Severe Persistent Asthma: Inflammatory and Immune Pathways

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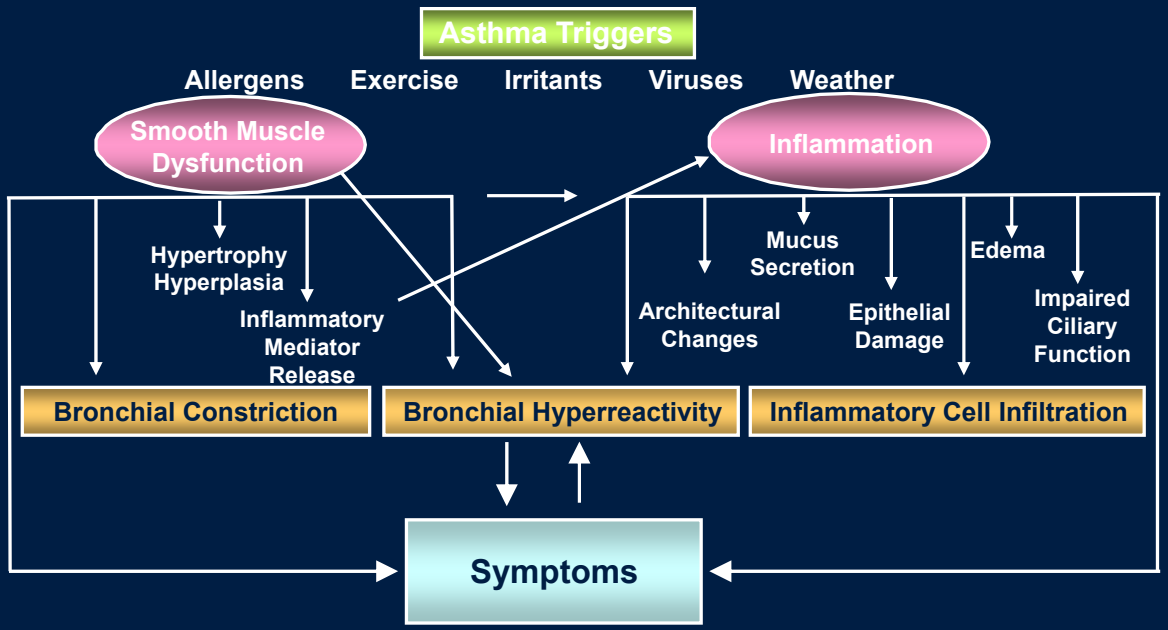
Disclosures

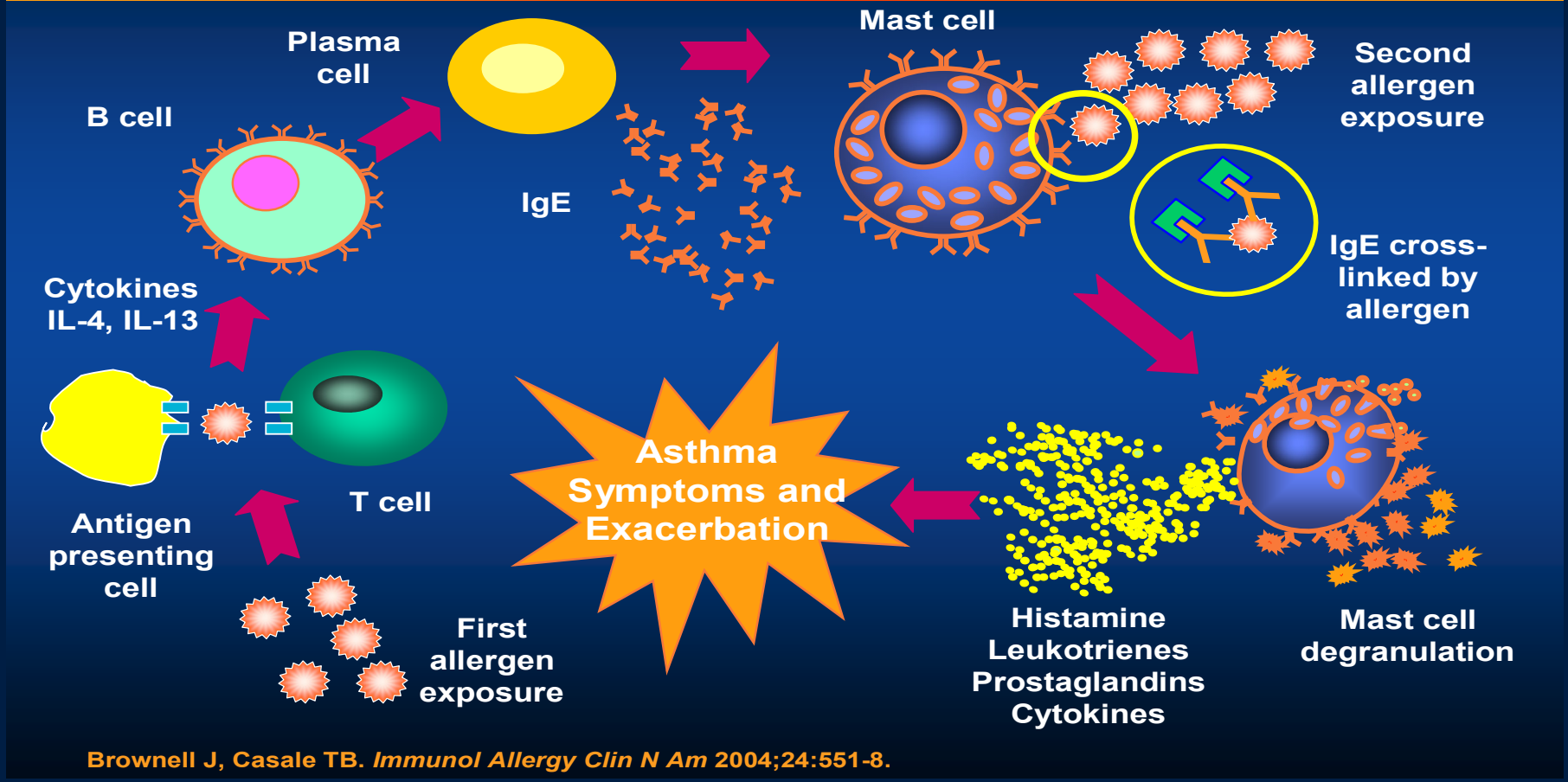
- Professor of Medicine; National Jewish Health; University of Colorado, Denver
- Appeals Committee, Accreditation Council of Graduate Medical Education
- “Opinions and assertions herein are not representative of any of the named entities but are of my own opinion”
- AstraZeneca: Employment ended 8/2022, honoraria

Mechanisms of Asthma Leading to Symptoms



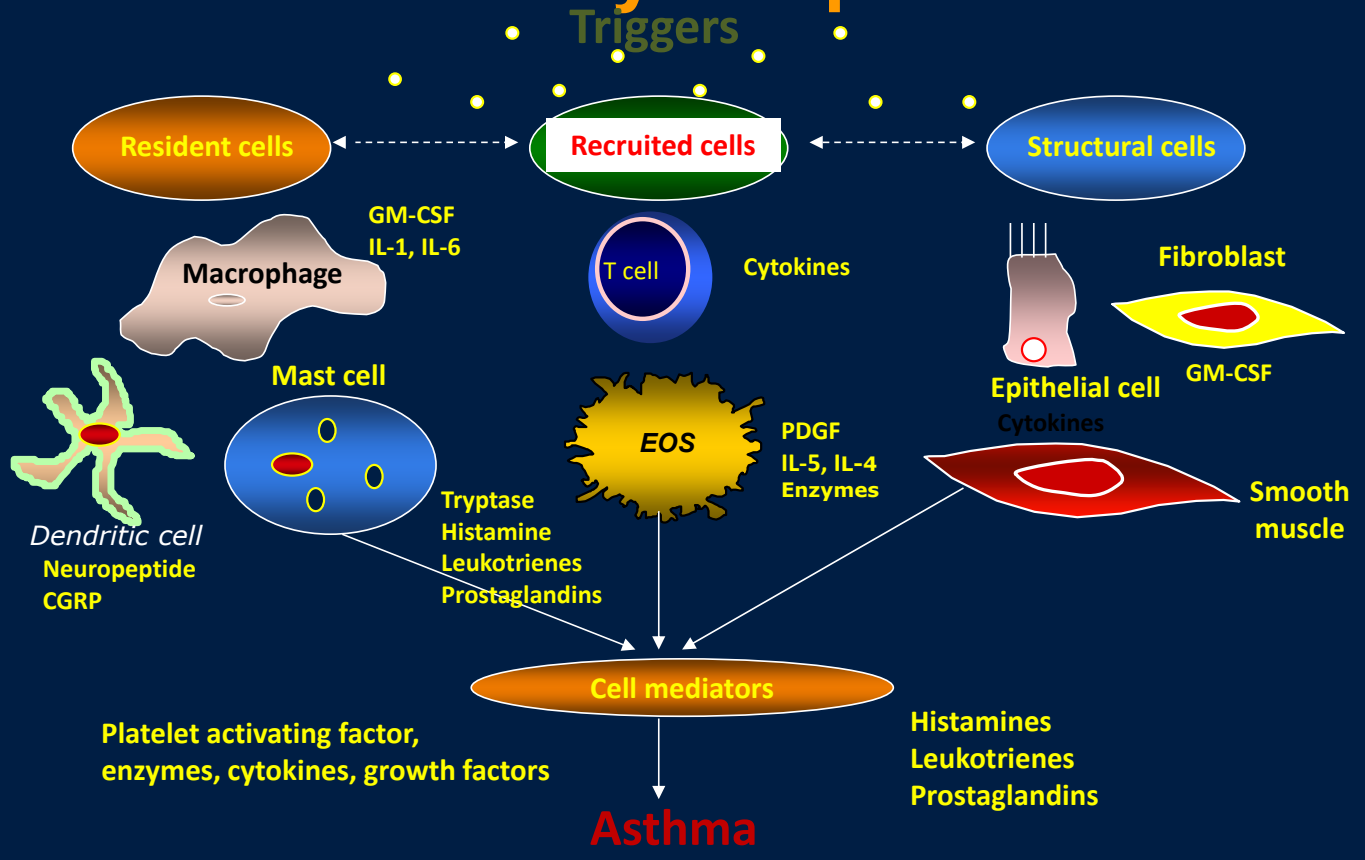
Adapted from Creticos. *Adv Stud Med*. 2002;2:499-503.

Overview of the Inflammatory Cascade



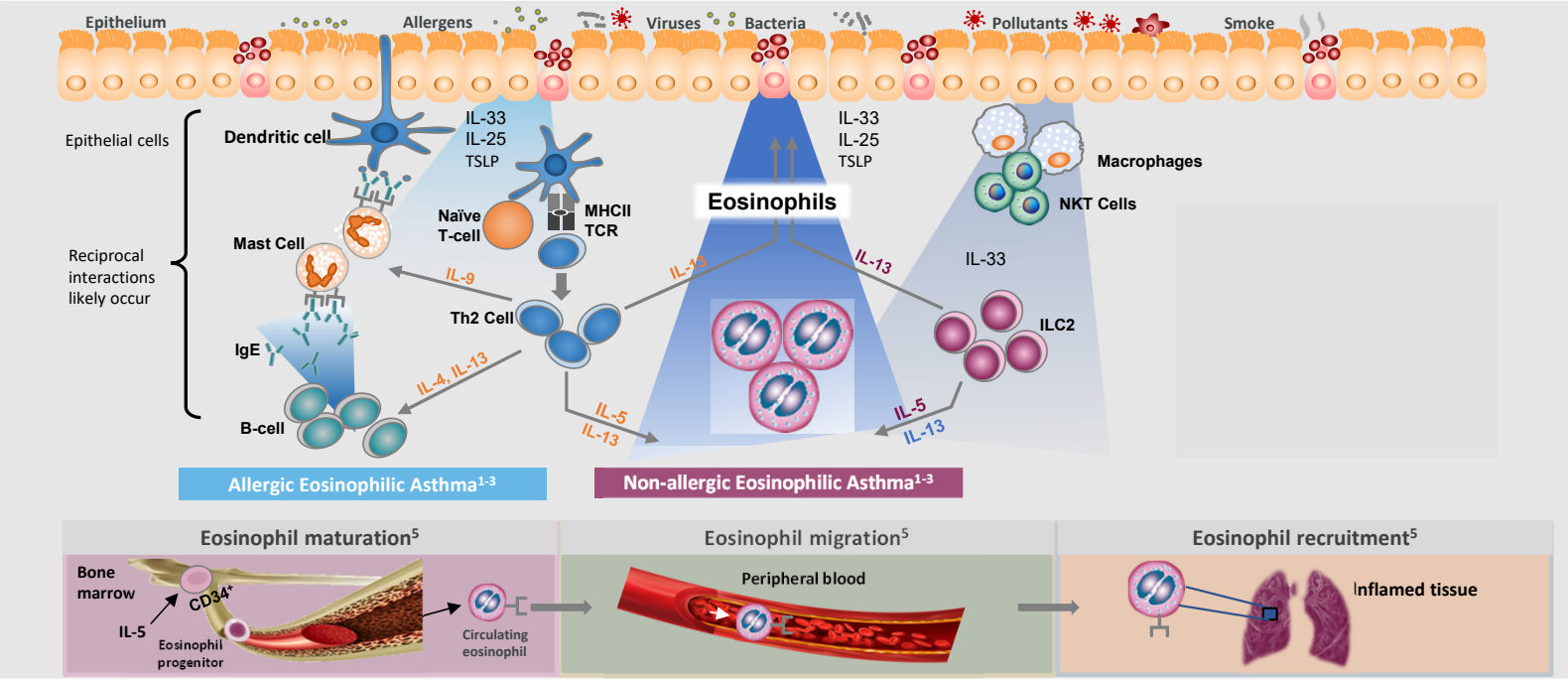
Brownell J, Casale TB. *Immunol Allergy Clin N Am* 2004;24:551-8.

Cells and Cell Mediators Involved in the Asthmatic Inflammatory Response



Barnes PJ, et al. *Am J Respir Crit Care Med*. 1998;157:S1-S53; Spina D. *J Pharm Pharmacol*. 2000;52:125-145.

Eosinophils Can Have Reciprocal Interactions With Other Immune Cells to Perpetuate Chronic Inflammation[1]



1. Jacobsen EA et al. *Annu. Rev. Immunol.* 2021. 39:719-757; 2. Brusselle GG et al. *Nat Med.* 2013;19:977-979; 3. Lambrecht BN et al. *Nat Immunol.* 2015;16:45-56; 4. Peters MC et al. *Lancet.* 2020;1;395:371-383; 5. Pelaia C et al. *Front. Physiol.* 2019.

Inflammation Is Central to the Key Pathological Features and Clinical Consequences of Asthma

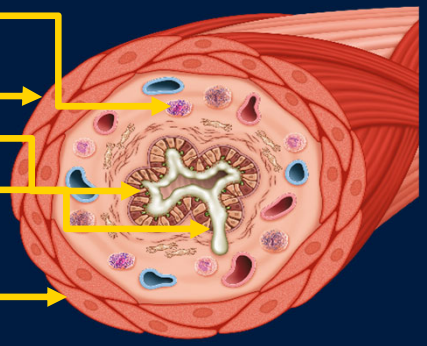
"Studies support a crucial role for eosinophils as drivers of type 2 immune-inflammatory responses in asthma"[1]

Micheal C Peters, Sally E Wenzel

Eosinophil activation

Asthma airway pathology

- **Eosinophil-rich airway inflammation (Type 2)**[2,3]
- **Epithelial damage**[4]
- Goblet cell hyperplasia[5]
- Mucus production[3]
- Smooth muscle contractility and hypertrophy[3]
- **Airway remodelling**[2,3]



Clinical consequences

- ✓ Exacerbations[2,3]
- ✓ Airway obstruction[2,3] (eg, ↓ lung function)
- ✓ Symptoms[2,3]
- ✓ Airway hyperresponsiveness[2,3]

EOS = eosinophils.

1. Peters MC et al. *Lancet.* 2020;395;371-383; 2. The Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention 2021; 3. Israel E et al. *N Engl J Med.* 2017;377:965-976; 4. Gandhi NA et al. *Nat Rev Drug Discov.* 2016;15:35-50; 5. McBrien et al. *Front Med.* 2017;4.

Airway Remodeling: Sub-epithelial Fibrosis

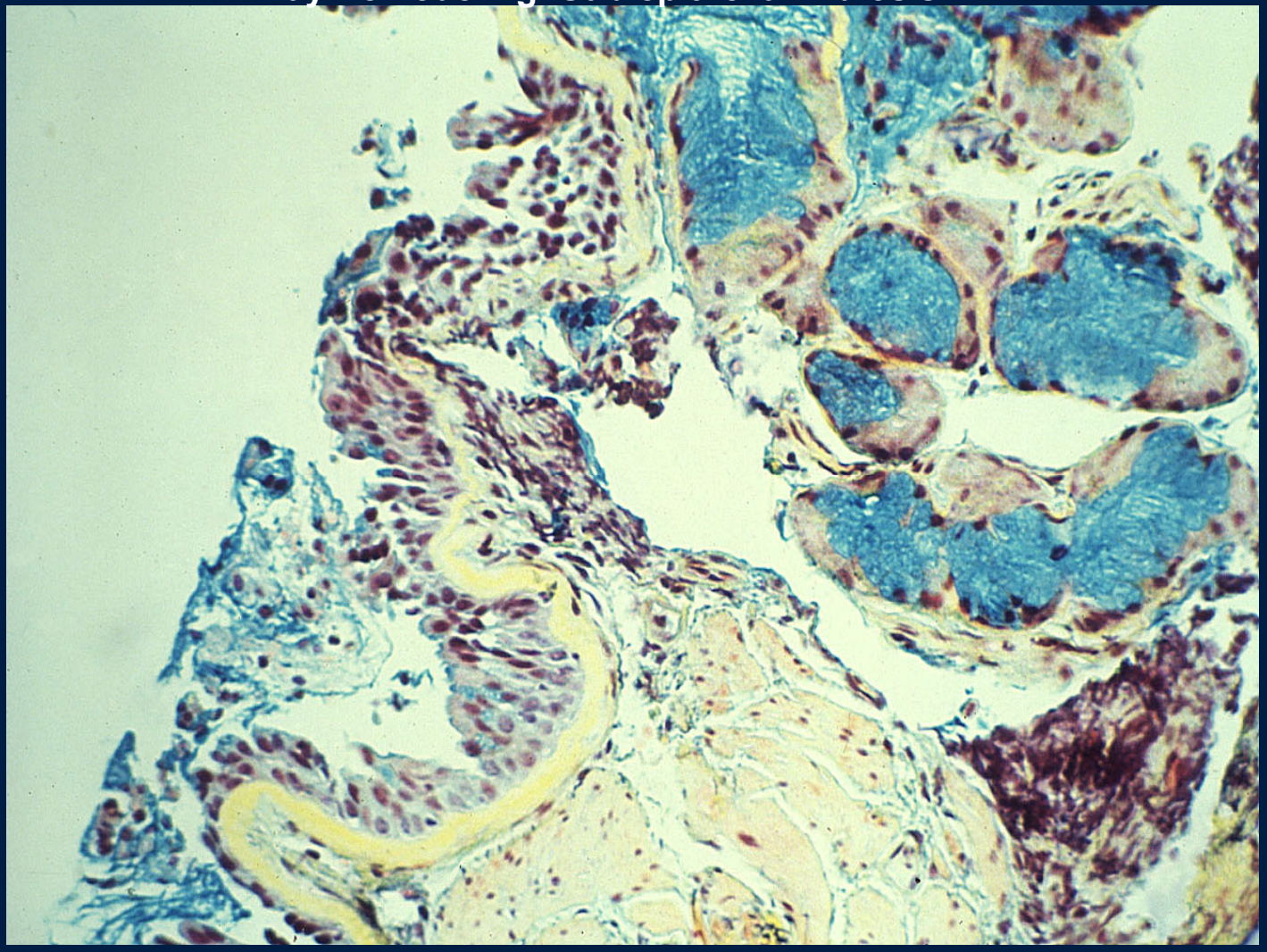
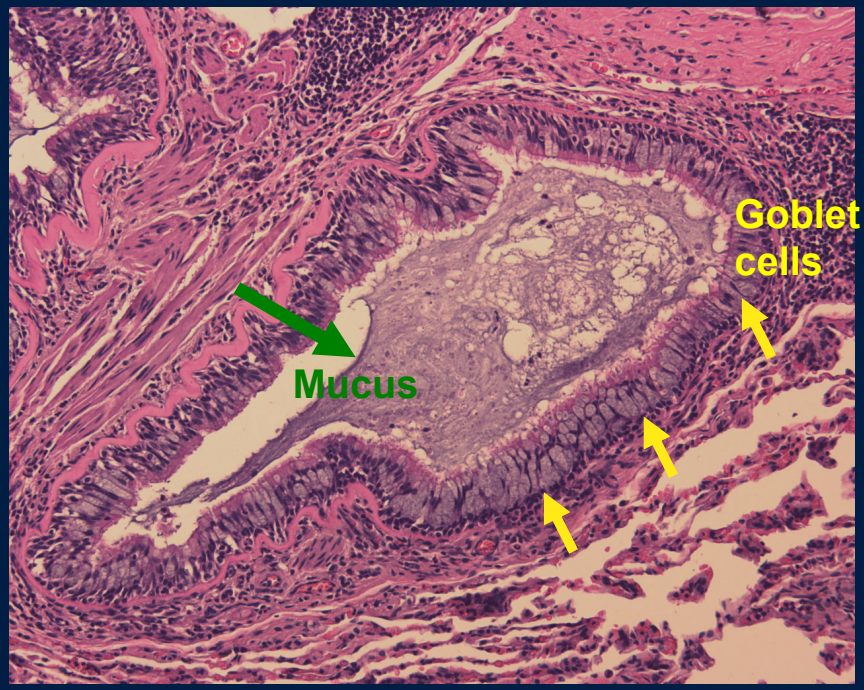


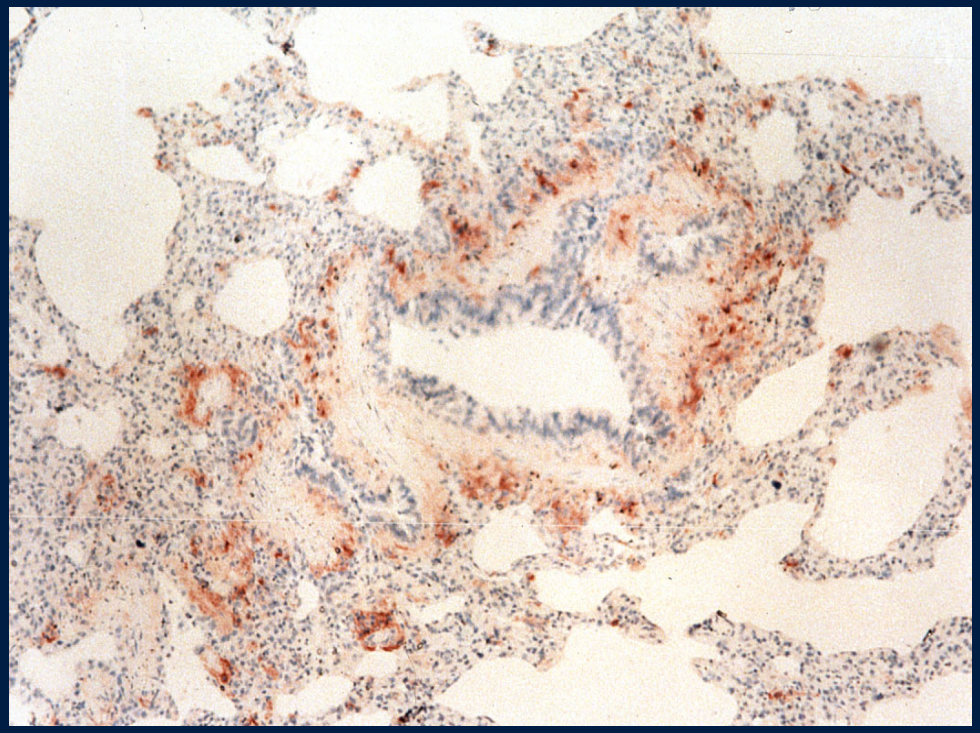
Image courtesy of Rohit Katial, MD.

Epithelial cells: goblet cell increases

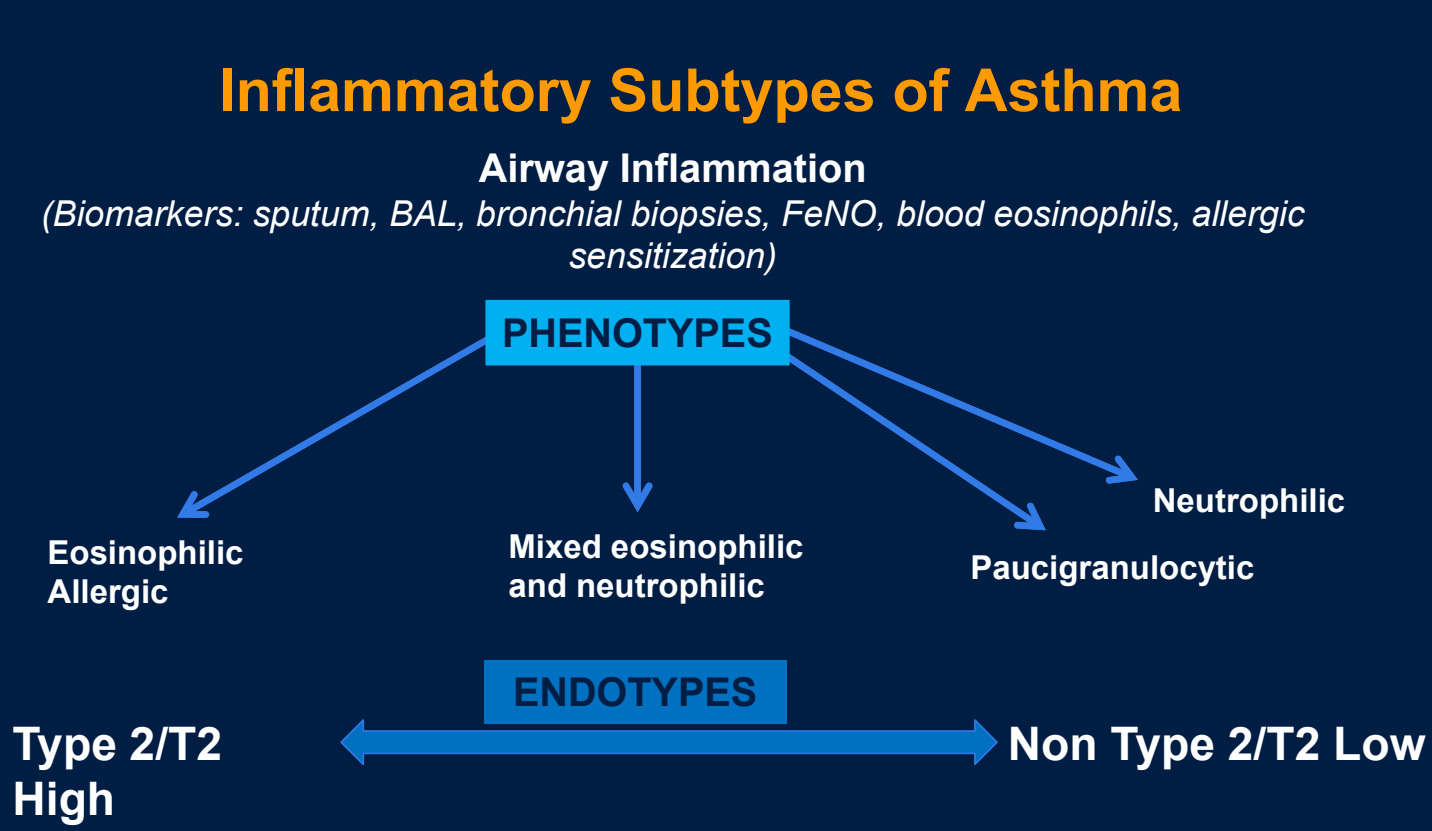


Courtesy of Hong Wei Chu

Different distribution of inflammatory cells: Mast cells



Inflammatory Subtypes of Asthma



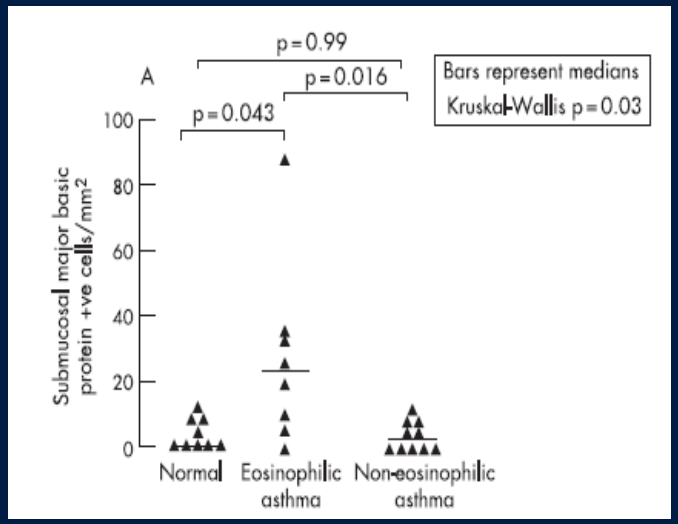
Sputum Cell Counts

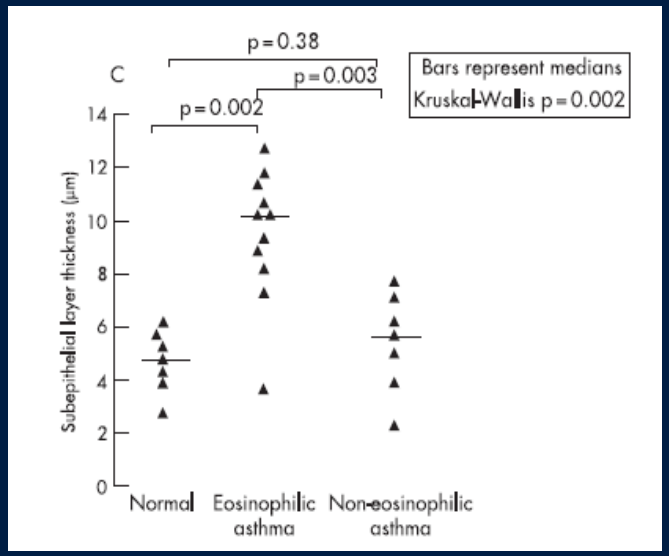
- **Average cell counts (partial data – subset of baseline samples from each group):**

	Total cell counts (million)	Eos percent	Neut percent
Non-asthmatic controls	4.63	2.4%	44.4%
Well-controlled asthmatics	2.75	0.9%	19.1%
Not well-controlled asthmatics	4.08	1.7%	40.8%
Poorly controlled asthmatics	4.43	2.5%	74.8%

Characteristics of Non-Eos Asthma

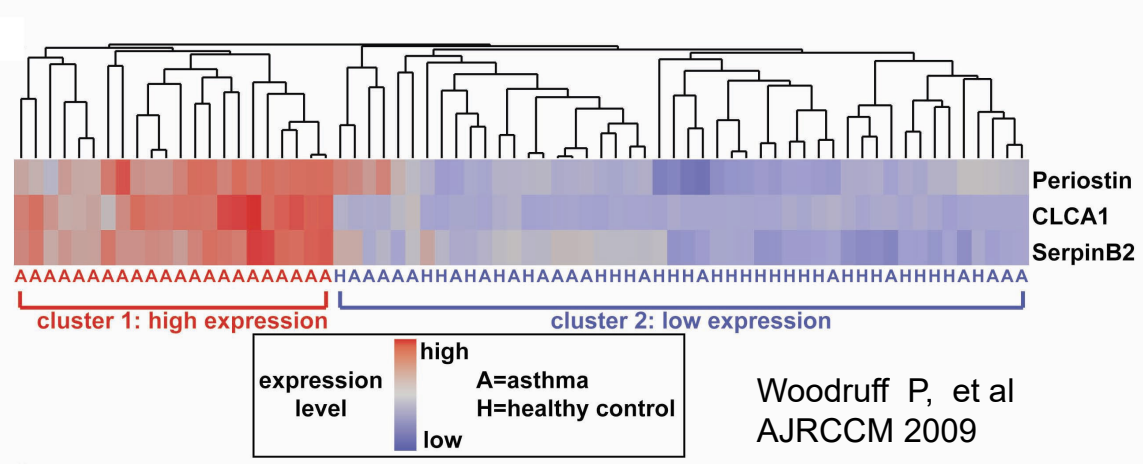
- Distinct Phenotype
- Absence of remodeling
- Absence of Eos
- Poor response to corticosteroids





Berry M, et al. Thorax 2007;62: 1043

Molecular phenotyping supports a Th2/atopic phenotype



3 genes expressed *in vitro* in epithelial cells in response to IL-13 applied to *ex vivo* epithelial cells with:

- More BHR, atopy, eosinophils
- Identified by increases in epithelial periostin in particular

Woodruff et al. AJRCCM 2009

Features of Molecular Phenotypes

Both Th2 High and Low has:

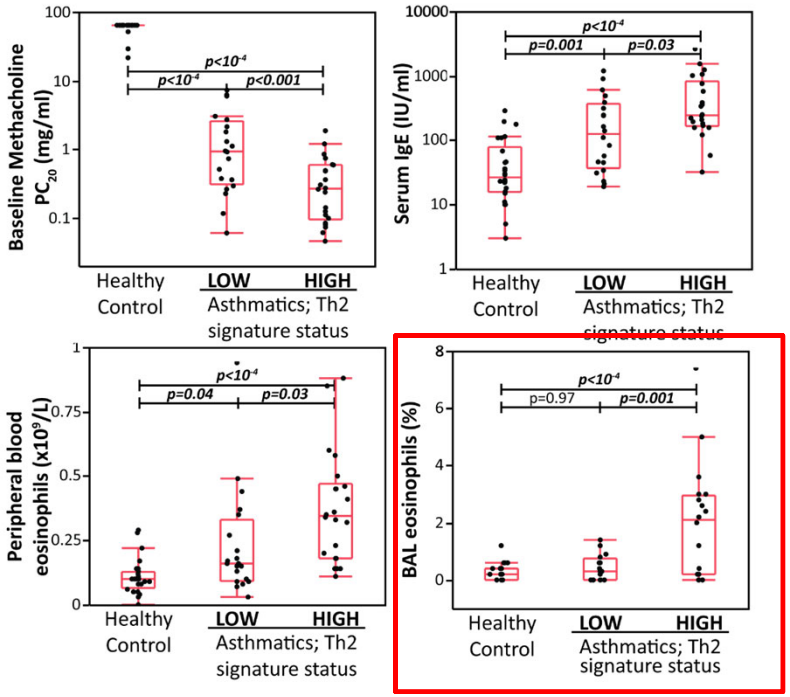
- Decrements in FEV1
- Bronchodilator responsiveness
- Skin Prcik test reactivity

Th2 High has greater:

- AHR
- IgE
- Blood and BAL Eosinophilia

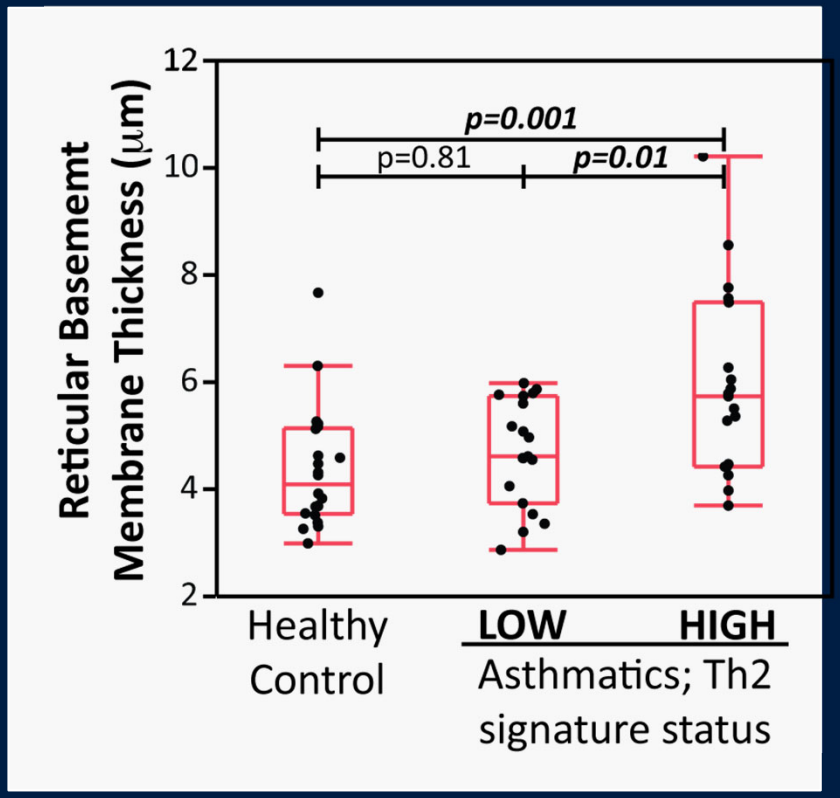
Woodruff et al. AJRCCM 2009

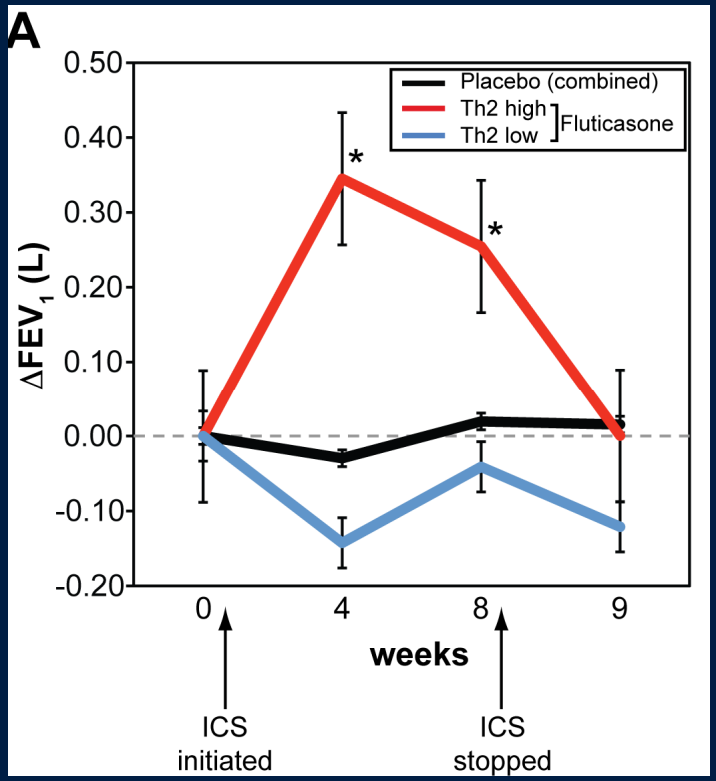
Clinical Features: Th2 High asthma has greater AHR, IgE levels and Eosinophilia



Woodruff et al. AJRCCM 2009

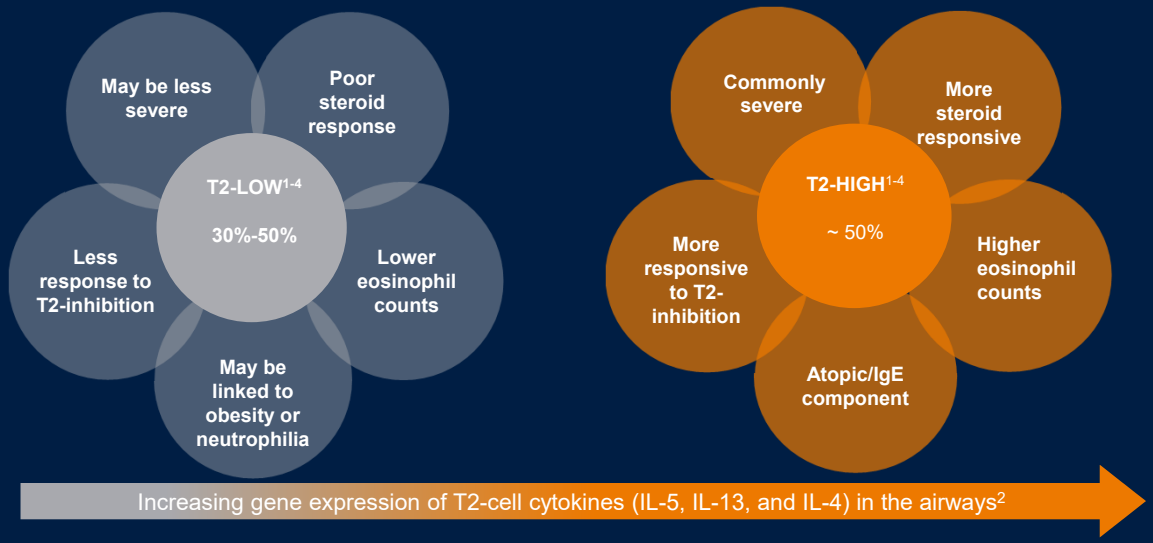
Th2 Hi: Thick SBM and Robust CS Response





Woodruff P, et al AJRCCM 2009

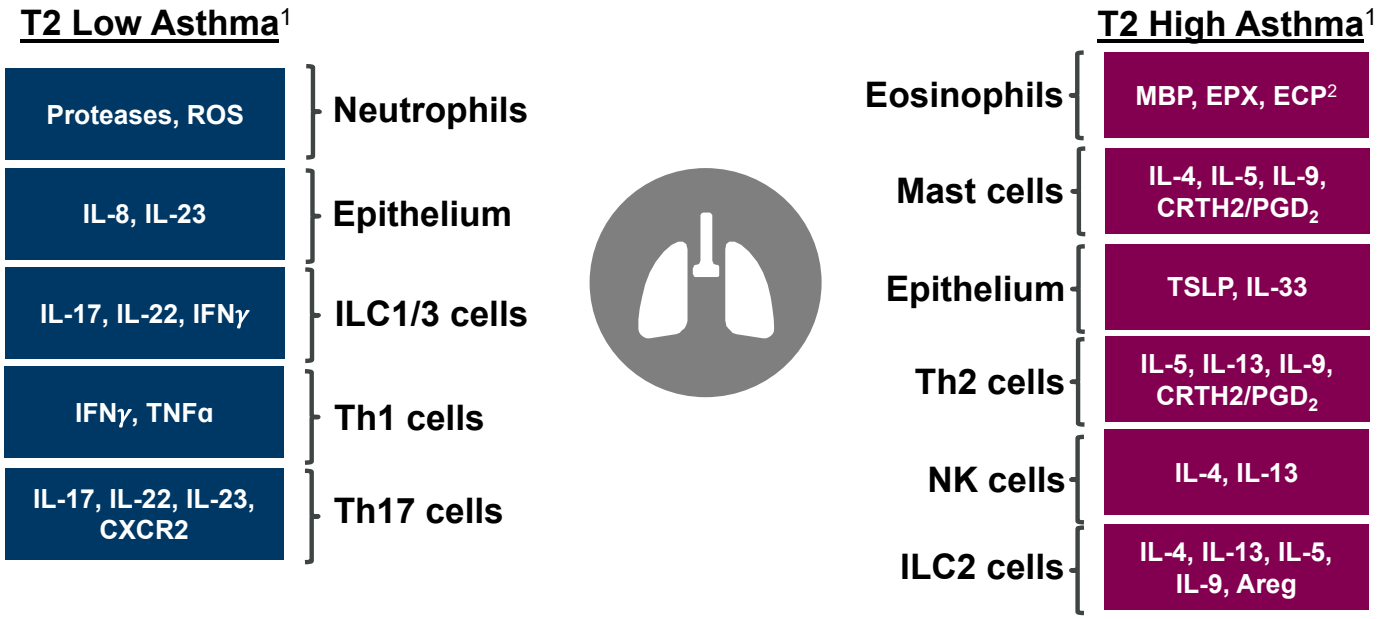
T2-Low and T2-High: Examples of Asthma Phenotypes[1,2]



IgE=immunoglobulin E; T2=T-helper cell type 2.

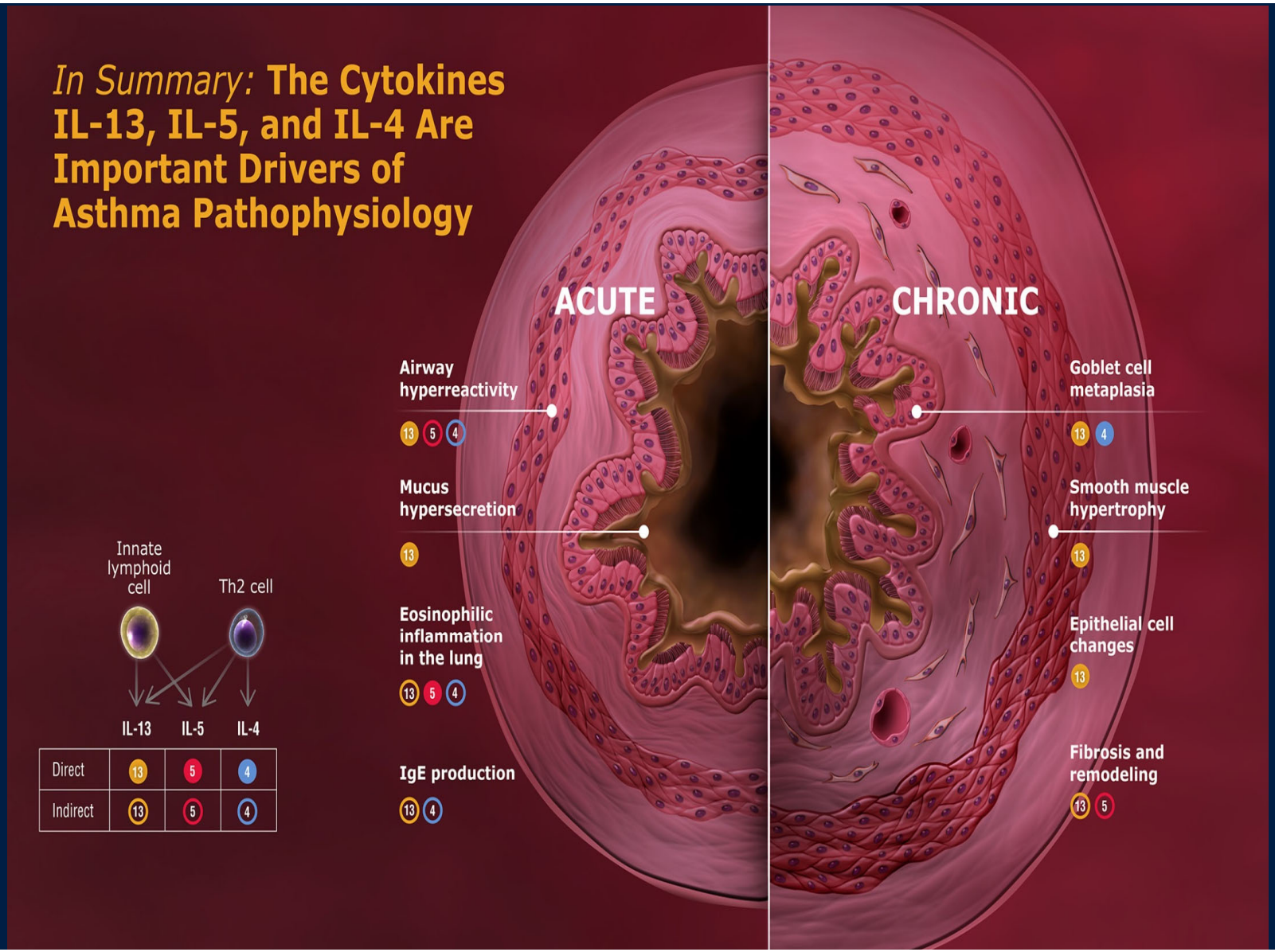
1. Fahy JV. *Nat Rev Immunol.* 2015;15(1):57-65.
2. Woodruff PG, et al. *Am J Respir Crit Care Med.* 2009;180:388-395.
3. Wenzel SE. *Nat. Med.* 2012;18(5):716-725.
4. Peters MC, et al. *J Allergy Clin Immunol.* 2014;133(2):388-394.

Different Patterns of Inflammation Can Drive Asthma

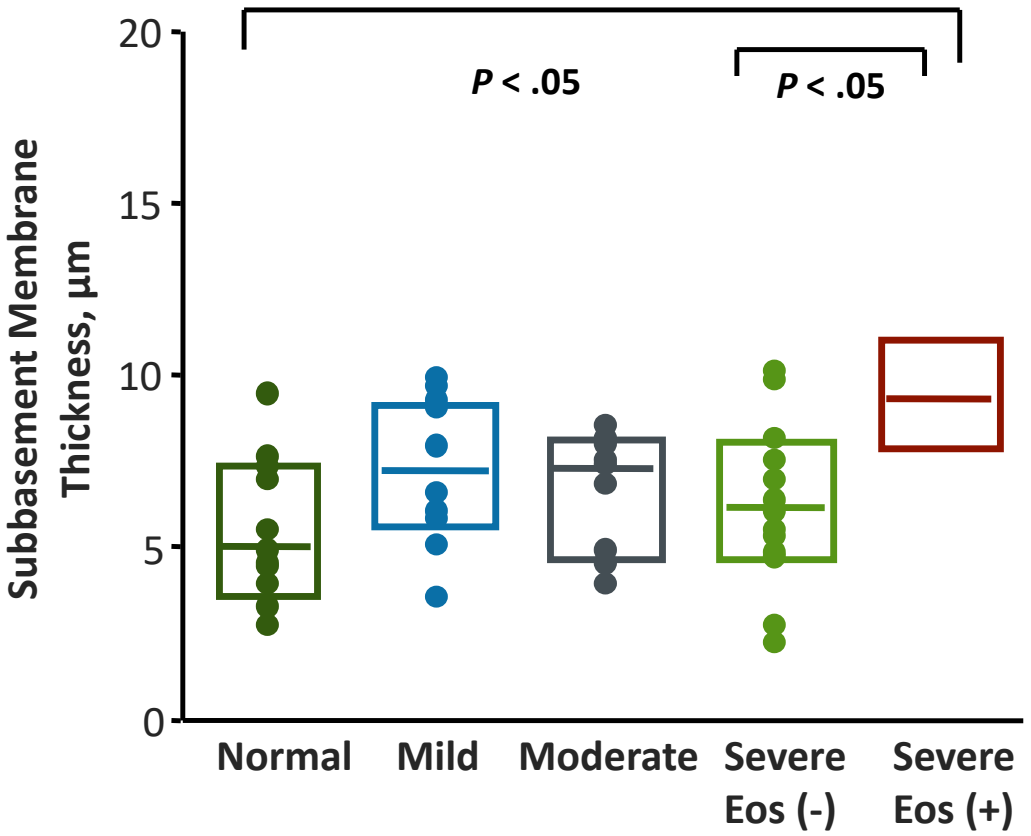


Areg=amphiregulin; CRTH2=chemoattractant receptor homologous molecule on T2 cells; CXCR2=C-X-C motif chemokine receptor 2; ECP=eosinophil cationic protein; EPX=eosinophil peroxidase; IFN=interferon; ILC1=type 1 innate lymphoid cells; ILC2=type 2 innate lymphoid cells; ILC3=type 3 innate lymphoid cells; MBP=major basic protein; NK=natural killer; PGD_2=prostaglandin D_2; ROS=reactive oxygen species; Th=T helper cells; TNF=tumor necrosis factor; TSLP=thymic stromal lymphopoietin.

1. Adapted from Tabatabaian F, et al. *Immunol Allergy Clin North Am.* 2017;37:329-343. 2. Carr TF, et al. *World Allergy Organ J.* 2016;9:21.



SBM Thickness Associated With Eosinophilic Phenotype



Wenzel SE, et al. *Am J Respir Crit Care Med*. 1999;160:1001-1008.

Increased Eosinophils in Sputum Are Strongly Associated With Increased Asthma Severity

	Eosinophils ($x10^3$/g)	
Group	**Median**	**Range**
Control	9	0-135
Intermittent asthma	45	4-220
Mild to moderate persistent asthma	199	4-2,227
Severe persistent asthma	305	12-10,800

p<0.05
p<0.001
p<0.001

- **Patients with severe asthma not treated with an OCS had a 44-fold higher median eosinophil count (p<0.0001) compared with individuals in the control group**
- **Despite treatment with high doses of ICS or with OCS, prominent eosinophilic inflammation was noted**

ICS = inhaled corticosteroids; OCS = oral corticosteroids.
Louis R et al. *Am J Respir Crit Care Med*. 2000;161:9-16.

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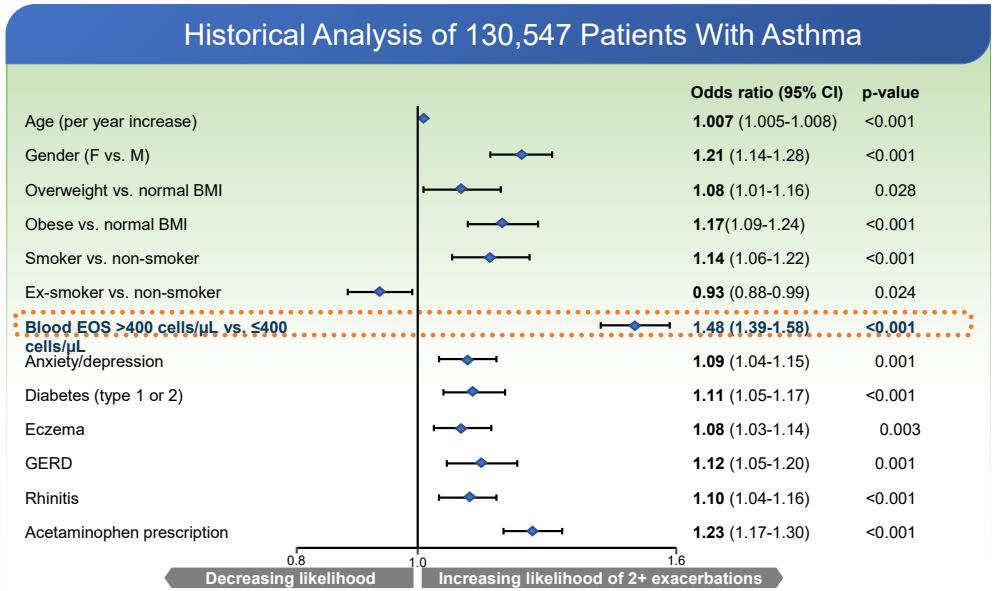
Increased Eosinophils in Asthma: Major Risk Factor for Exacerbations



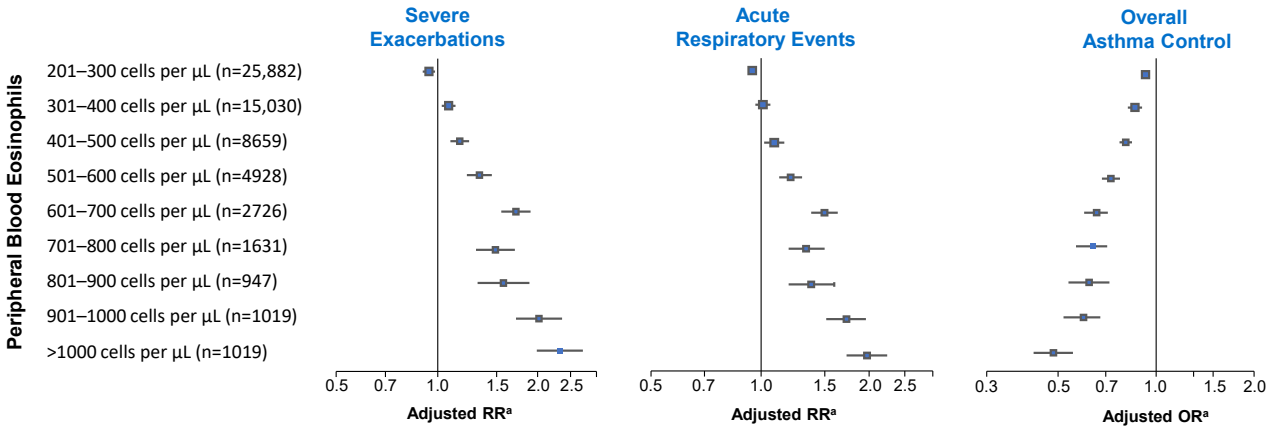
Adapted from Price D et al. *J Asthma Allergy*. 2016;9:1-12.

Blood eosinophil >400 cells/µL:

- **Single best predictor of multiple exacerbations**
- **↑ likelihood of ≥2 exacerbations by 1.5-fold**

BMI = body mass index; CI = confidence interval; EOS = eosinophils; F = female; GERD = gastroesophageal reflux disease; M = male.
Price D et al. J *Asthma Allergy*. 2016;9:1-12.

Peripheral Blood Eosinophil Levels Have Been Correlated With Both Asthma Severity and Control



Severe Exacerbation - an asthma-related hospitalization, attendance at an accident and an emergency department, or a prescription for acute oral OCS

Acute Respiratory Event - defined more broadly as an asthma-related hospital attendance or admission, or accident and an emergency department attendance, a prescription for acute OCS, or prescription for antibiotics in conjunction with an asthma-related primary care consultation

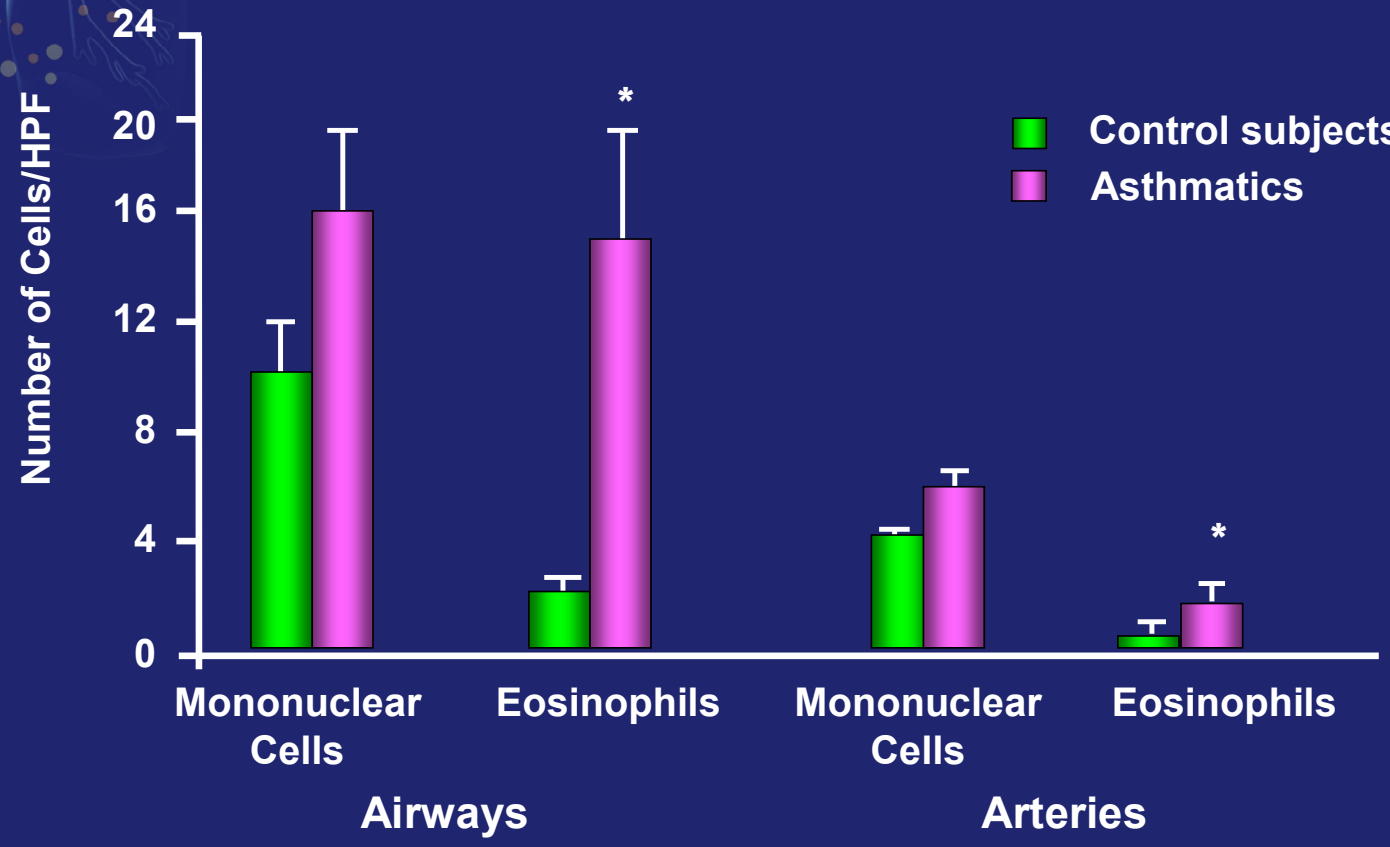
Overall Asthma Control - the absence of any acute respiratory event (as defined above) or asthma-related outpatient department visit with an average daily dose of 200 µg or less of salbutamol or 500 µg or less of terbutaline

[a]Data from medical records of patients with asthma who were aged 12-80 years and had 2 years of continuous records, including 1 year before (baseline) and 1 year after (outcome) their most recent eosinophil count. Patients were assigned to 9 eosinophil count categories and were compared with a reference category of ≤200 cells/µL (n=68,407). Data adjusted for age, gender, body mass index, smoking status, and Charlson comorbidity index score.

OCS = oral corticosteroids; OR = odds ratio; RR = rate ratio.

Price DB et al. *Lancet Respir Med.* 2015;3:849-858.

Eosinophils in Small Airways (Autopsies)

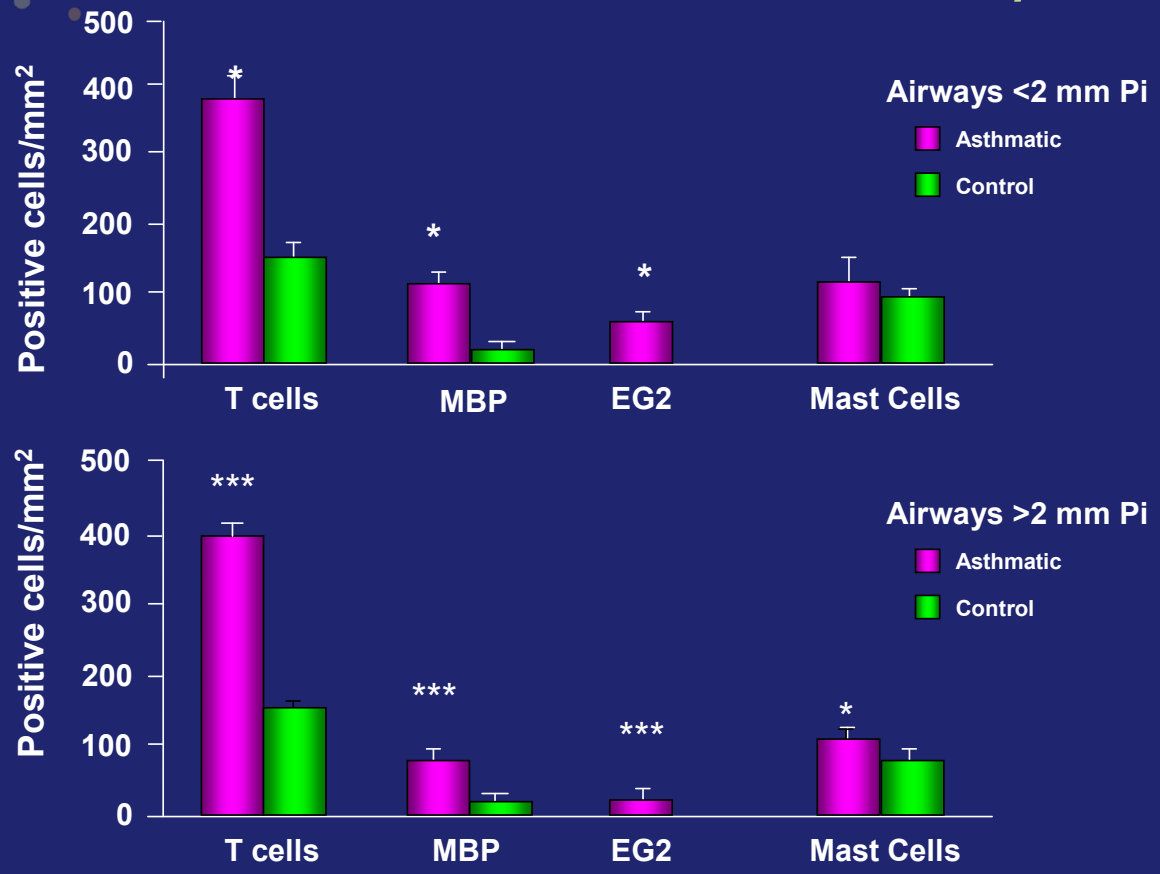


*$P<0.05$ vs. control subjects

HPF = High Power Field

Saetta M, et al. *Am Rev Respir Dis.* 1991;143:138-143.

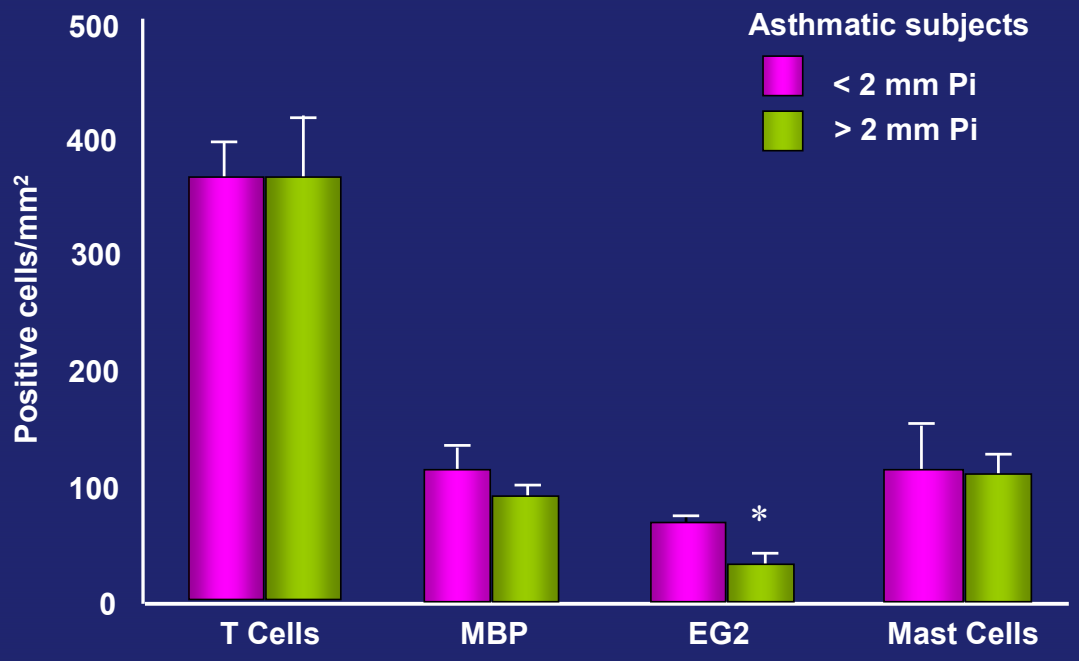
The Inflammatory Response in Asthma Is ***Not*** Restricted to the Proximal Airways



Pi: internal perimeter; MBP: major basic protein (total eosinophils); EG2: activated eosinophils

Tulic MK. *Chest.* 2003;123:348-355. Hamid Q, et al. *J Allergy Clin Immunol*. 1997;100:44-51.

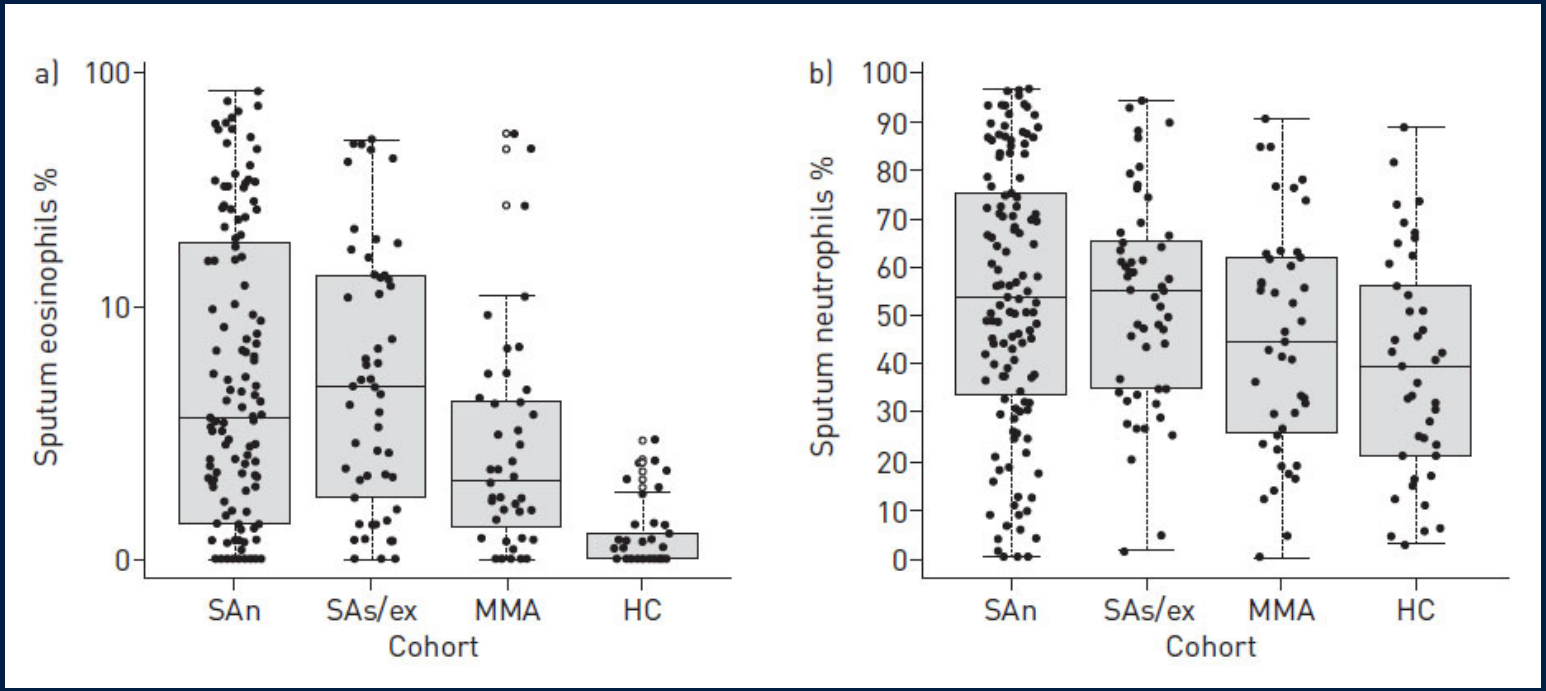
The Inflammatory Response in Asthma Is ***Not*** Restricted to the Proximal Airways



MBP: major basic protein; EG2: activated eosinophils
*$P < 0.05$ vs airways >2 mm Pi (internal perimeter)

Tulic MK, et al. *Respir Res.* 2001;2:333-339.

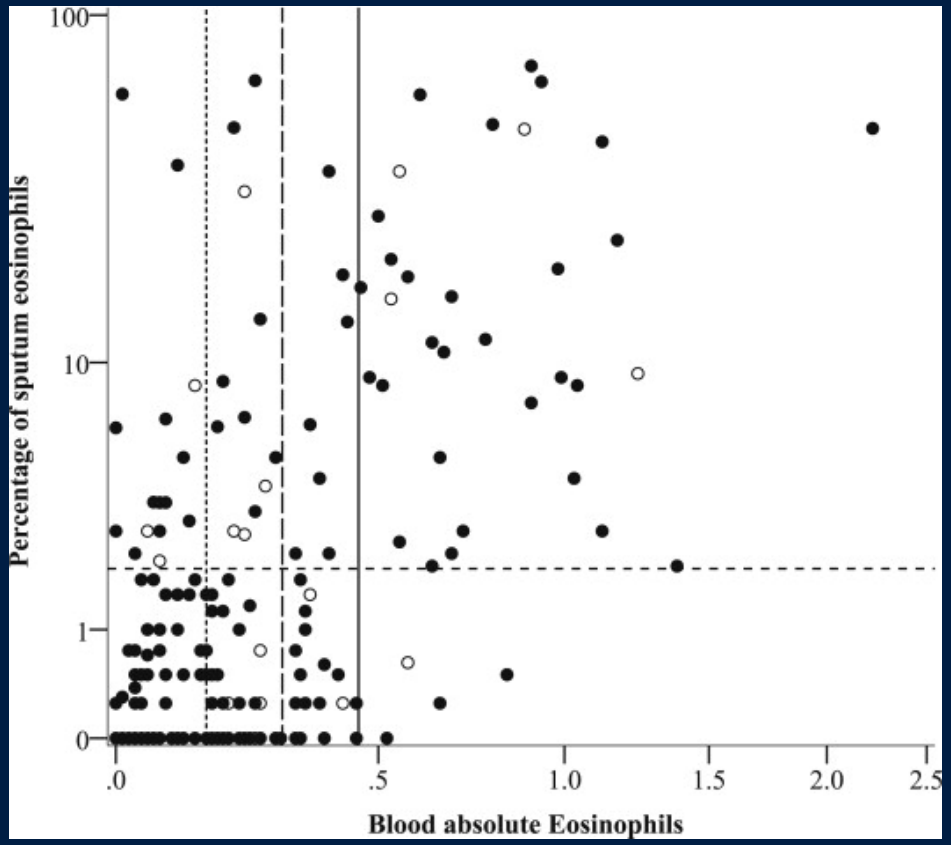
A) Sputum Eosinophil Count and B) Sputum Neutrophil Count, By Cohort



Shaw, DE et al. Eur Respir J 2015; 46: 1227-1231.

High Blood Eosinophil Counts Predict Sputum Eosinophilia In Patients With Severe Asthma

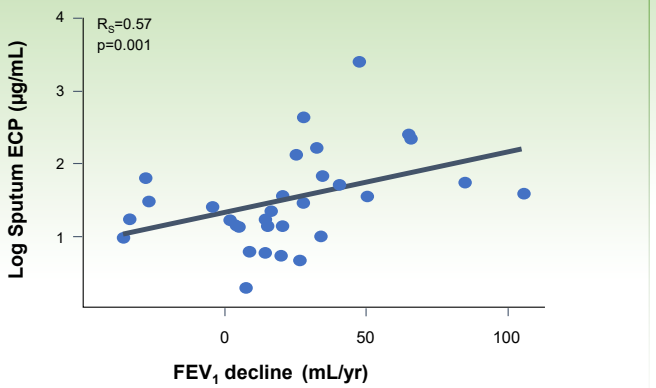
N=202



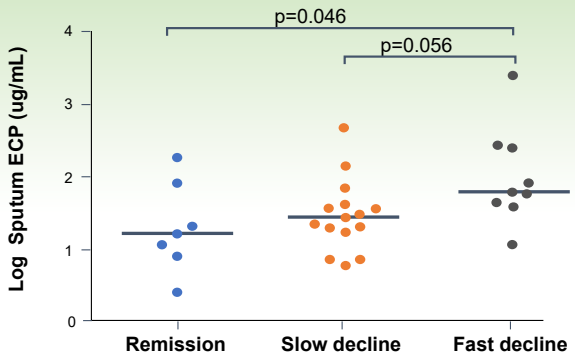
Fowler S, et al JACI; 2015: 135: 822–824.

Eosinophilic Inflammation Correlates With Deterioration in Lung Function

Correlation of Sputum ECP Concentration With FEV_1 Decline[1]



Adapted from Broekema M et al. *Respir Med.* 2010;104:1254-1262.



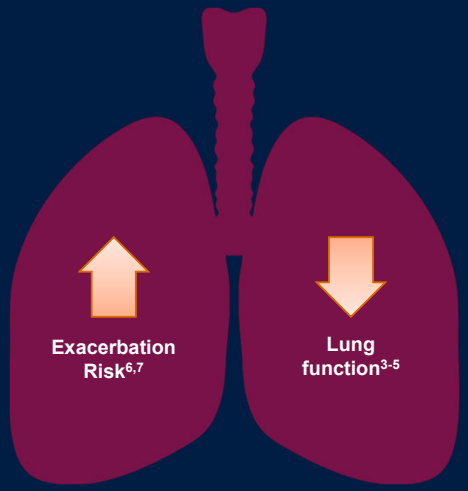
Adapted from Broekema M et al. *Respir Med.* 2010;104:1254-1262.
Note: Horizontal bars represent median values.

ECP is a protein released during degranulation of eosinophils that can be used as a biomarker of eosinophilic activity.[2]

ECP = eosinophil cationic protein; FEV_1 = forced expiratory volume in 1 second.

1. Broekema M et al. *Respir Med.* 2010;104:1254-1262; 2. Björk A et al. *Allergy.* 2000;55:442-448.

Summary: Increased Eosinophils in Asthma

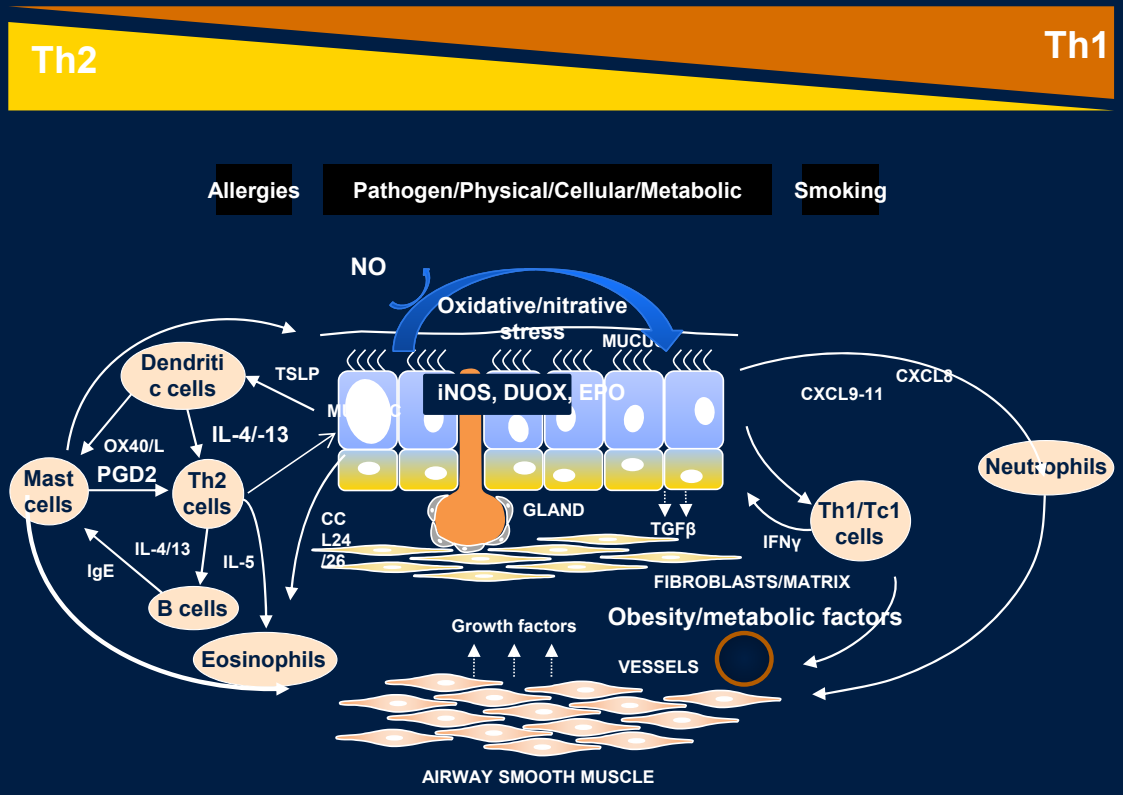


Elevated eosinophils were correlated with:

- Increased asthma severity[1,2]
- Worsening lung function[3-5]
- Increased risk of exacerbations[6,7]
- Increased rates of hospitalizations and ED visits[6]

1. Bousquet J et al. *N Engl J Med.* 1990;323;1033-1039; 2. Louis R et al. *Am J Respir Crit Care Med.* 2000;161;9-16; 3. Broekema M et al. *Respir Med.* 2010;104:1254-1262; 4. Woodruff PG et al. *J Allergy Clin Immunol.* 2001;108:753-758; 5. McGrath KW et al. *Am J Respir Crit Care Med.* 2012;185:612-619; 6. Zeiger RS et al. *J Allergy Clin Immunol Pract.* 2014;2:741-750; 7. Price D et al. *J Asthma Allergy.* 2016;9:1-12.

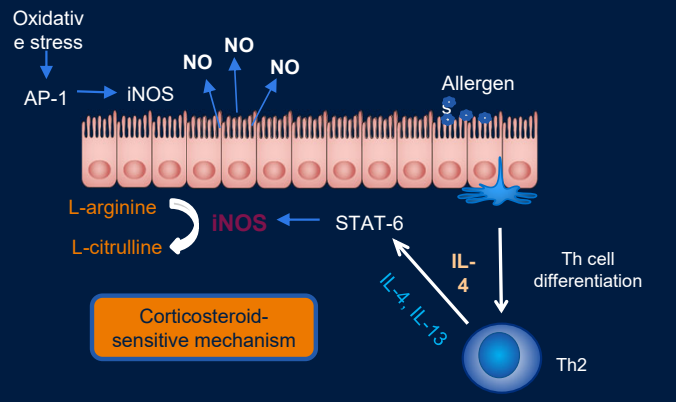
Pathobiology may involve multiple different immune/cellular pathways



Nitric oxide production regulated by NOS enzymes, IL-4, and IL-13

- Derived endogenously from L-arginine[1]
- Synthesis catalyzed by 3 forms of the NO synthetase (NOS) enzyme: 2 constitutive forms and 1 inducible form[1]
- In asthma, iNOS expression is upregulated by IL-4 and IL-13, leading to increased levels of FeNO[1,2]
  - NO production by iNOS is corticosteroid sensitive[2]

NOS forms[1]	Expresed in:	Role
Constitutive NOS (eNOS, cNOS)	Platelets, neuronal, epithelial and endotelial cells	Physiological regulation of airway function
Inducible NOS (iNOS)	Macrophages, neutrophils, hepatocytes, epithelial, mesangial, endothelial and vascular smooth muscle cells	Produced in response to airway inflammation and in host defense against infection

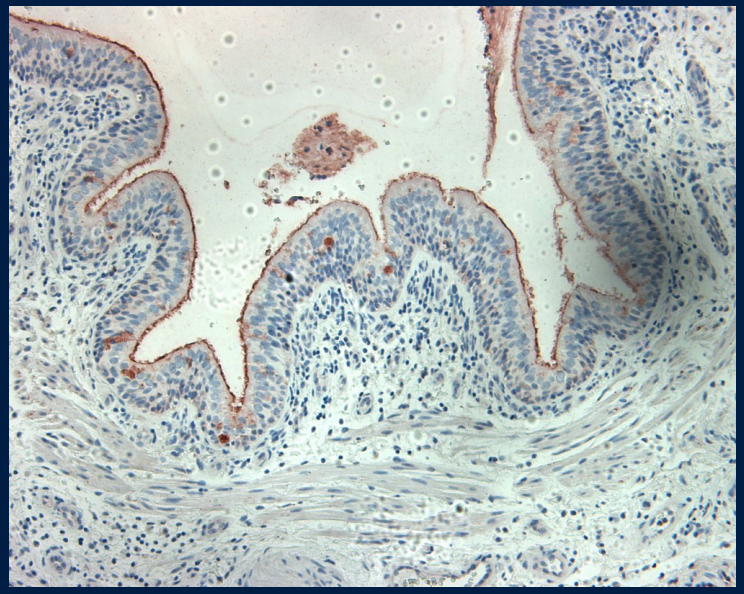


Adapted from Menzies-Gow,[1] Alving et al,[3] and Duong-Quy et al[4]

NO=nitic oxide; AP=activator protein; IL-interleukin; STAT=signal transducer and activator of transcription; Th2= T-helper cell type 2

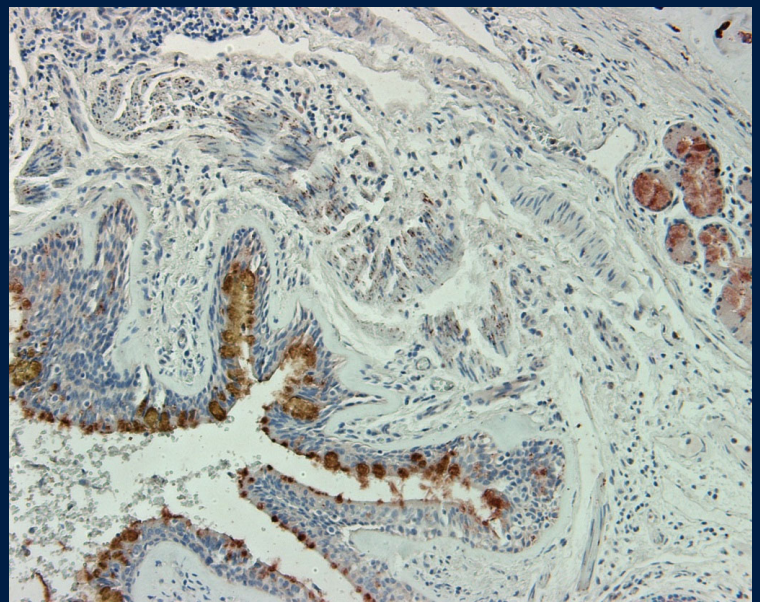
1. Menzies-Gow A et al. *Eur Respir J.* 2020; doi.org/10.1183/13993003.01633-2019. 2 Hoyte et al. *Immunol Allergy Clin N Am.* 2018; 38:573–585. 3. Alving K, Malinosvchi A. *Eur Respir Mon.* 2010;49: 1–31. 4. Duong-Quy C. *J Asthma Allergy.* 2019:12 331–341.

iNOS Staining



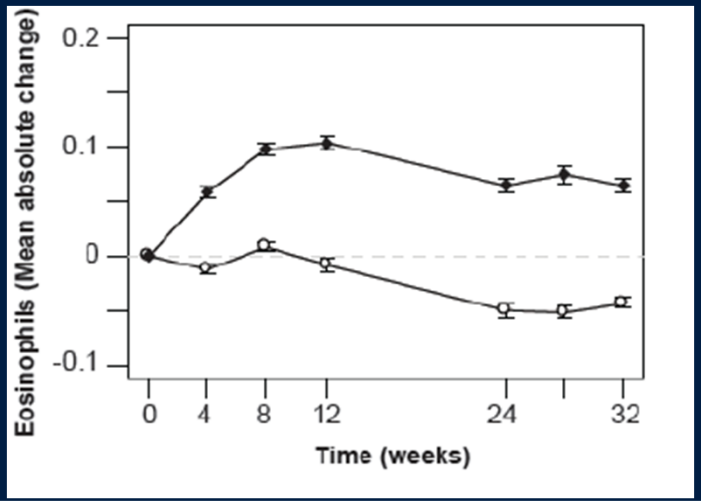
iNOS Stain in a Normal Subject

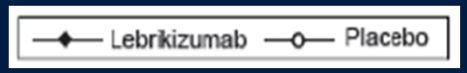
iNOS Stain in a Severe Asthmatic

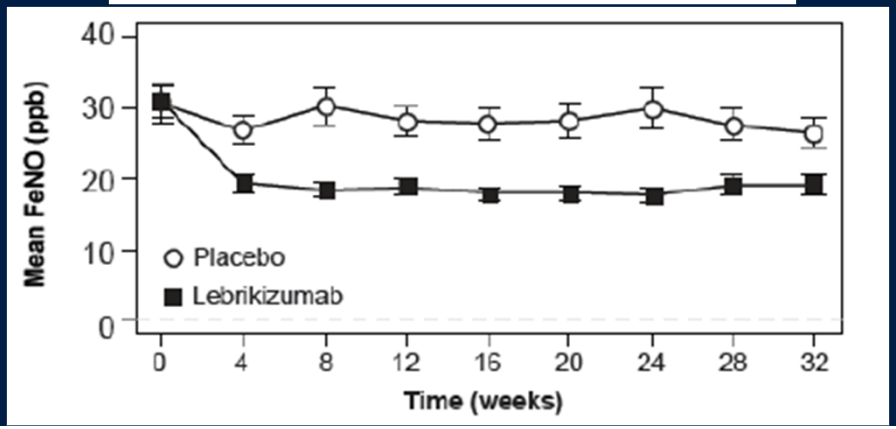


Courtesy Sally Wenzel and Silvana Balzar

FeNO and Eosinophils After Anti-IL-13 Treatment

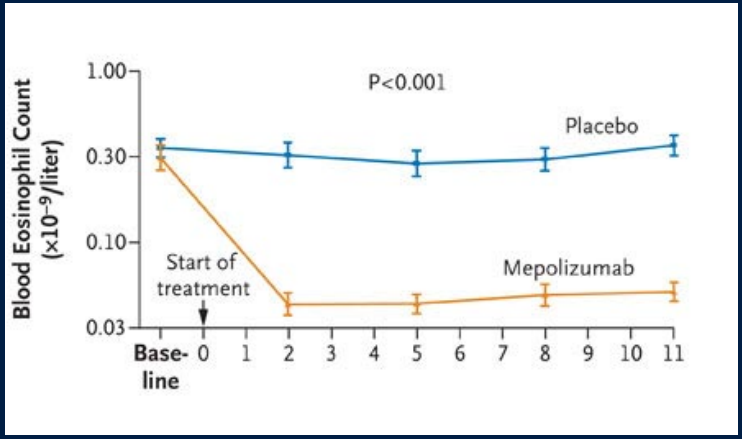


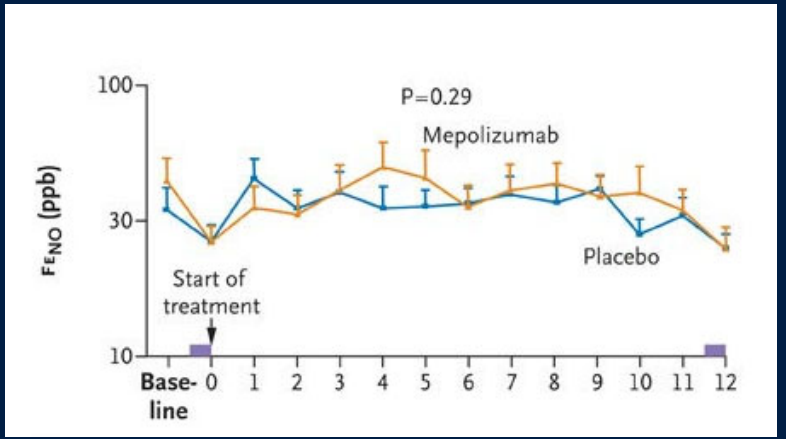




Adapted from Corren *et al* *NEJM* 2011; 365: 1088-98

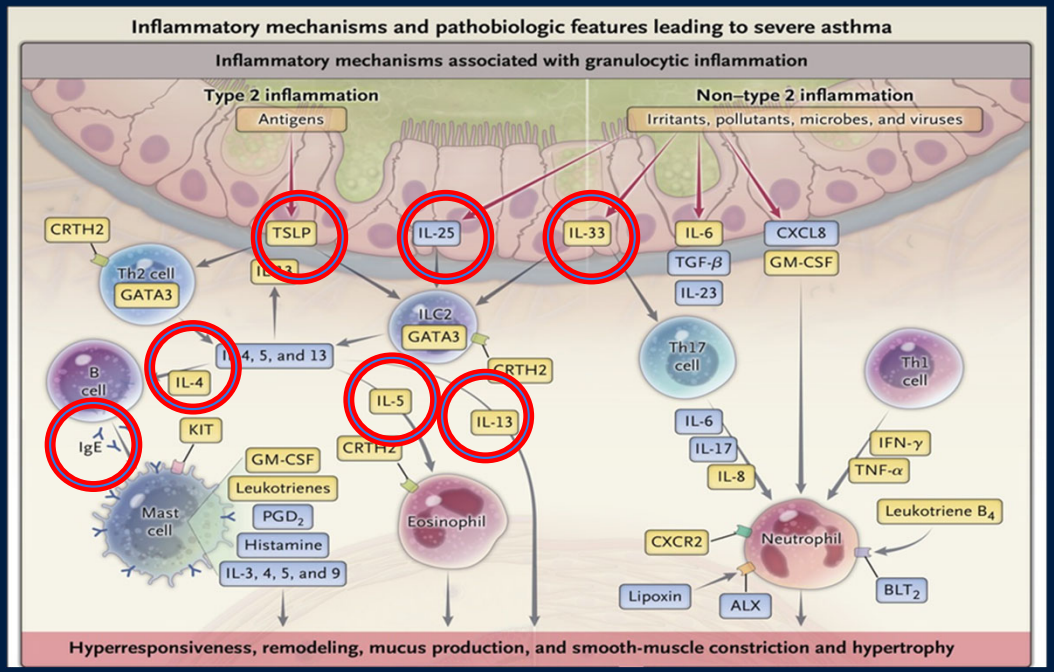
FeNO and Eosinophils After Anti-IL-5 Treatment





Adapted from Haldar et al; NEJM 2009; 360: 973-84

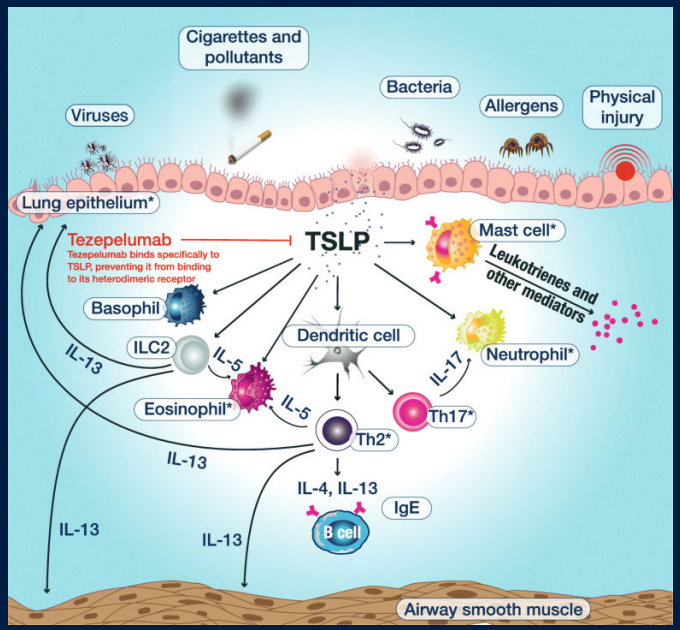
There are Many Potential Targets in Severe Asthma



From Israel E, Reddel HK. Severe and difficult-to-treat asthma in adults. Drazen JM, ed. *N Engl J Med*. 377(10):965-976. Copyright© 2017 Massachusetts Medical Society. Reprinted with permission from Massachusetts Medical Society.

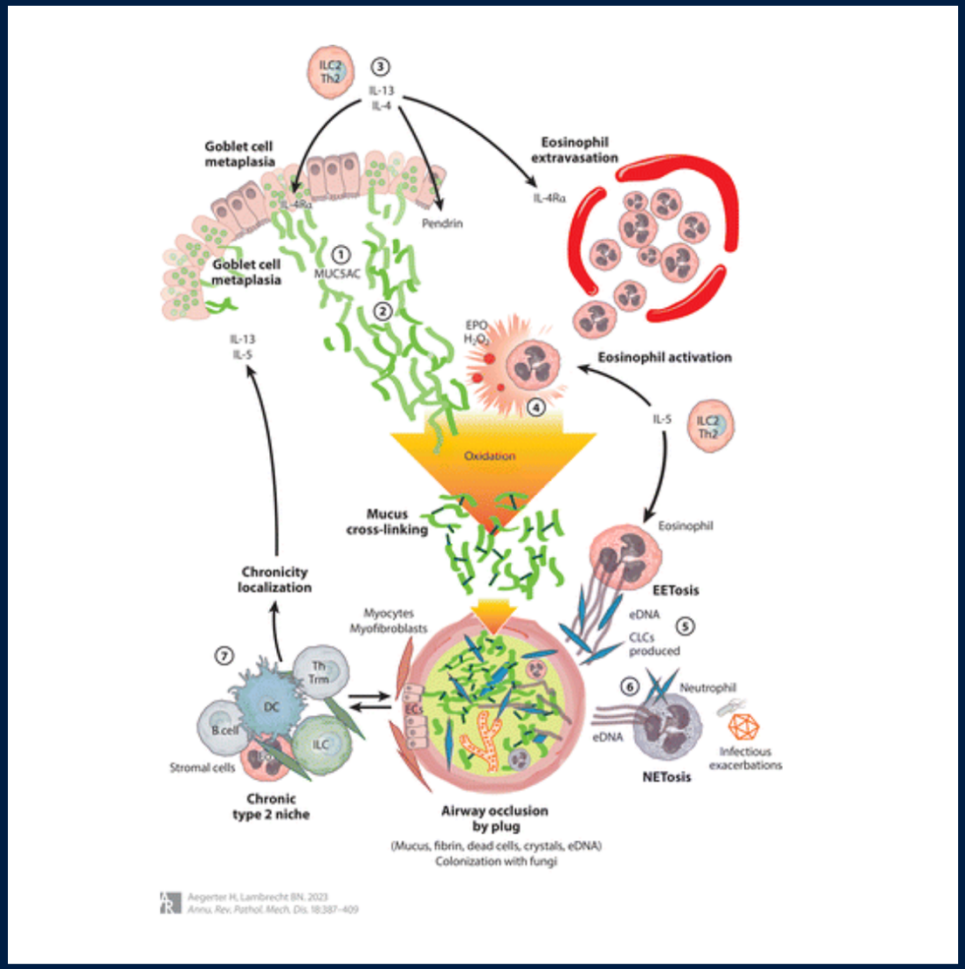
TSLP: A key epithelial cytokine in asthma

- Human TSLP is an epithelial-derived cytokine first described in 2001
- Variants at the TSLP gene loci have been associated with asthma risk
- TSLP expression:
  - higher in the airways of patients with asthma
  - correlates with Th2 cytokine and chemokine expression
  - correlates with disease severity

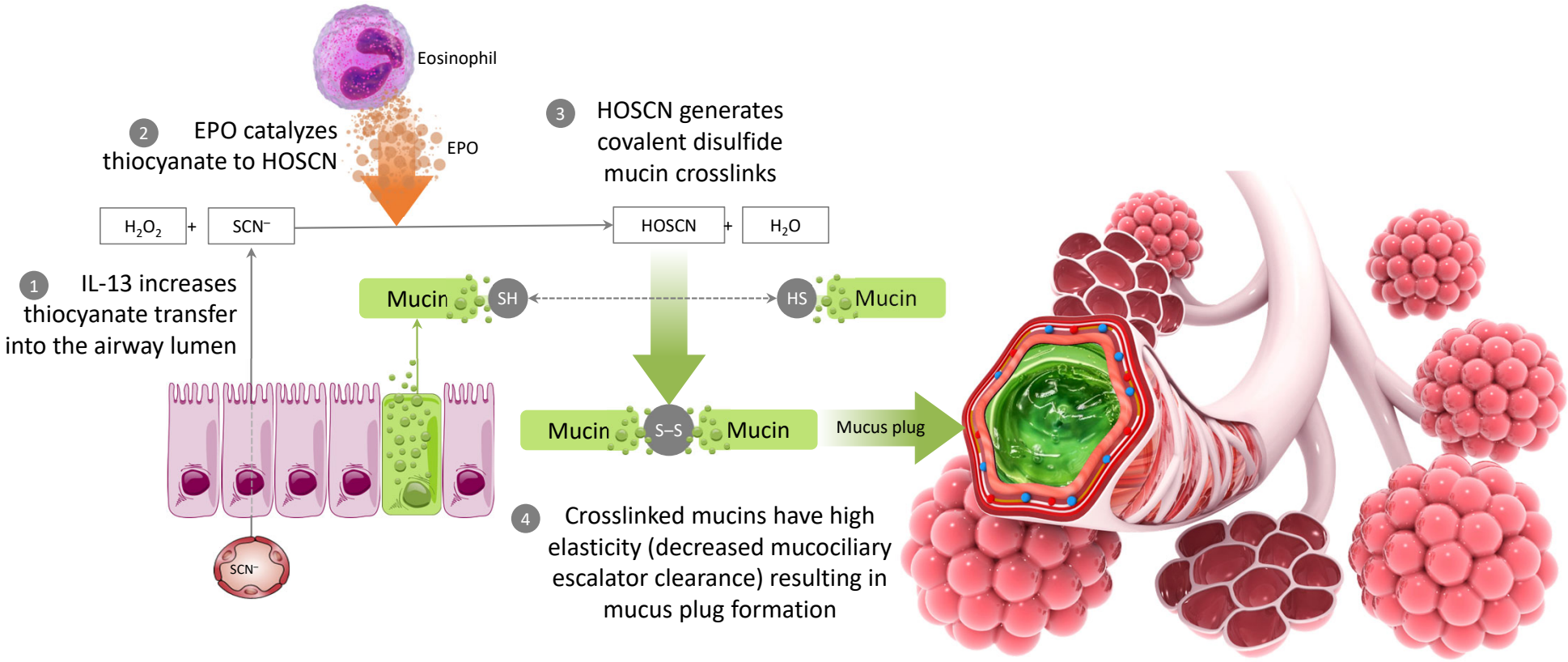


Emson, C., Diver, S., Chachi, L. *et al*. CASCADE: a phase 2, randomized, double-blind, placebo-controlled, parallel-group trial to evaluate the effect of tezepelumab on airway inflammation in patients with uncontrolled asthma. *Respir Res* **21,** 265 (2020). https://doi.org/10.1186/s12931-020-01513-x

1. Ziegler and Artis. *Nat Immunol* 2010;11:289–293. 2. Soumelis, et al. *Nat Immunol* 2002;3:673–680. 3. Allakhverdi, et al. *J Exp Med* 2007;204:253–258. Ziegler, et al. *Adv Pharmacol* 2013;66:129–155. 4. Shikotra, et al. *J Allergy Clin Immunol* 2012;129:104-11 e1-9. 5. Ying, et al. *J Immunol* 2005;174:8183–8190. 6. Ying, O'Connor B, et al. *J Immunol* 2008;181:2790–2798.



Eosinophils Promote Mucus Plug Formation



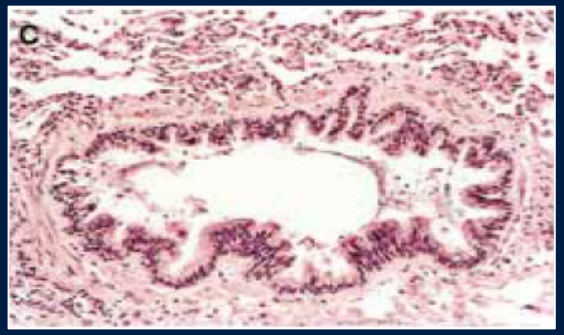
- EPO, eosinophil peroxidase; HOSCN, hypothiocyanous acid; SCN, thiocyanate

Dunican EM, et al. *J Clin Invest* 2018;128:997–1009.

Increased Eosinophils Contribute to Mucus Plugging

Elevated eosinophils in the lungs cause airway inflammation and damage

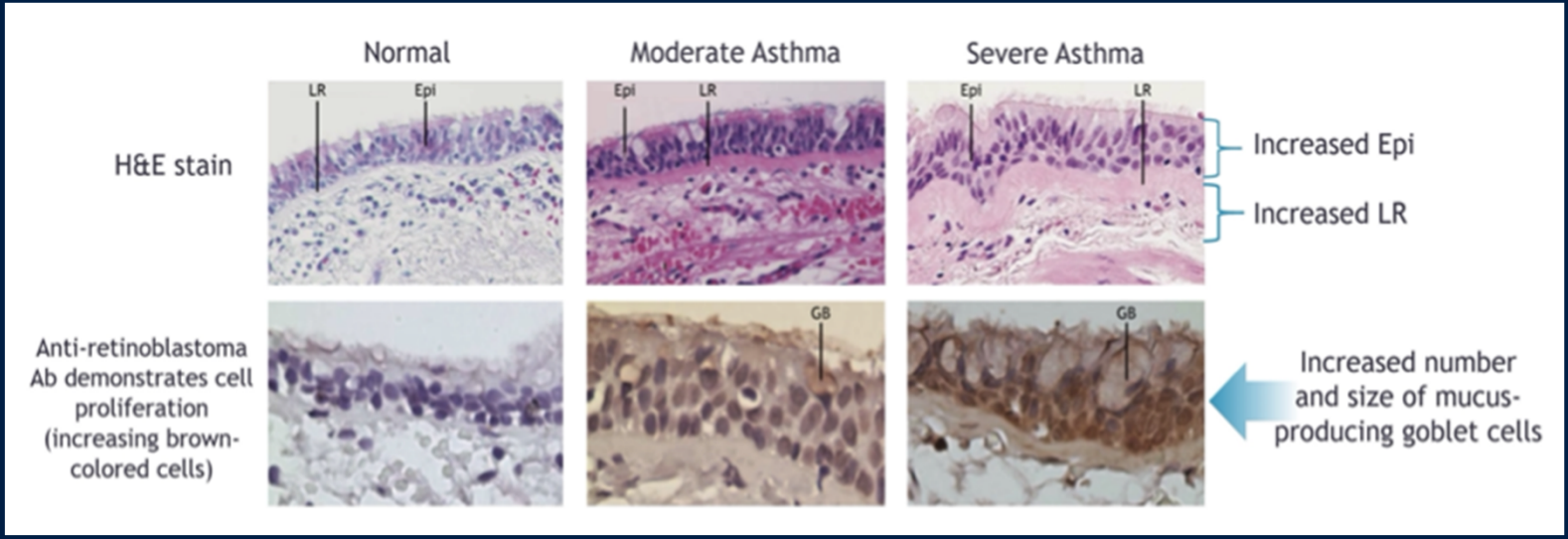
Normal airway[1]



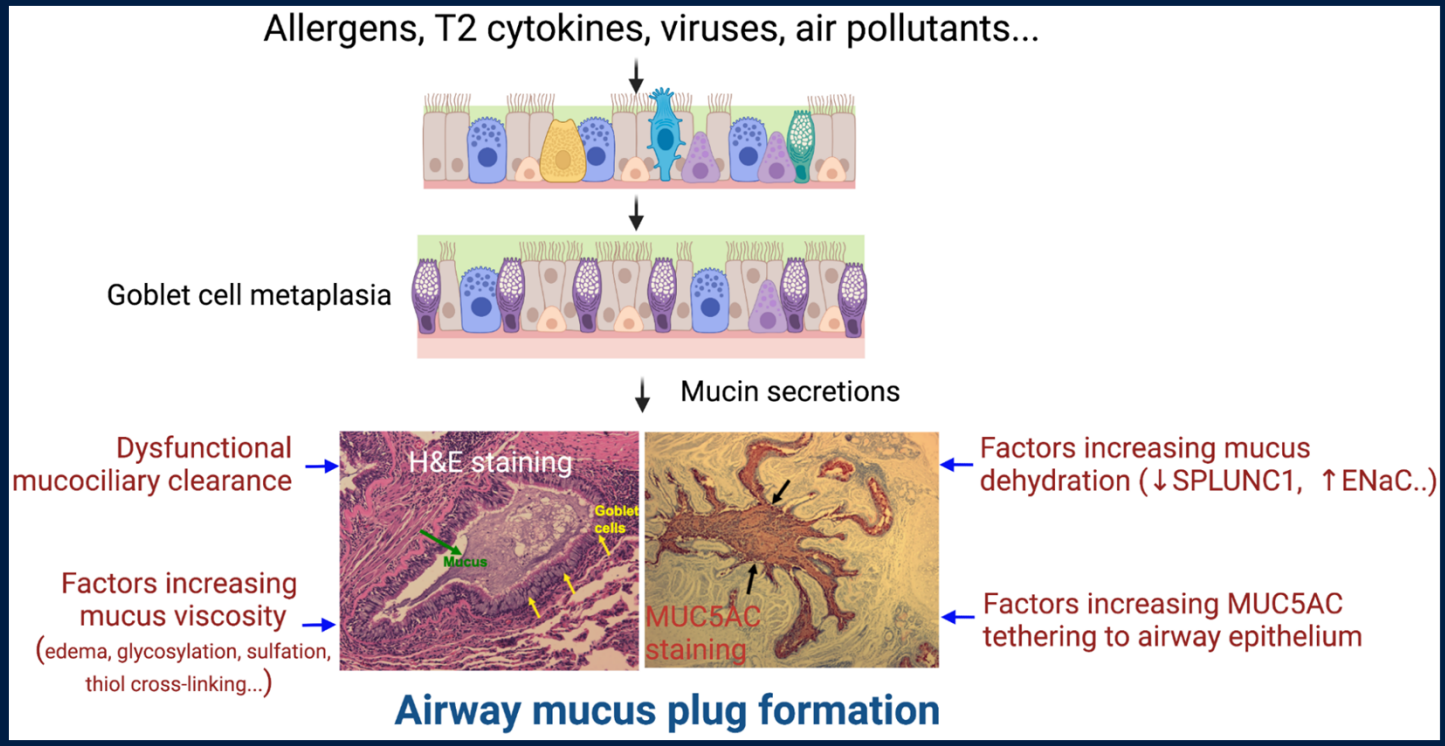
Airway of patient with fatal asthma[1]

Reprinted from J Allergy Clin Immunol, Vol 106/issue 6,William W. Busse MDa, Susan Banks-Schlegel PhDb, Sally E. Wenzel MDc , Pathophysiology of severe asthma;1033-1042;2000, with permission from Elsevier.

Mucus is Produced by Goblet Cells and Can Lead to Remodeling in Severe Asthma

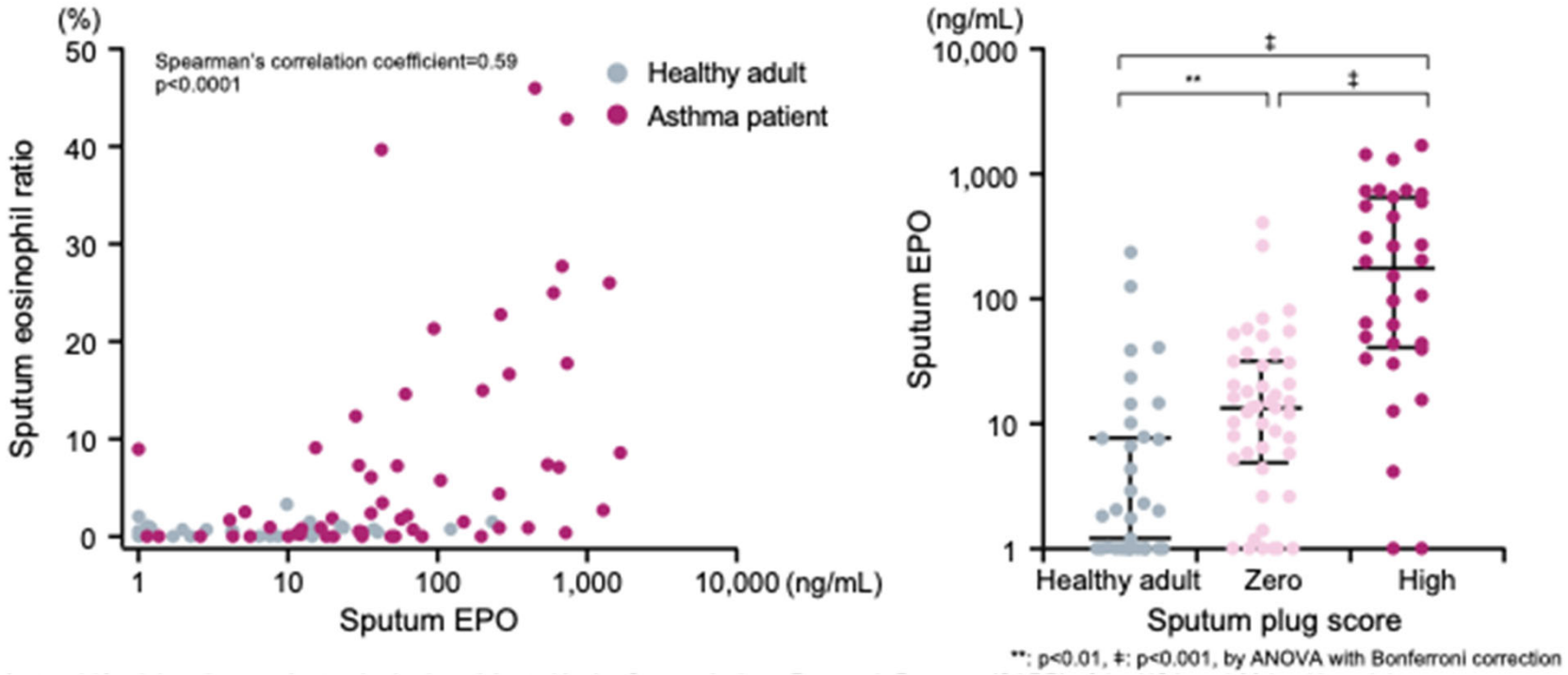


Shifren A et al. *J Allergy* (Cairo). 2012; 2012-316049.

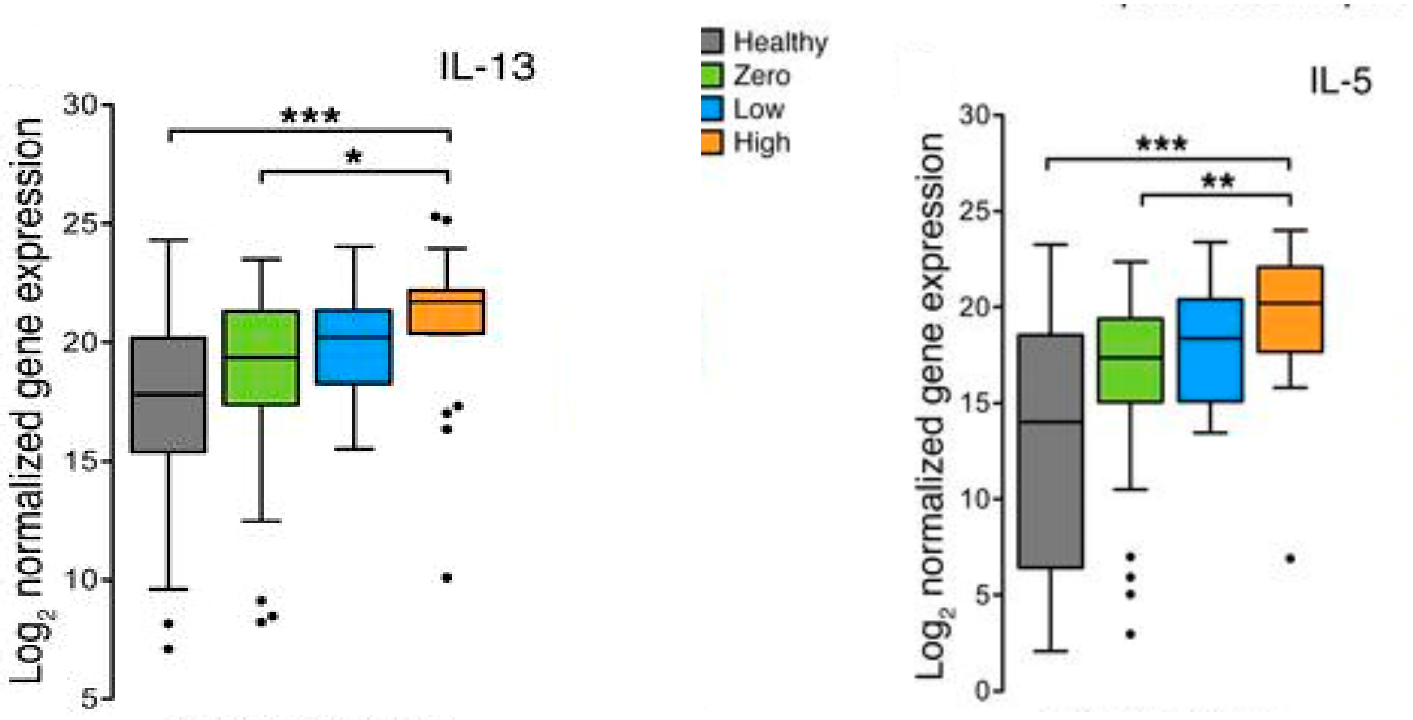


Eosinophil products are associated with mucus plugging

- Patients with a higher mucus plug score had larger amounts of EPO in the sputum



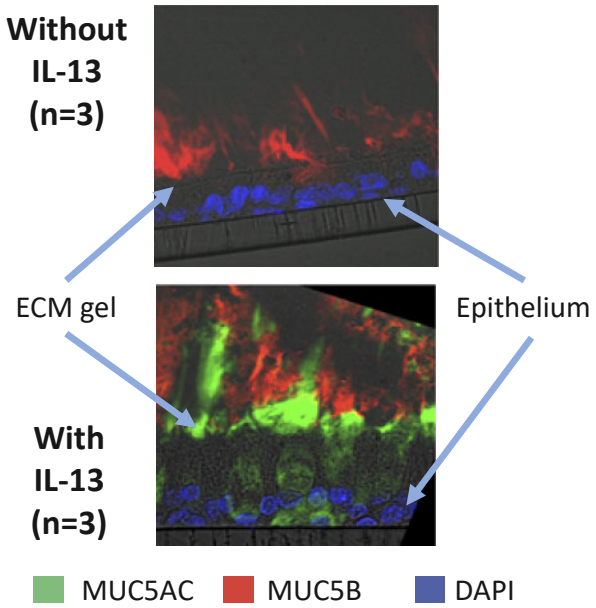
Type 2 Cytokine Expression is Associated with High Mucus Plug Scores



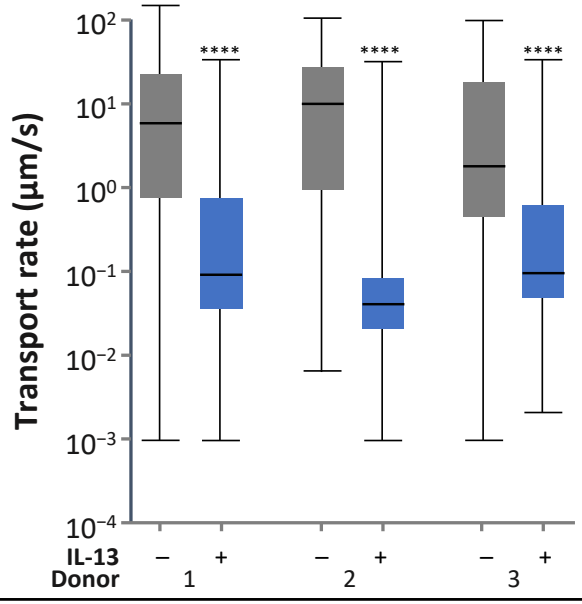
***P < 0.001; **P < 0.01; *P < 0.05. P values were determined by Kruskal-Wallis test with Dunn's correction

IL-13 Is a Key and Central Driver of Excess Mucus Production and Plugging

IL-13 Increases MUC5AC Expression (Cross-sectional Staining From HBE Cultures)[1]



Mucus Transport Is Impaired When MUC5AC Is Overexpressed (IL-13 Stimulated HBE Cultures)[1]



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p<0.05. ***p*<0.001. ****p*<0.0001. ECM, extracellular matrix; HBE, human bronchial epithelial; IL, interleukin.

Used with permission of American Society for Clinical Investigation, from Mucus plugs in patients with asthma linked to eosinophilia and airflow obstruction, Eleanor M Dunican, J Clin Invest 2018;128:997–1009 permission conveyed through Copyright Clearance Center, Inc.

Summary

- "Guideline" guided treatment is not optimal in at least 50% of patients
- Step 3 and above assessment of role of TH2 mechanism to guide therapy
- Recognition and treatment of co-morbidities

Summary

- Biologics are currently directed to TH2 pathways
- Macrolides may reduce exacerbation in non-eosinophilic asthma
- Biomarkers currently being studied most extensively are Th2 based in relation to current therapeutics