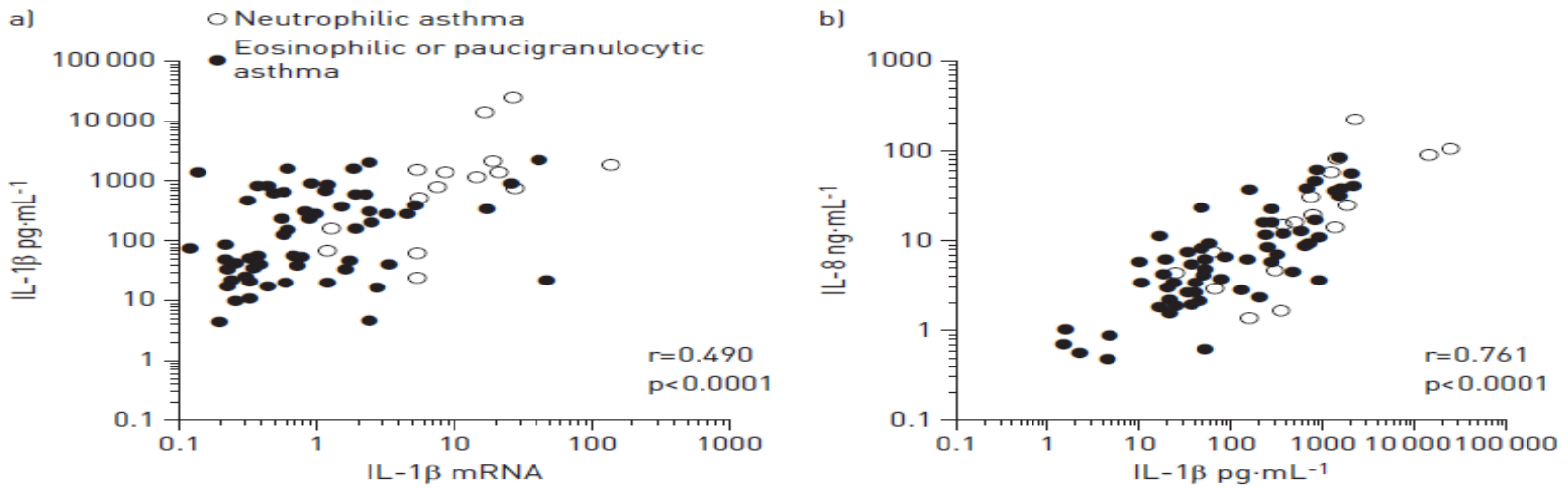

FIGURE 5 Scatter plots of independent predictors of sputum interleukin (IL)-1β protein levels as a) IL-1β mRNA and b) IL-8 protein.

In conclusion, we have shown the presence of increased gene expression for the NLRP3 inflammasome in mixed sputum cells from patients with neutrophilic asthma and have further demonstrated the presence of NLRP3 and caspase-1 protein in both sputum macrophages and neutrophils in neutrophilic asthma.

Eur Respir J 2014; 43: 1067–1076

NLRP3 inflammasome is required in murine asthma in the absence of aluminum adjuvant. A.-G. Besnard AG et al. Allergy 2011; 66: 1047–1057.

Hyaluronan Activation of the Nlrp3 Inflammasome Contributes to the Development of Airway Hyperresponsiveness. Feng F et al. Environ Health Perspect 2012;120:1692–1698.

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Serum Amyloid A Activates the NLRP3 Inflammasome and Promotes Th17 Allergic Asthma in Mice. Ather JL et al. J Immunol 2011; 187: 64–73.

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Interleukin-1 Receptor and Caspase-1 Are Required for the Th17 Response in **Nitrogen Dioxide**–Promoted Allergic Airway Disease. Martin RA et al. Am J Respir Cell Mol Biol 2013;48:655–664

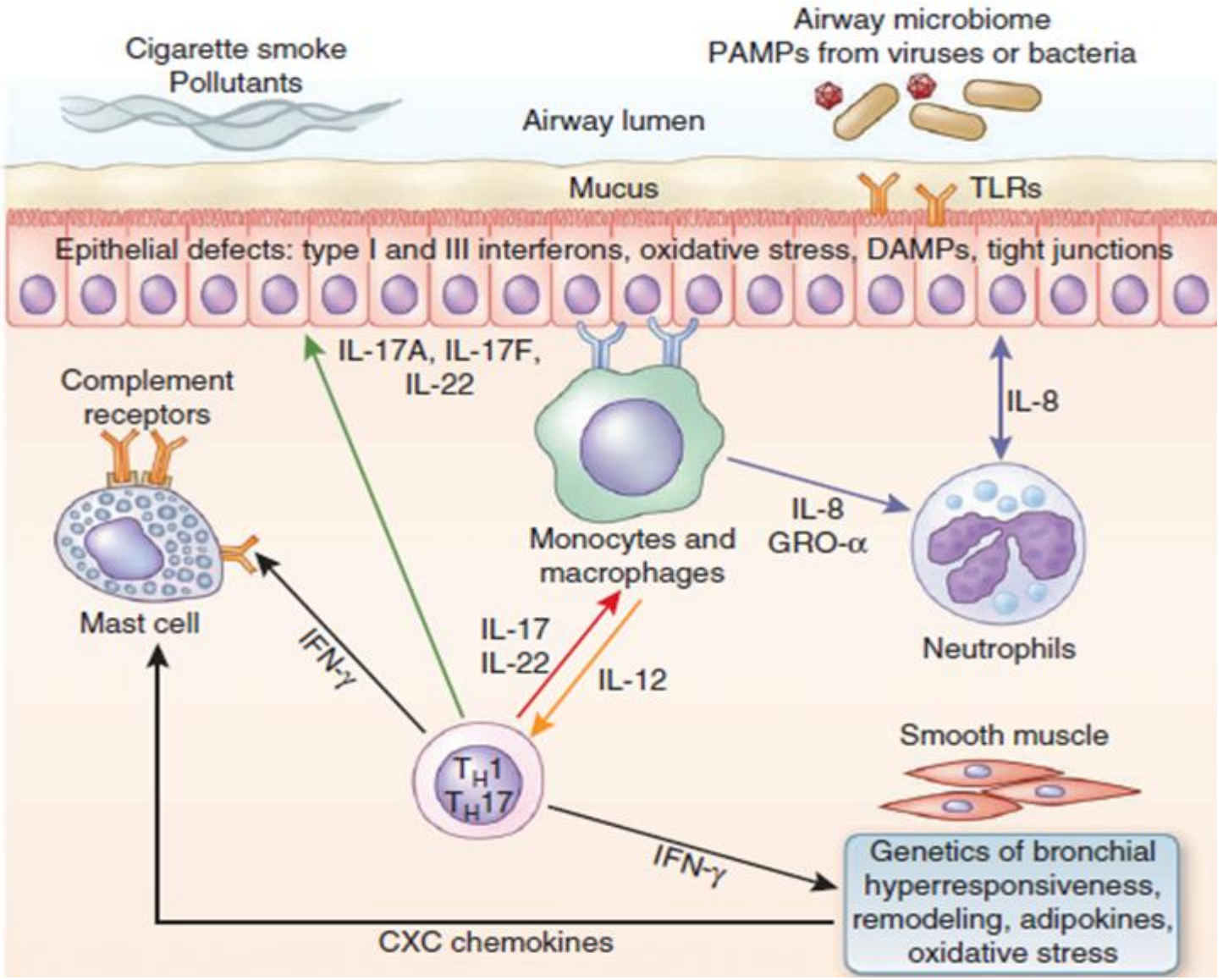
Rhinovirus-Induced Calcium Flux Triggers NLRP3 and NLRC5 Activation in Bronchial Cells. Triantafilou K et al. Am J Respir Cell Mol Biol 2013;49: 923–934

Asthma phenotypes

Table 1 Asthma phenotypes in relation to characteristics

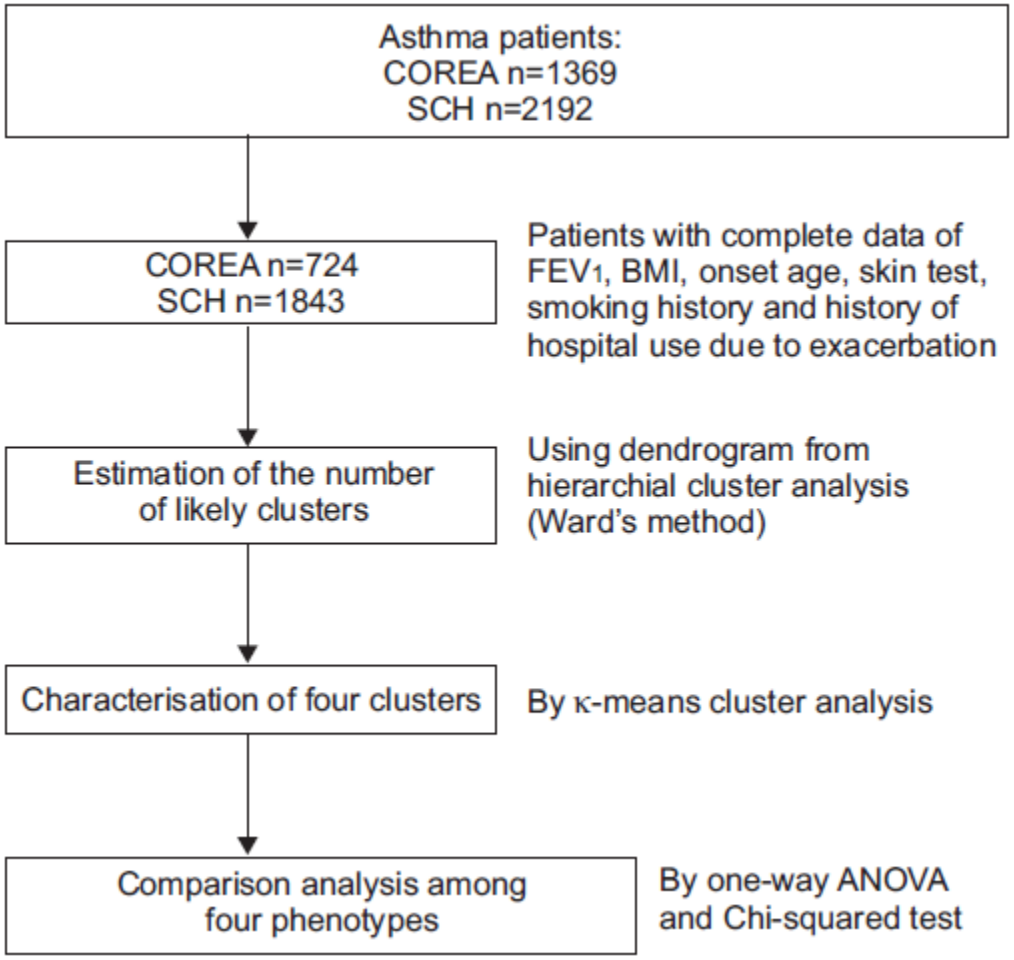
	Natural history	Clinical and physiological features	Pathobiology and biomarkers	Genetics	Response to therapy
Early-onset allergic	Early onset; mild to severe	Allergic symptoms and other diseases	Specific IgE; T_H2 cytokines; thick SBM	17q12; T_H2-related genes	Corticosteroid-responsive; T_H2-targeted
Late-onset eosinophilic	Adult onset; often severe	Sinusitis; less allergic	Corticosteroid-refractory eosinophilia; IL-5		Responsive to antibody to IL-5 and cysteinyl leukotriene modifiers; corticosteroid-refractory
Exercise-induced		Mild; intermittent with exercise	Mast-cell activation; T_H2 cytokines; cysteinyl leukotrienes		Responsive to cysteinyl leukotriene modifiers, beta agonists and antibody to IL-9
Obesity-related	Adult onset	Women are primarily affected; very symptomatic; airway hyperresponsiveness less clear	Lack of T_H2 biomarkers; oxidative stress		Responsive to weight loss, antioxidants and possibly to hormonal therapy
Neutrophilic		Low FEV1; more air trapping	Sputum neutrophilia; T_H17 pathways; IL-8		Possibly responsive to macrolide antibiotics

Nat Med 2012 ;18(5):716-25



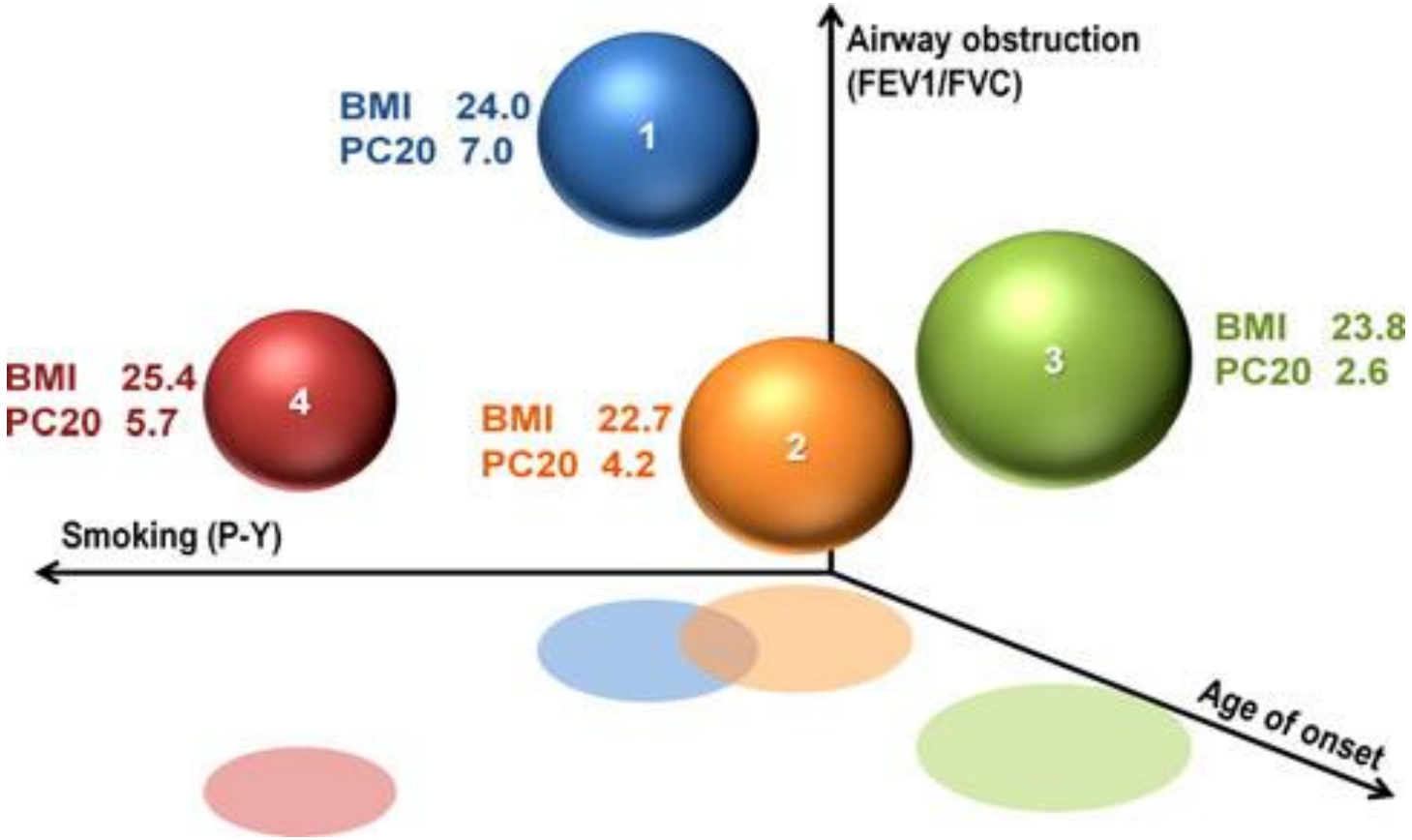
Nat Med 2012 ;18(5):716-25

Identification of asthma clusters in two independent Korean adult asthma cohorts



Eur Respir J 2013; 41: 1308–1314

Identification of Subtypes of Bronchial Asthma in Korean Patients by Cluster Analysis



Lung 2013;191:87–93

Table 1 Demographic and clinical characteristics of asthma patients with mild, moderate, and severe asthma and those with refractory asthma

	Mild persistent	Moderate persistent	Severe persistent	Refractory
Sex, M/F (*n*)	447/756	347/407	116/114	33/53
BMI (kg/mL)	24.4 ± 3.5	24.1 ± 3.6	24.0 ± 3.7	23.3 ± 3.7
Age of onset (years)	37.2 ± 17.0	39.8 ± 17.6	41.0 ± 17.3	39.9 ± 17.3
Symptom duration (years)	6.5 ± 8.8	7.7 ± 10.9*	10.2 ± 12.1*	11.7 ± 14.6*,‡
Atopy (% positive)	61.7	57.8	56.5	42*,‡,†
Smoking status [*n* (%)]				
Smoking	173 (14)	140 (19)	52 (23)	14(16)*
Ex-smoking	169 (14)	171 (22)	66 (29)	20(23)*
Nonsmoking	861 (72)	442 (59)	111 (48)	52(60)*
Cigarettes (pack years)	5.0 ± 11.2	9.7 ± 15.5*	13.8 ± 17.7*,‡	8.4 ± 15.6*,†
FEV_1 (%)	96.1 ± 12.0	66.2 ± 8.5*	38.5 ± 8.8*,‡	62.1 ± 22.2*,‡,†
FVC (%)	92.9 ± 12.9	73.6 ± 11.2*	54.8 ± 13.4*,‡	72.7 ± 20.5*,‡,†
FEV_1/FVC (%)	80.2 ± 8.1	70.8 ± 11.7*	55.5 ± 13.1*	63.8 ± 13.1*,‡
Log PC_{20} M (mg/mL)	0.38 ± 0.7	0.22 ± 0.7*	0.01 ± 0.8*	0.01 ± 0.8*
Log IgE (ng/mL)	2.13 ± 0.6	2.16 ± 0.7	2.20 ± 0.7*	2.22 ± 0.6†
Log blood eosinophil (/μL)	2.39 ± 0.42	2.46 ± 0.46*	2.51 ± 0.49	2.46 ± 0.53*
Blood eosinophil (%)	5.0 ± 4.8	5.6 ± 5.2*	5.6 ± 5.7*	5.9 ± 5.8*
Sputum analysis (*n*)	409	337	122	65
Total cells (× 10^6 cells/mL)	10.8 ± 30.2	14.0 ± 30.4	16.0 ± 36.8*	9.6 ± 20.6
Macrophage %	27.8 ± 31.3	19.2 ± 21.5*	14.1 ± 16.9*,‡	22.4 ± 35.5†
Neutrophil %	55.8 ± 29.2	64.9 ± 27.8	63.2 ± 28.3	61.2 ± 25.5*
Eosinophil %	9.8 ± 22.4	10.8 ± 20.0	14.5 ± 22.1*,‡	13.6 ± 18.6*
Lymphocyte %	1.5 ± 2.9	1.6 ± 3.1	1.2 ± 2.1	2.0 ± 4.0‡,†

Table 3 Pulmonary function and laboratory results of refractory asthma patients in each cluster

	Cluster 1 ($n = 18$)	Cluster 2 ($n = 21$)	Cluster 3 ($n = 35$)	Cluster 4 ($n = 12$)
PC_{20} (mg/mL)	7.0 ± 10.1	4.2 ± 7.6	2.6 ± 5.8	5.7 ± 9.1
PFT, at the time of enrollment				
FEV_1 % predicted	79.5 ± 24.3	48.6 ± 19.2*	61.4 ± 17.4*,‡	61.5 ± 21.1*
FVC % predicted	77.9 ± 22.8	69.2 ± 22.6	70.9 ± 16.4	75.9 ± 24.2
FEV1/FVC (%)	80.0 ± 6.8	52.3 ± 6.2*	63.9 ± 10.4*,‡	58.7 ± 11.6*
Log IgE (IU/mL)	2.1 ± .4	2.0 ± 0.7*	2.2 ± 0.6*	2.4 ± 0.5*,‡
Log blood eosinophil counts (/μL)	2.7 ± 0.4	2.3 ± 0.6*	2.4 ± 0.5*	2.3 ± 0.7
Sputum analysis (n)	15	16	25	9
Total cells ($\times 10^6$ cells/mL)	2.9 ± 1.3	15.0 ± 20.3*	11.1 ± 26.5*	3.0 ± 1.7‡,†
Macrophage %	16.2 ± 15.4	14.6 ± 23.6	20.5 ± 22.5	18.0 ± 15.2
Neutrophil %	49.1 ± 21.1	64.6 ± 31.5	55.0 ± 22.8	48.5 ± 21.7
Eosinophil %	18.6 ± 18.4	7.9 ± 12.2	11.2 ± 15.4	18.1 ± 28.6
Lymphocyte %	1.9 ± 3.0	2.1 ± 3.3	1.9 ± 4.0	0.8 ± 1.5

Comparison between study groups is based on an independent t test for continuous variables and a χ^2 test for proportions. Data are given as mean ± SD

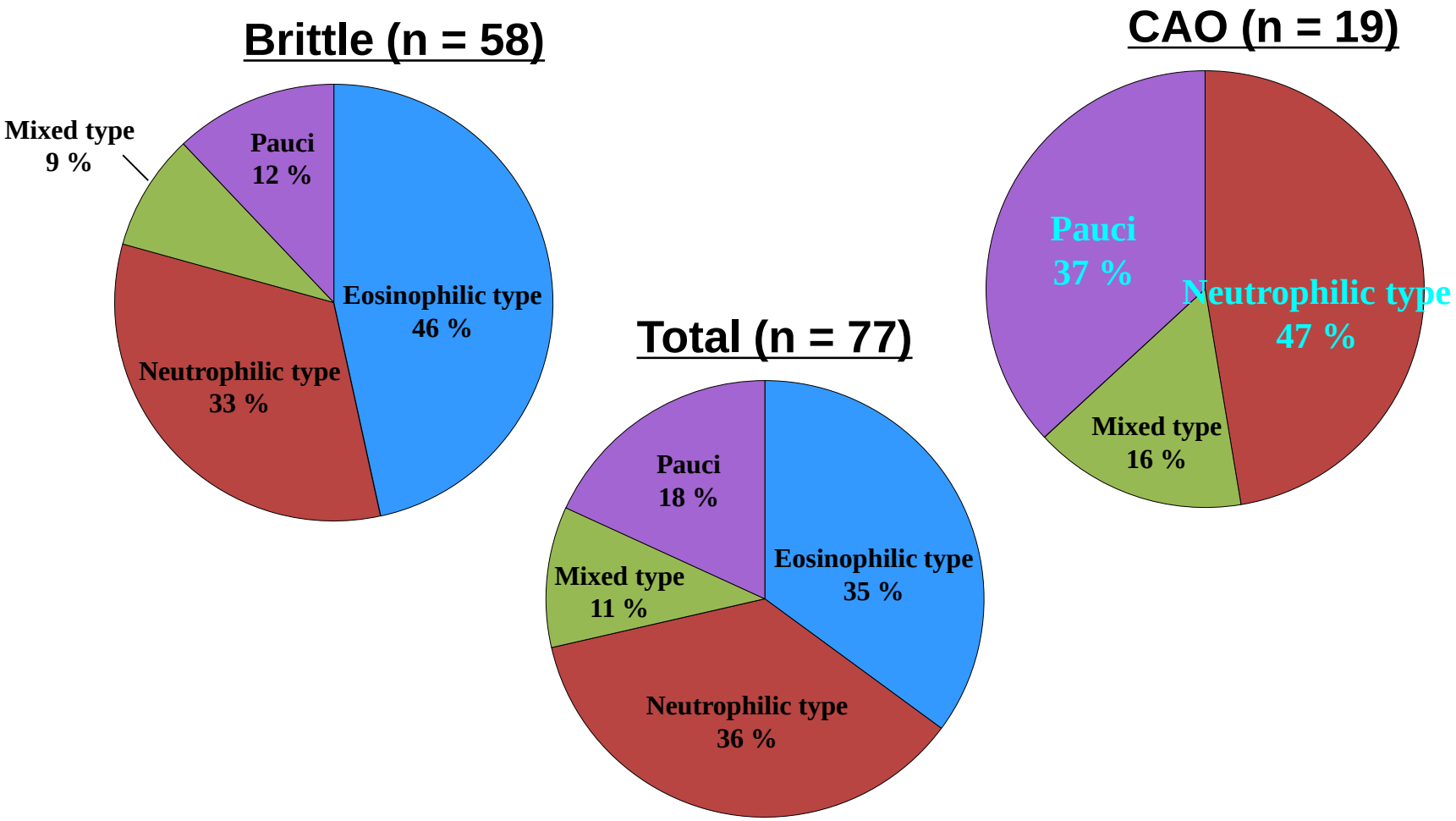
FEV$_1$ forced expiratory volume in 1 s, *FVC* forced vital capacity, *PFT* pulmonary function test, *PC*$_{20}$ *M* provocation concentration that caused a decrease in FEV$_1$ of 20 %

* $p < 0.05$ versus Cluster 1

‡ $p < 0.05$ versus Cluster 2

† $p < 0.05$ versus Cluster 3

Role of neutrophils in persistent airway obstruction due to refractory asthma



Respirology 2012; 17: 322–329

Elevation of S100 calcium binding protein A9 in sputum of neutrophilic inflammation in severe uncontrolled asthma

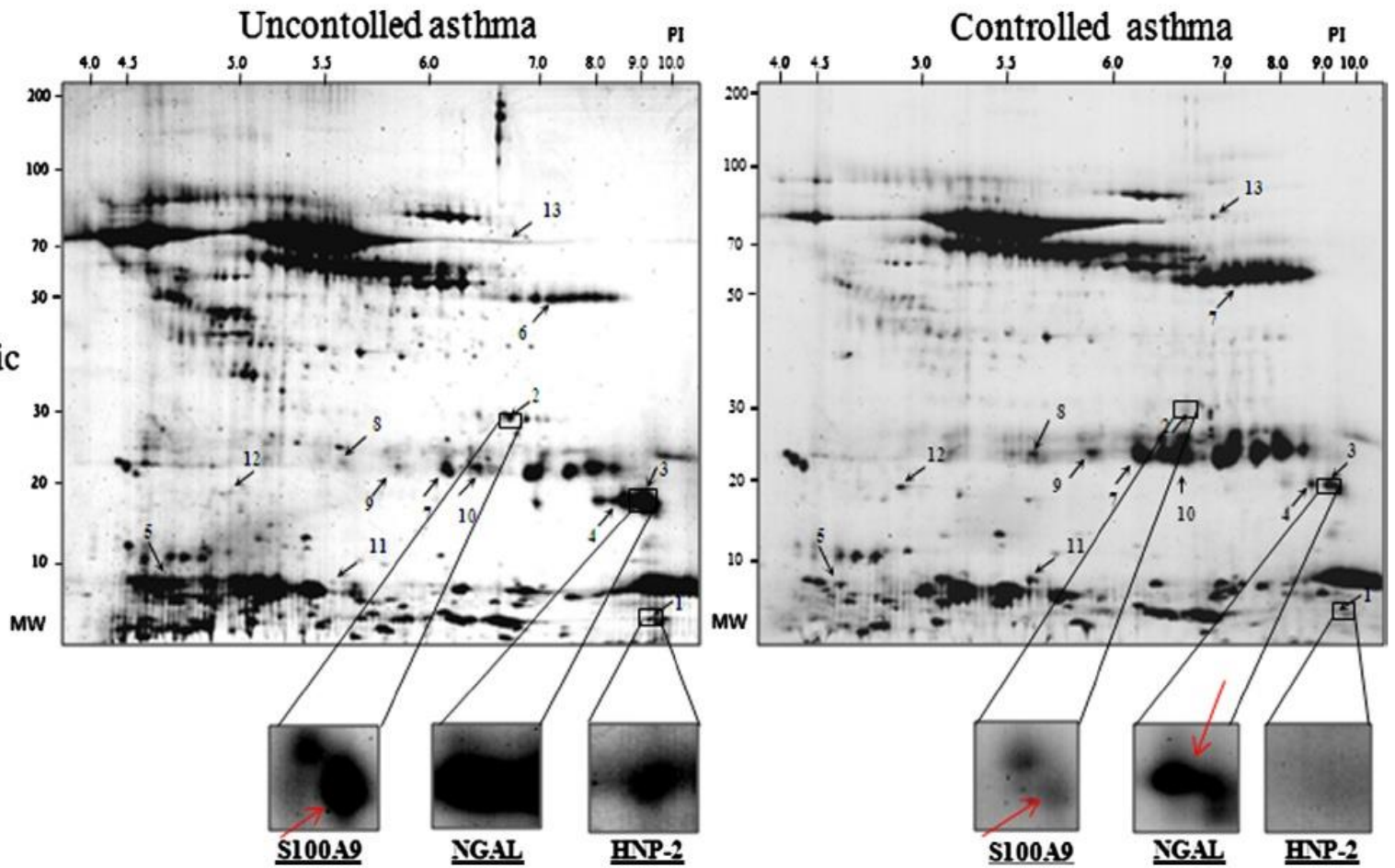
Table 2
List of differentially expressed proteins identified by matrix-assisted laser adsorption/ionization–time of flight/time of flight

Number	Protein name	Accession number	Determined sequence	MW (kDa)/PI	Relative intensity			Fold change	Function
					UA		CA		
1	human neutrophil peptide-2	75765819	R.YXTCIYQGR.L	34.28/8.67	0.175	>	0.028	6.23	anti-inflammatory
2	S100 calcium binding protein A9	4506773	R.NIETIINTFHQYSVK.L	13.234/5.71	0.124	>	0.02	6.2	inflammatory
3	neutrophil gelatinase-associated lipocalin	300181	K.WYVVGLAGNAILR.E	20.536/8.87	1.831	>	0.32	5.718	inflammatory
4	4-aminobutyrate transaminase	84702675	K.GLIFLTAGRK.A	46.748/8.63	0.04	>	0.007	5.399	enzyme activity
5	cystatin SA	359513	R.EQIVGGVNYFFDIEVGR.T	13.843/4.85	0.597	>	0.111	5.383	cysteine protease inhibitor
6	Plunc	7706119	K.VTDPQLLELGLVQSPDGHR.L	26.696/5.41	0.086	>	0.021	3.97	inflammatory
7	complement component C3	179665	K.VQLSNDFDEYIMAIEQTIK.S	18.704/6.02	0.016	<	0.123	0.13	immunity
8	immunoglobulin heavy chain variable region	194719561	_.EVQLVESGGGLVQPGGVLR.L	12.965/6.45	0.005	<	0.03	0.167	immunity
9	glial fibrillary acidic protein isoform 1	4503979	K.LALDIEIATYR.K	49.850/5.42	0.009	<	0.044	0.204	inflammatory
10	IgM κIIIb SON	224377	R.TVAAPSVFIFPPSDEQL.K	13.756/8.65	0.076	<	0.352	0.215	immunity
11	MLL-AF4 der(11) fusion protein	347377	K.HFESSSKVAQAPSPCIAR.S	24.986/10.07	0.026	<	0.12	0.216	signaling
12	cytokeratin 8	30313	K.LSELEAALQR.A	30.840/4.91	0.009	<	0.041	0.219	cytoskeleton
13	recombinant IgG4 heavy chain	9857759	K.GFYPSDIAVEWESNGQPENNYK.T	42.895/5.72	0.112	<	0.475	0.235	immunity

Abbreviations: CA, controlled neutrophilic asthma; MW, molecular weight; PI, isoelectric point; UA, sever uncontrolled neutrophilic asthma.

Ann Allergy Asthma Immunol 2013;111:268-275

Neutrophilic



S100 proteins: functions and association with human diseases

Protein	Postulated functions	Disease association
S100A1	Regulation of cell motility, muscle contraction, phosphorylation, Ca^{2+}-release channel, transcription	Cardiomyopathies
S100A2	Tumour suppression, nuclear functions, chemotaxis	Cancer, tumour suppression
S100A3	Hair shaft formation, tumour suppression, secretion, and extracellular functions	Hair damage, cancer
S100A4	Regulation of cell motility, secretion and extracellular functions, angiogenesis	Cancer (metastasis)
S100A5	Ca^{2+}, Zn^{2+}, and Cu^{2+}-binding protein in the CNS and other tissues; unknown function	Not known
S100A6	Regulation of insulin release, prolactin secretion, Ca^{2+} homeostasis, tumour progression	Amyotrophical lateral sclerosis
S100A7	S100A7-fatty acid binding protein complex regulates differentiation of keratinocytes	Psoriasis, cancer
S100A8/ S100A9	Chemotactic activities, adhesion of neutrophils, myeloid cell differentiation, apoptosis, fatty acid metabolism	Inflammation, wound healing, cystic fibrosis
S100A10	Inhibition of phospholipase A2, neurotransmitter release, in connection with annexin II regulates membrane traffic, ion currents	Inflammation
S100A11	Organization of early endosomes, inhibition of annexin I function, regulation of phoshorylation, physiological role in keratinocyte cornified envelope	Skin diseases, ocular melanoma
S100A12	Host-parasite interaction, differentiation of squamous epithelial cells and extracellular functions	Mooren's ulcer (autoimmune disease), inflammation
S100A13	Regulation of FGF-1 and synaptotagmin-1 stress-induced release; involved in the formation of Cu^{2+}-dependent IL-1α:S100A13 heterotetramer that facilates the export of both proteins	Angiogenesis, vascular response to injury
S100A15	unknown	Psoriasis
S100A16	unknown	Malignant transformation
S100B	Cell motility, proliferation, inhibition of microtubule assembly, transcription, regulation of nuclear kinase, extracellular functions, e.g. neurite extension	Alzheimer's disease, Down syndrome, melanoma, amyotrophic lateral sclerosis, epilepsy
S100P	Function in the placenta	Malignant transformation
S100Z	Function in spleen and leukocytes	Aberrant in some tumours
Calbindin D9K	Ca^{2+} buffer and Ca^{2+} transport	Vitamin D deficiency, abnormal mineralization

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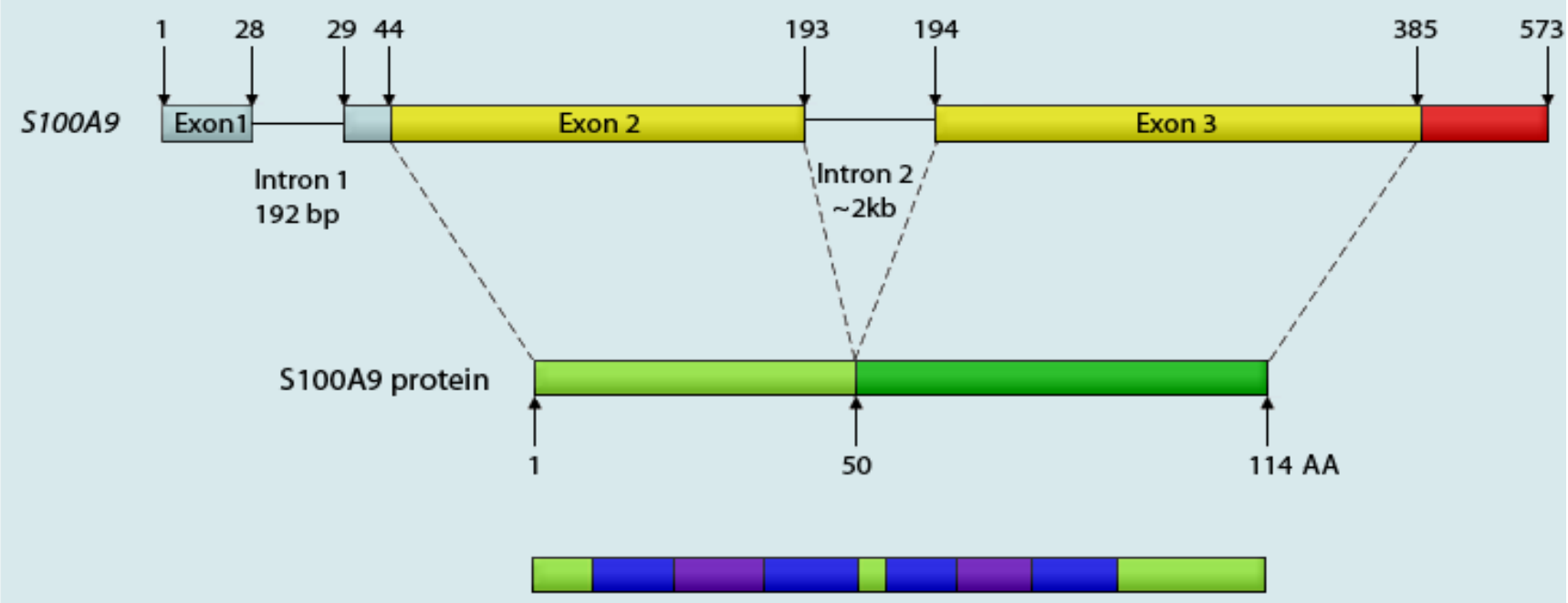
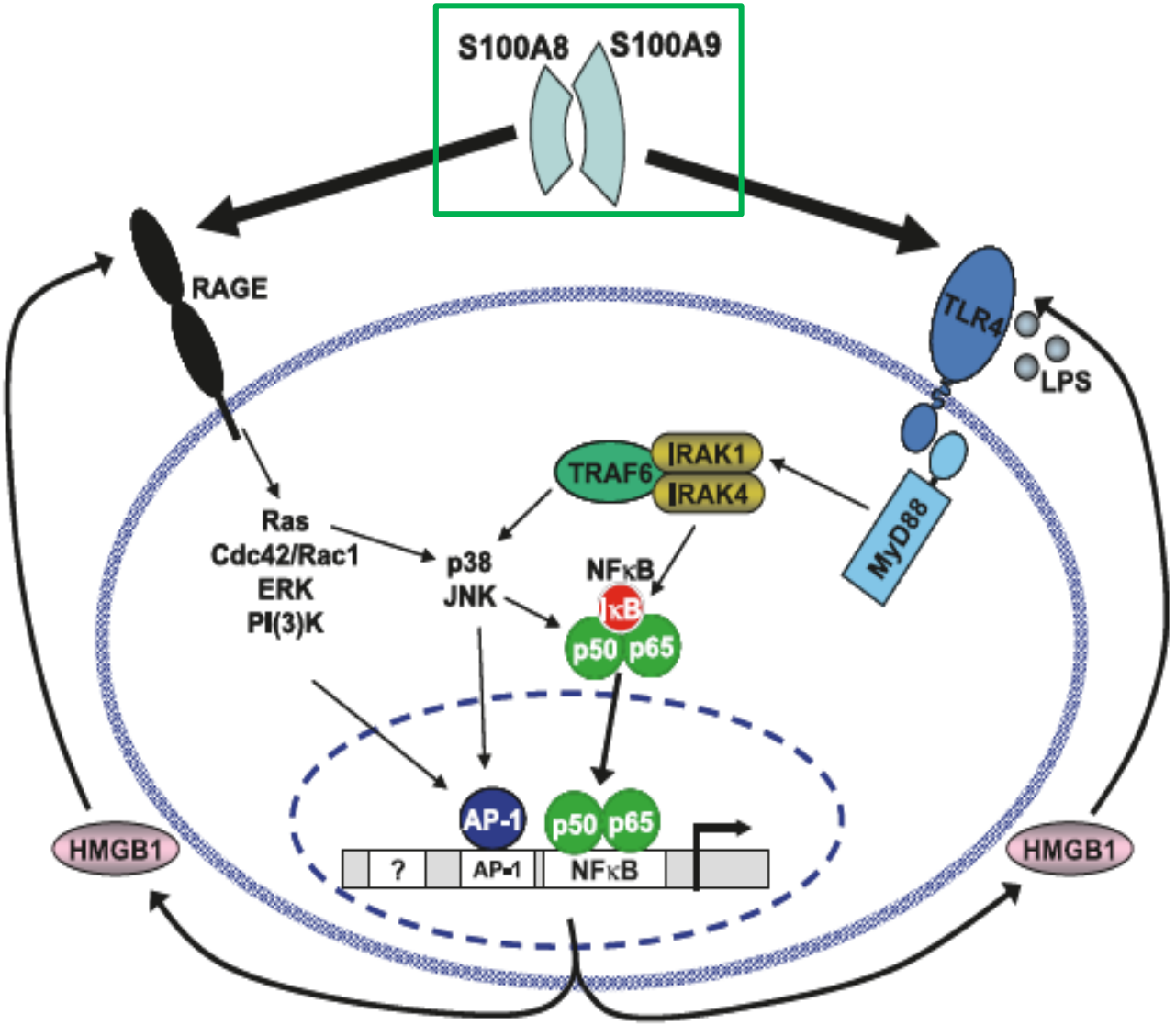


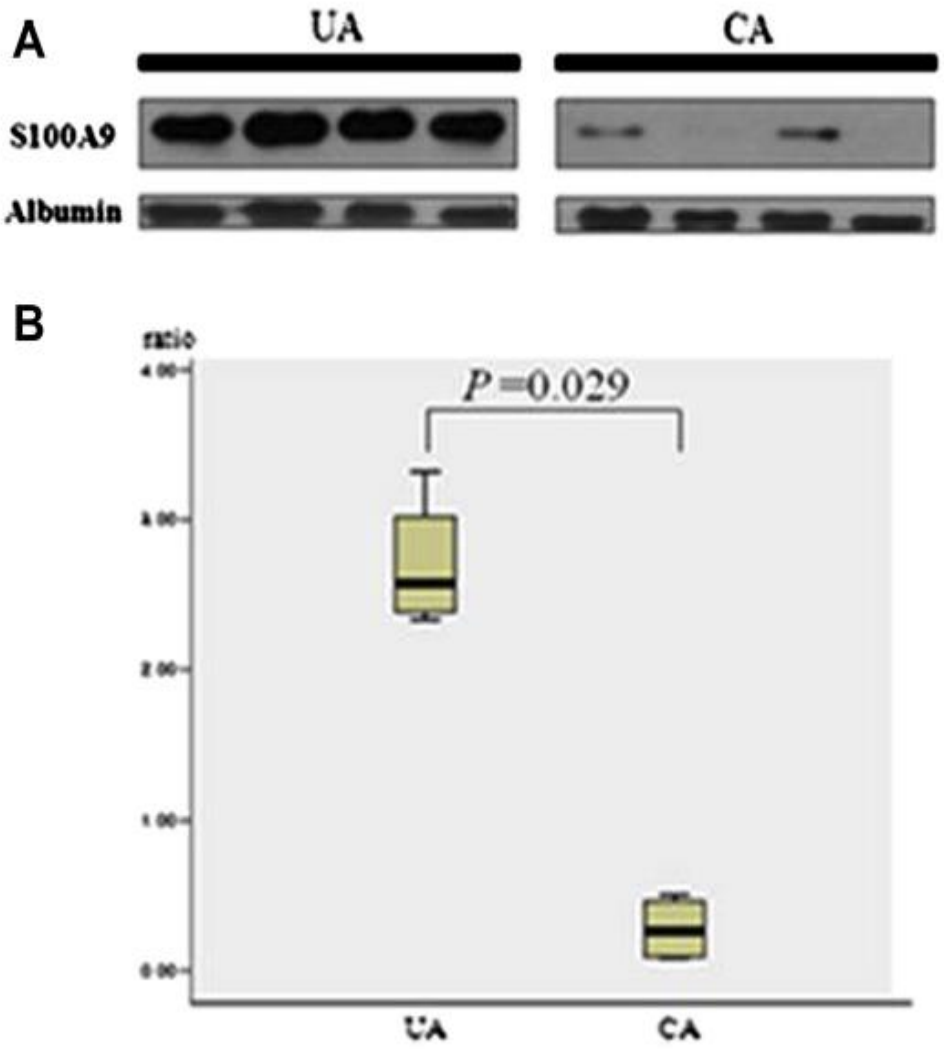
Gene: Box = exon (light blue = 5'UTR, yellow = CDS, red = 3'UTR); Line = intron.
Protein: Upper boxes, alternating colours: exons (coding part only). Lower boxes: protein domains. Green box = not structure; blue box = helix; violett box = calcium-binding domain

Official Symbol: S100A9 and **Name:** S100 calcium binding protein A9
Other Aliases: 60B8AG, CAGB, CFAG, CGLB, L1AG, LIAG, MAC387, MIF, **MRP14**, NIF, P14
Chromosome: 1; Location: 1q21
Total gene size: 3714bp
mRNA: 573bp
Protein:114aa



Schematic representation of the proposed major receptor-mediated signaling pathways induced by S100A8/A9 in airway mesenchymal cells.

Can. J. Physiol. Pharmacol. 2009;**87:743–755**


A
UA
CA
S100A9
Albumin
B
ratio
4.00
3.00
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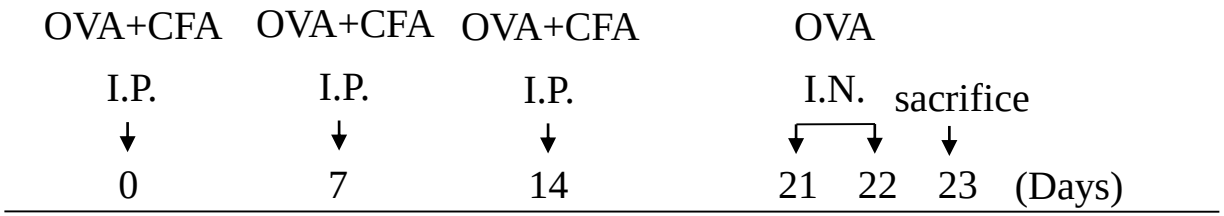
1.00
0.00
P=0.029
UA
CA

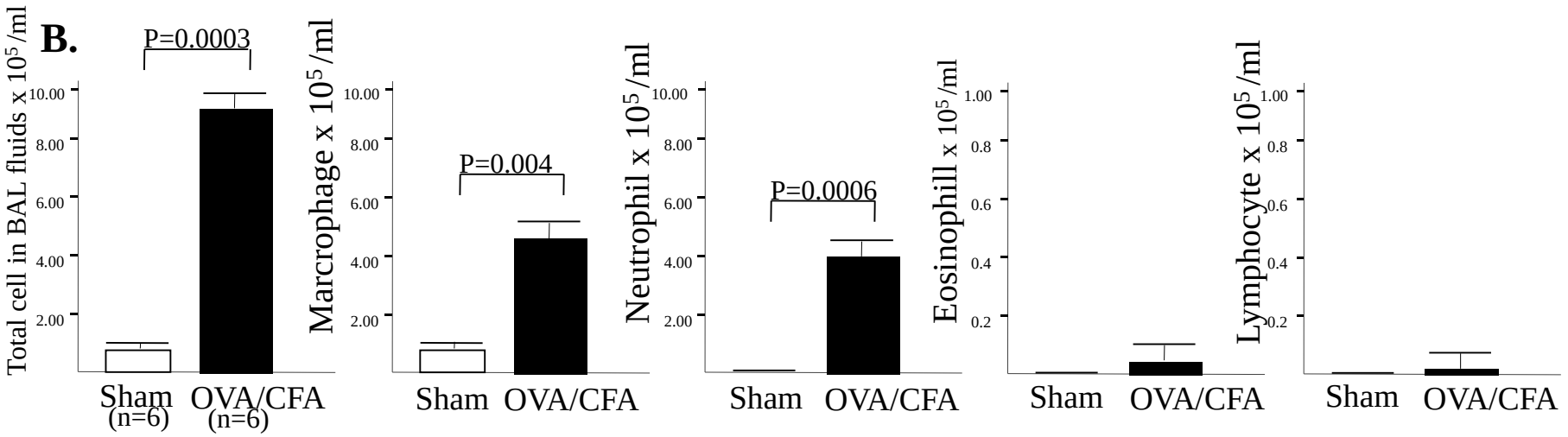
Does S100A9 induced neutrophilic inflammation in mice?

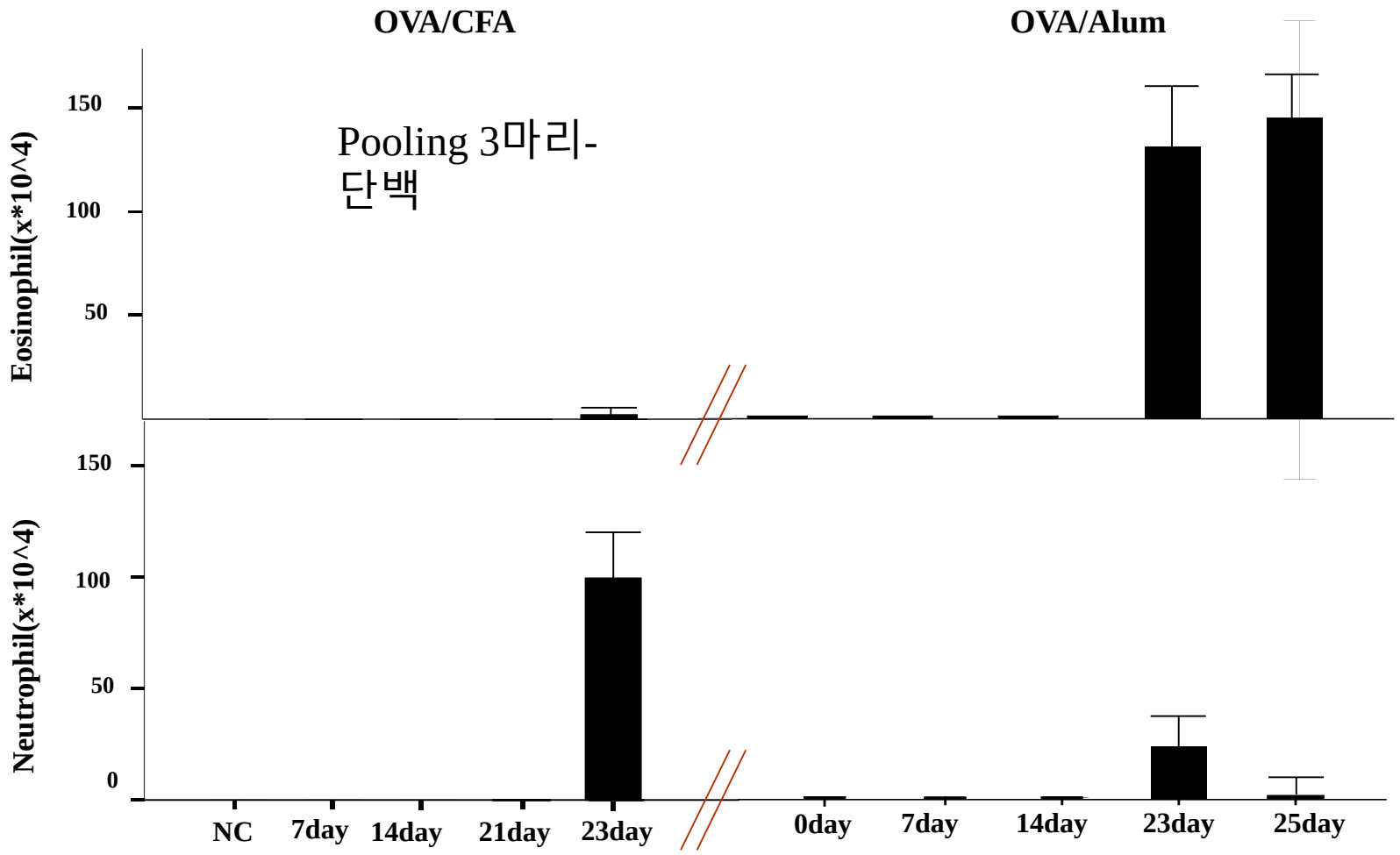
A.



Generation of neutrophilic mice model using OVA/Complete Freund's Ajuvants (CFA) – Treated mice (Am J Physiol Lung Cell Mol Physiol. 2011;300(5):L679-90).

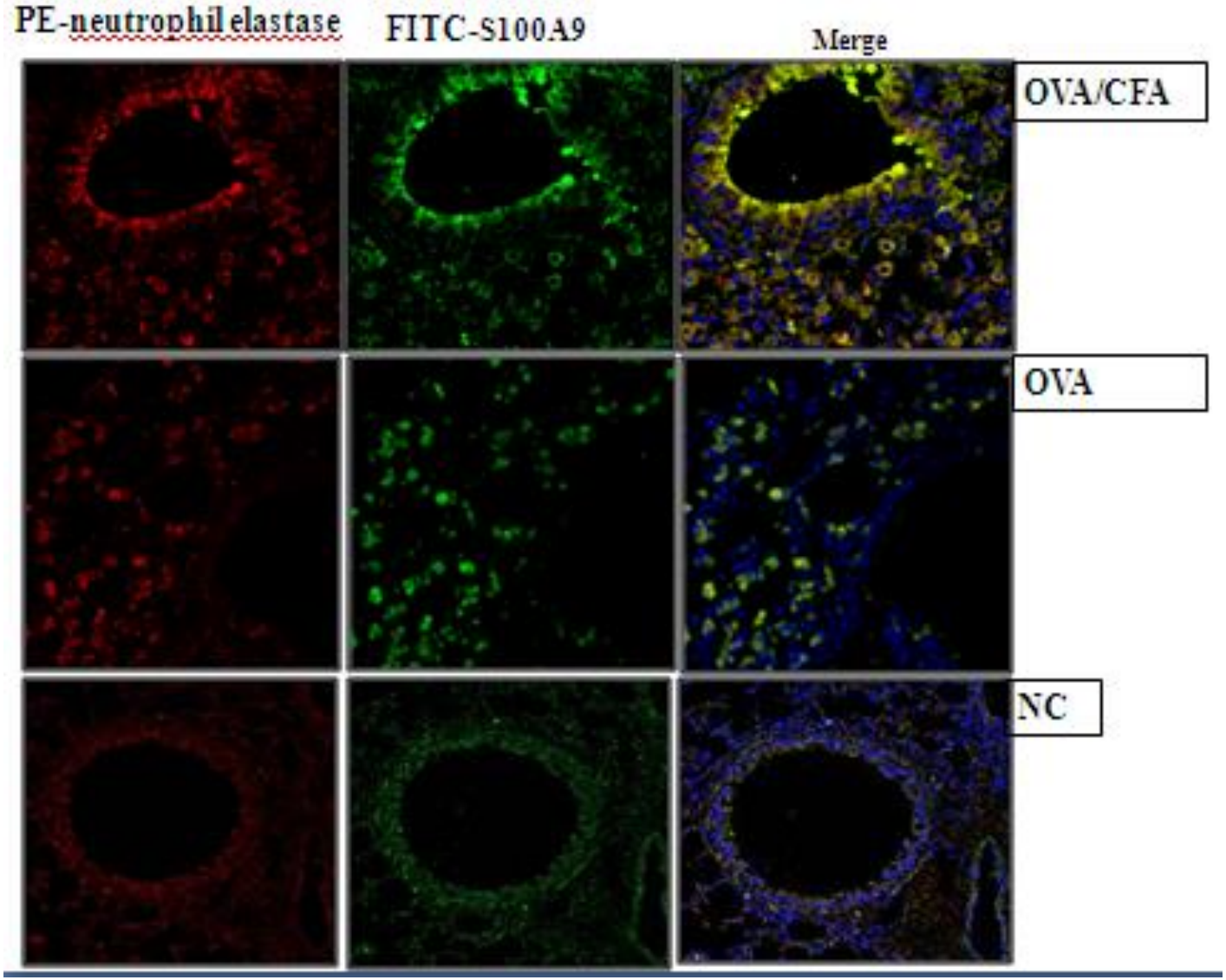
B.



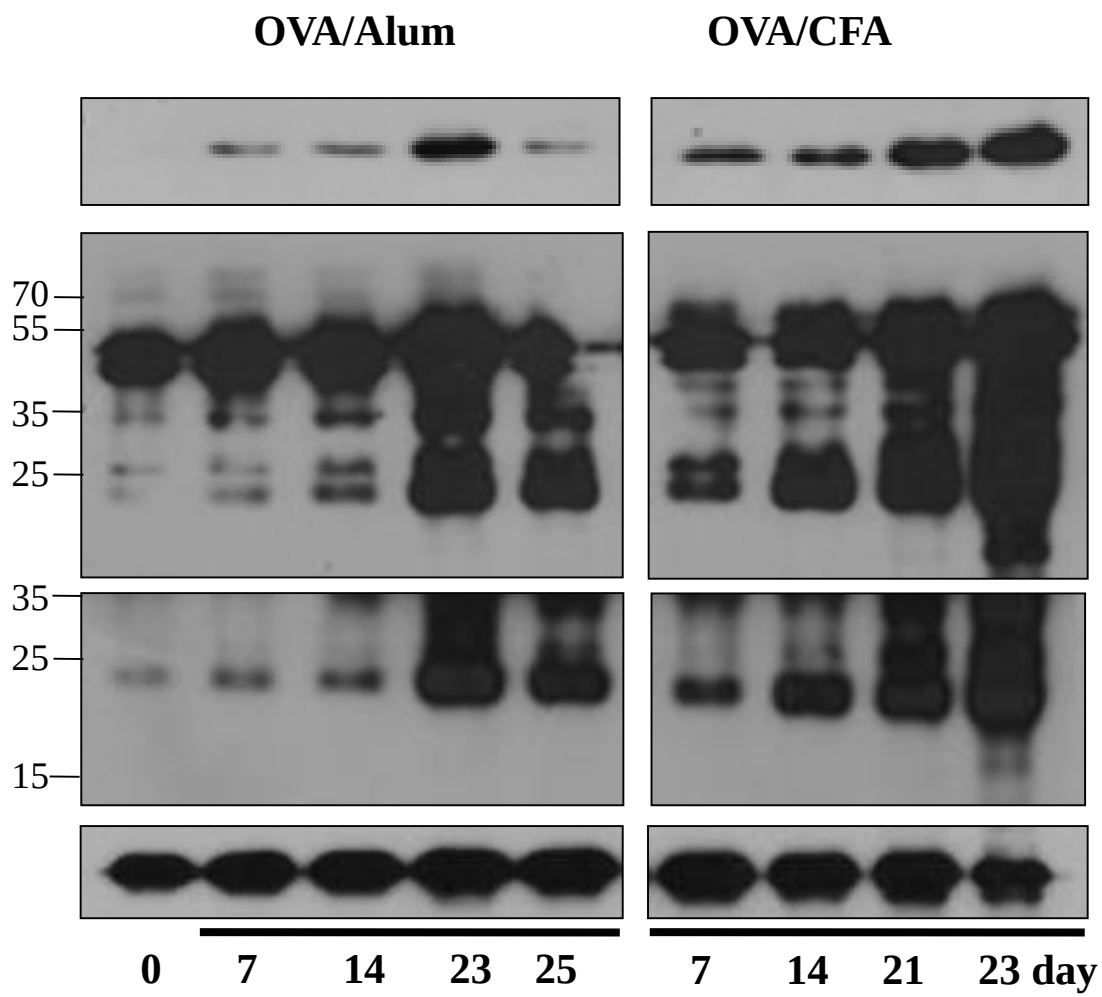

OVA/CFA
OVA/Alum
Pooling 3마리-
단백
Eosinophil(x*10^4)
150
100
50
Neutrophil(x*10^4)
150
100
50
0
NC
7day
14day
21day
23day
0day
7day
14day
23day
25day

S100A9

Expression of S100A8 and A9 in OVA/OVA and OVA/CFA model



Temporal expression of S100A9 expression and activation of caspase-1 in Ova/Alum sensitized/Ova challenged and OVA/CFA sensitized/OVA challenged mice.



➔ S100A9 (13KDa)

➔ Procaspase-1 (46kDa)

➔ Cleaved caspase-1 (30kDa)

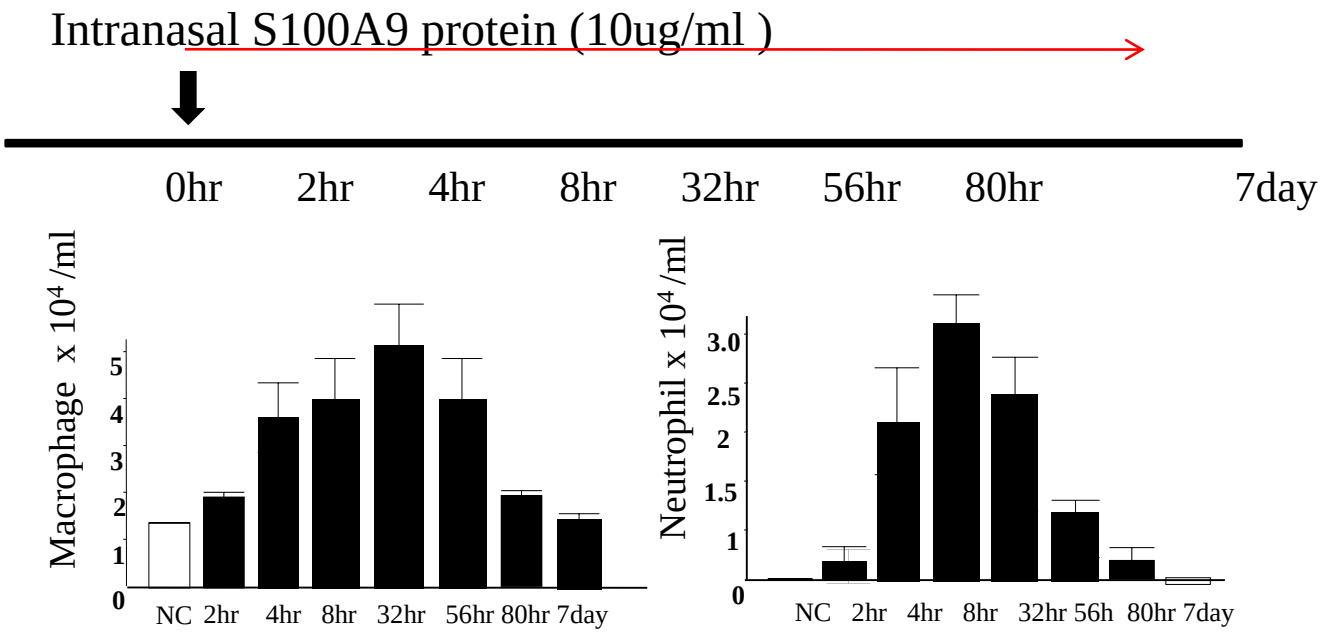
➔ P20 activated caspase-1 (20kDa)

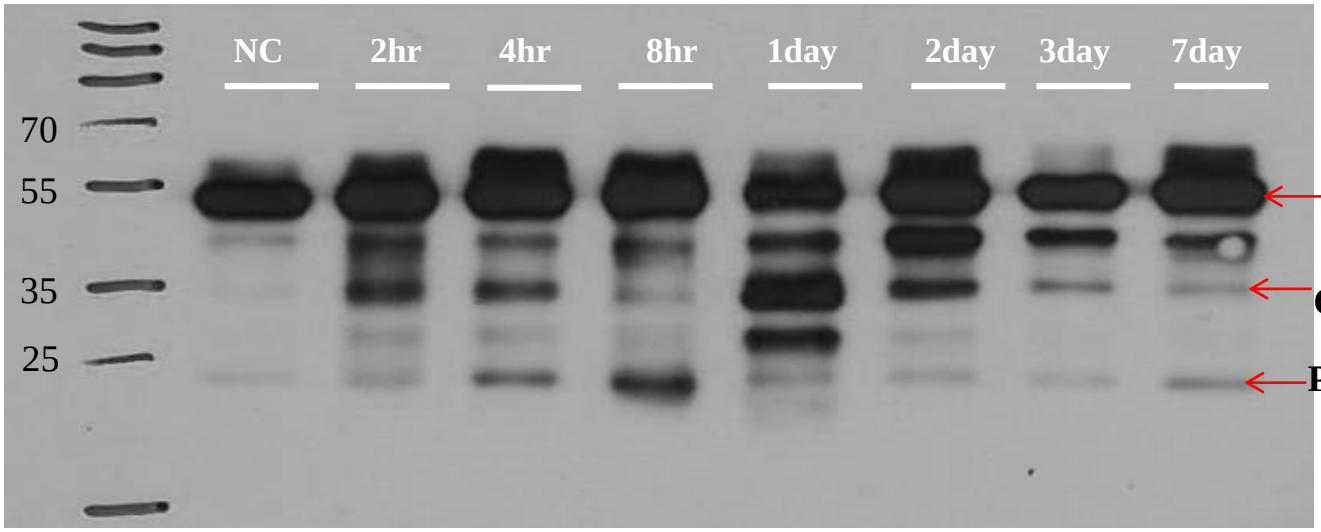
➔ Procursor IL-1β (31kDa)

➔ Mature form IL-1β (17~22kDa)

➔ β-actin (42KDa)

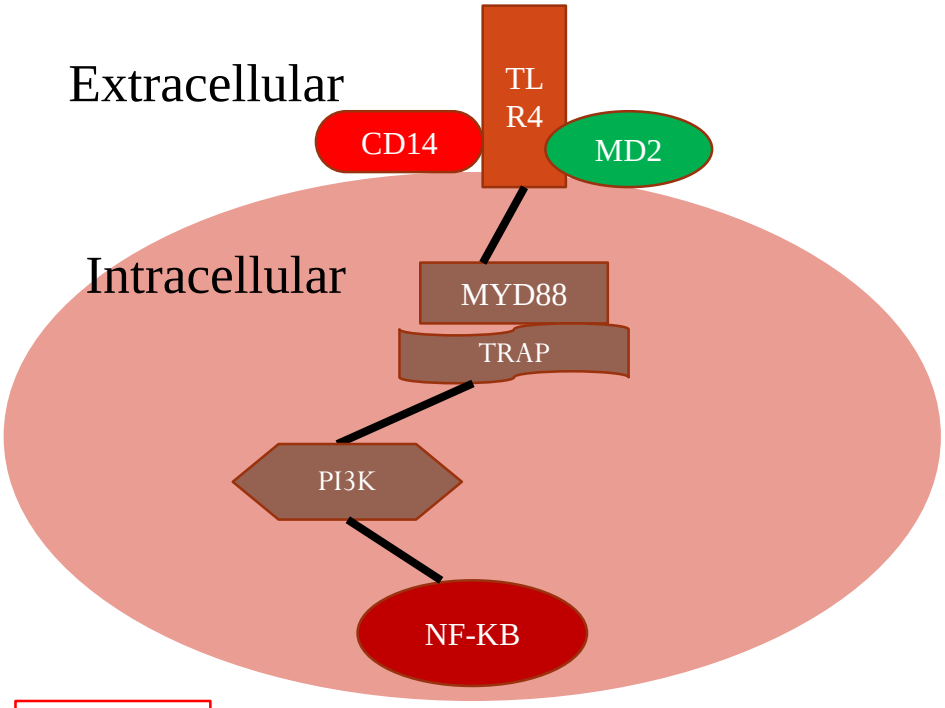
Induction of neutrophilic inflammation and inflammasome activation in airway of mice by intranasal administration of 100A9 in wild mice.

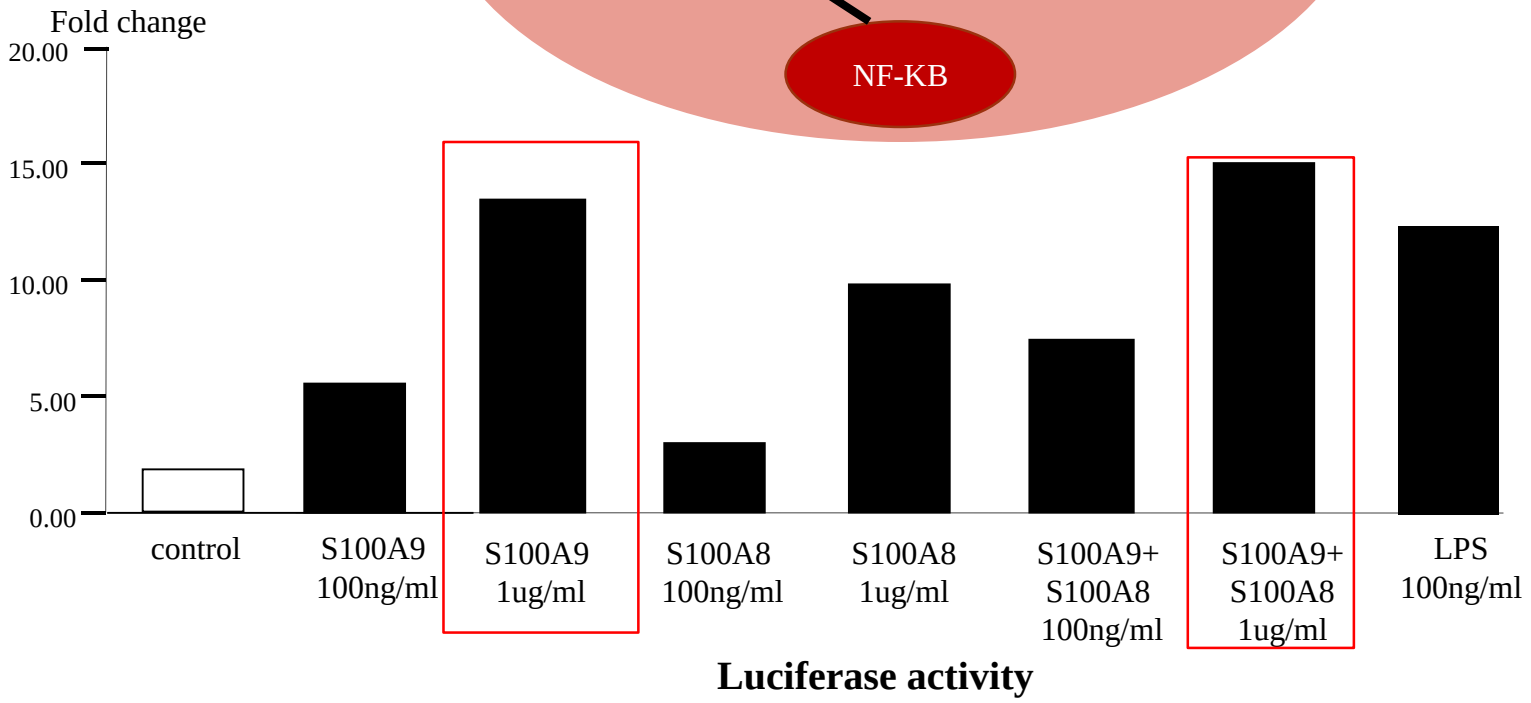




NF-kB activation by S100A9

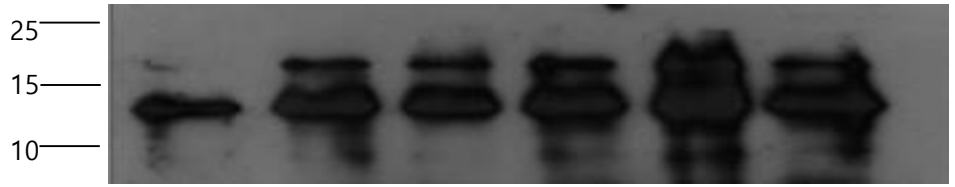
293TLR4 CELL LINE





Induction of S100A9 by IL-beta poster 14S178

THP-1
monocyte



S100A9

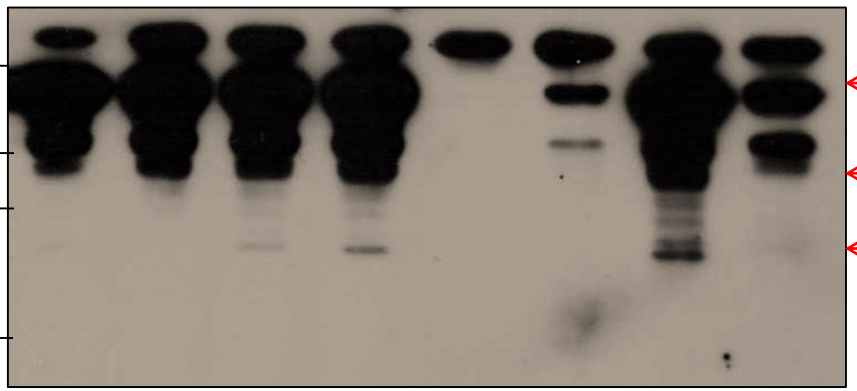
(1mM)$CaCl_2$ - + + + + -

(50ng) IL-1b - - - + + +

24h 96h 24h 96h 24h

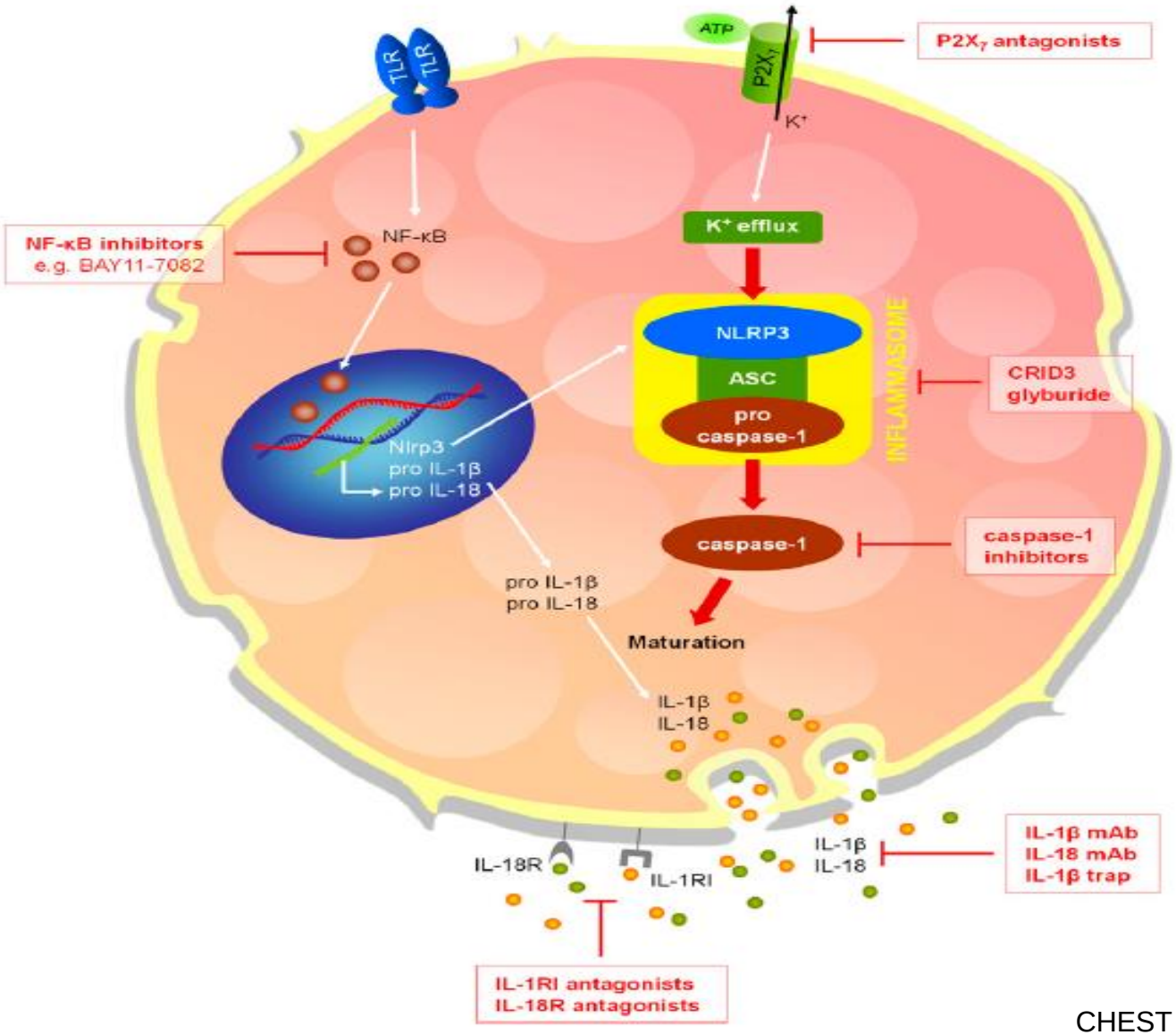
B-actin

Induction of inflammasome by S100A9



S100 A9(ug/ml)	**0**	**0.1**	**1**	**10**	**10**	-	-	-
Inflammasome inhibitor **Bay 110782(uM)**	-	-	-	-	**12**	-	-	-
Parthenolide(uM)	-	-	-	-	-	**10**	-	**10**
LPS (ug/ml)	-	-	-	-	-	-	**1**	-

Inhibitors for inflammasomes



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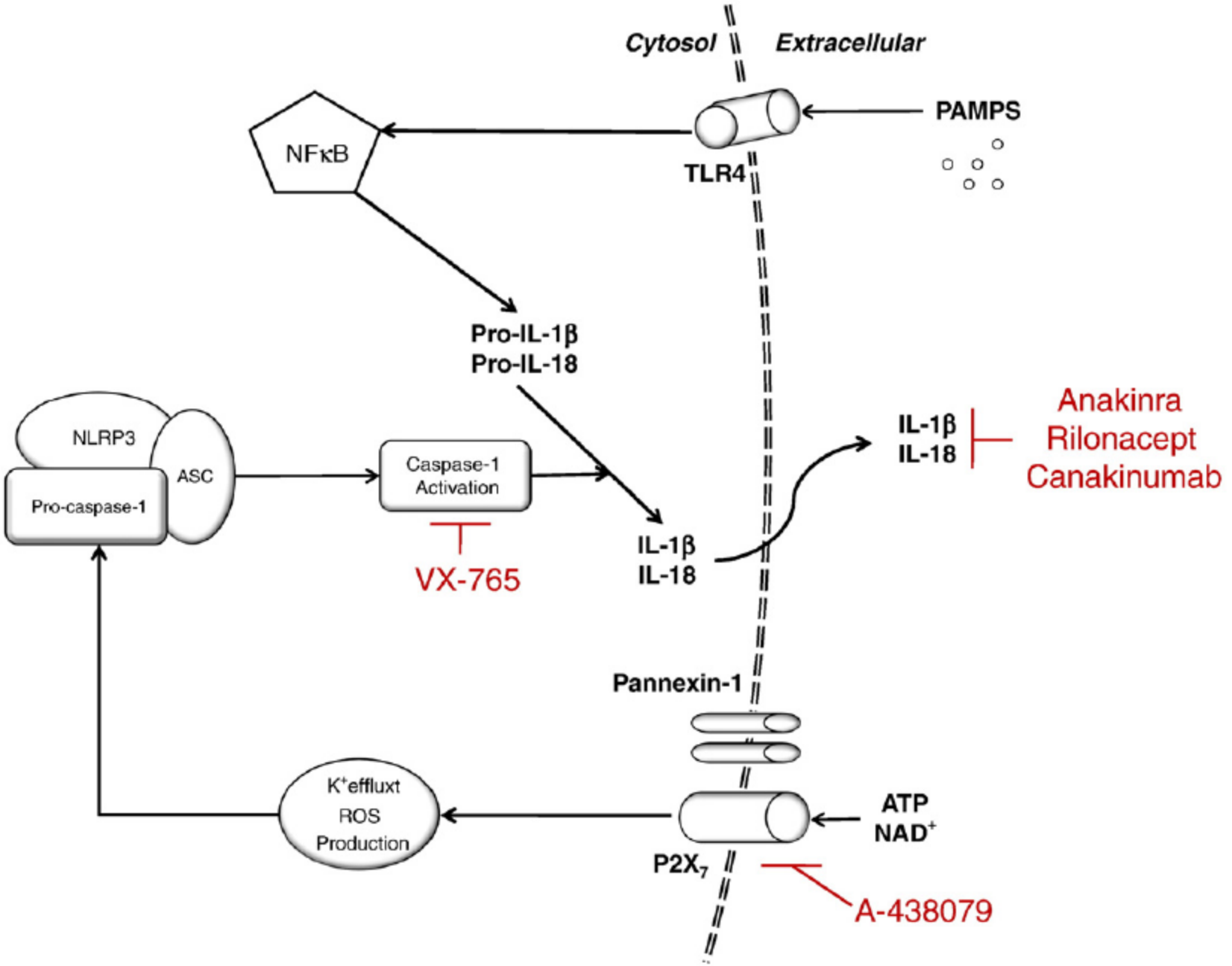

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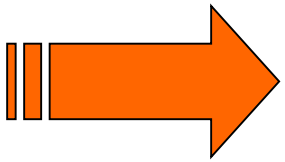
Future directions

- Inflammasome→ more information
- The role of Inflammasome on lung → further evaluation



Better understanding and treatement of lung diseases