



# Diagnosis and Management of Fungal Allergy

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# Pulmonary Disease due to *Aspergillus*

- Disseminated or invasive aspergillosis
- Aspergilloma or mycetoma
- Bronchial asthma
- Extrinsic allergic alveolitis (malt worker's lung)
- Allergic bronchopulmonary aspergillosis

# Diagnostic Criteria for ABPA

## ■ Major Criteria

- Bronchial obstruction – asthma, CF
- Pulmonary infiltrates
- Positive skin test to *Aspergillus*
- Precipitating (IgG) antibodies to *Aspergillus*
- Elevated serum IgG, IgA & IgE anti-*Aspergillus*
- Elevated IgE level >1000 ng/ml
- Blood eosinophilia
- Proximal or central bronchiectasis

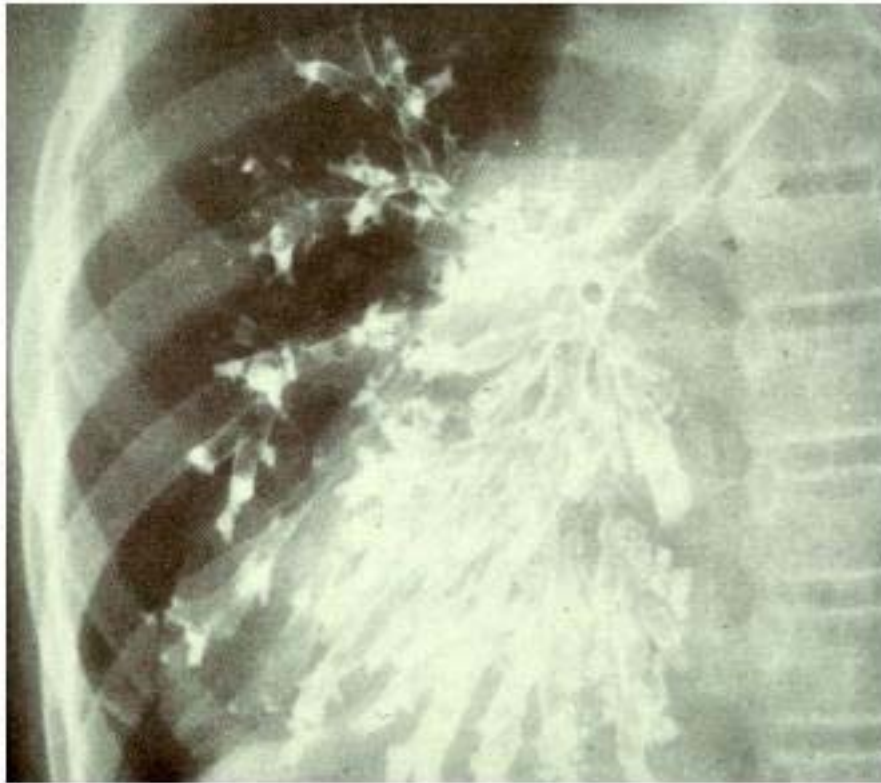
## ■ Minor Criteria

- Sputum culture positive for *A. fumigatus*
- Late-skin (Arthus) reactivity
- History of expectoration of brown plugs

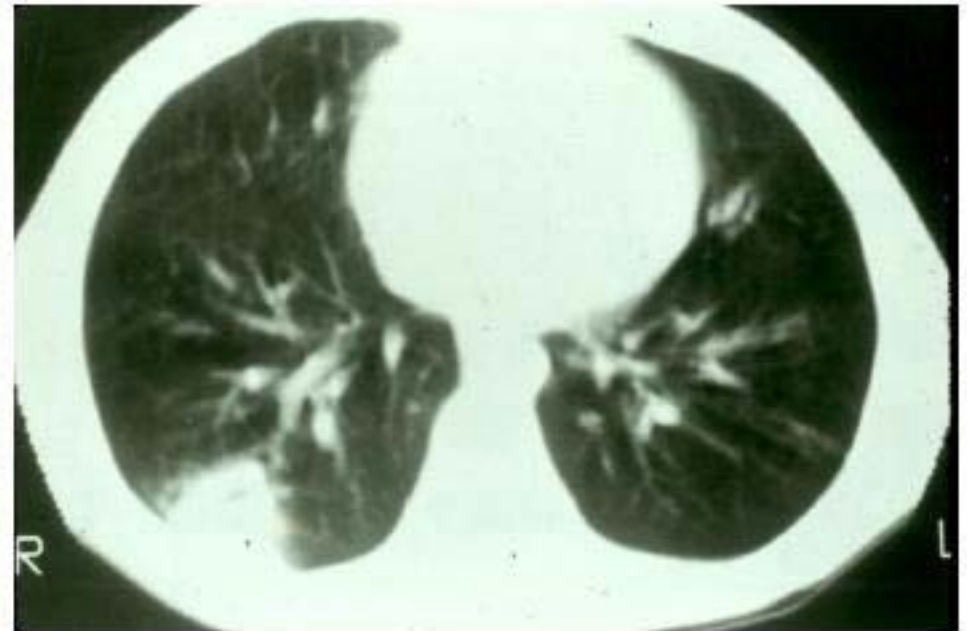
# Demographics of Patients with ABPA

Study	ABPA (41)	Non-ABPA (49)
CF	34%	50%
Asthma	66%	50%
Age, years	24 ± 18	17 ± 12
Sex, % male	58%	49%
IgE, IU/ml	2617 ± 2275	322 ± 1043
Reactivity to <i>A. fumigatus</i>		
Af skin test	100%	43%
IgG anti-Af antibody	100%	88%

# Bronchiectasis in ABPA

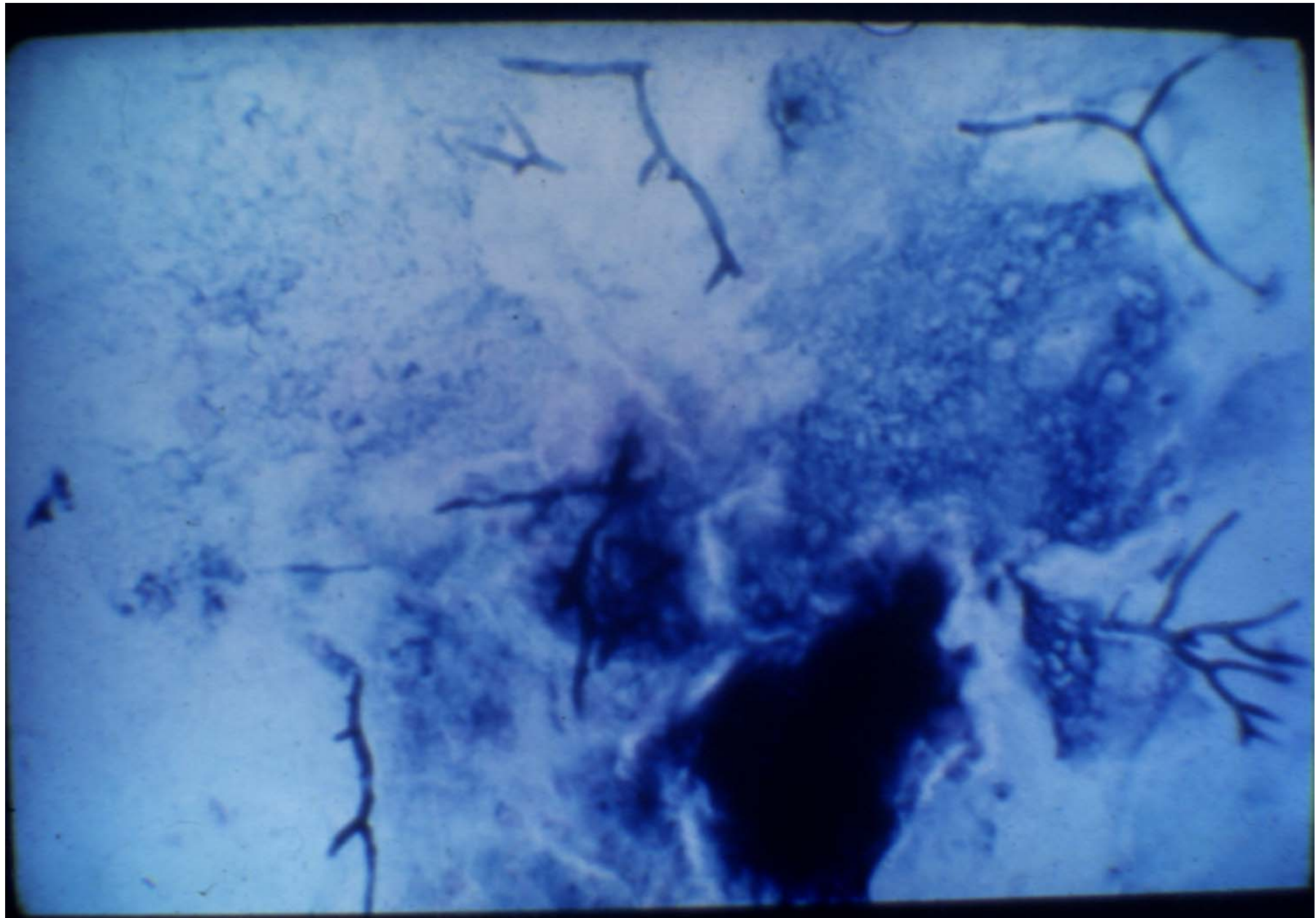


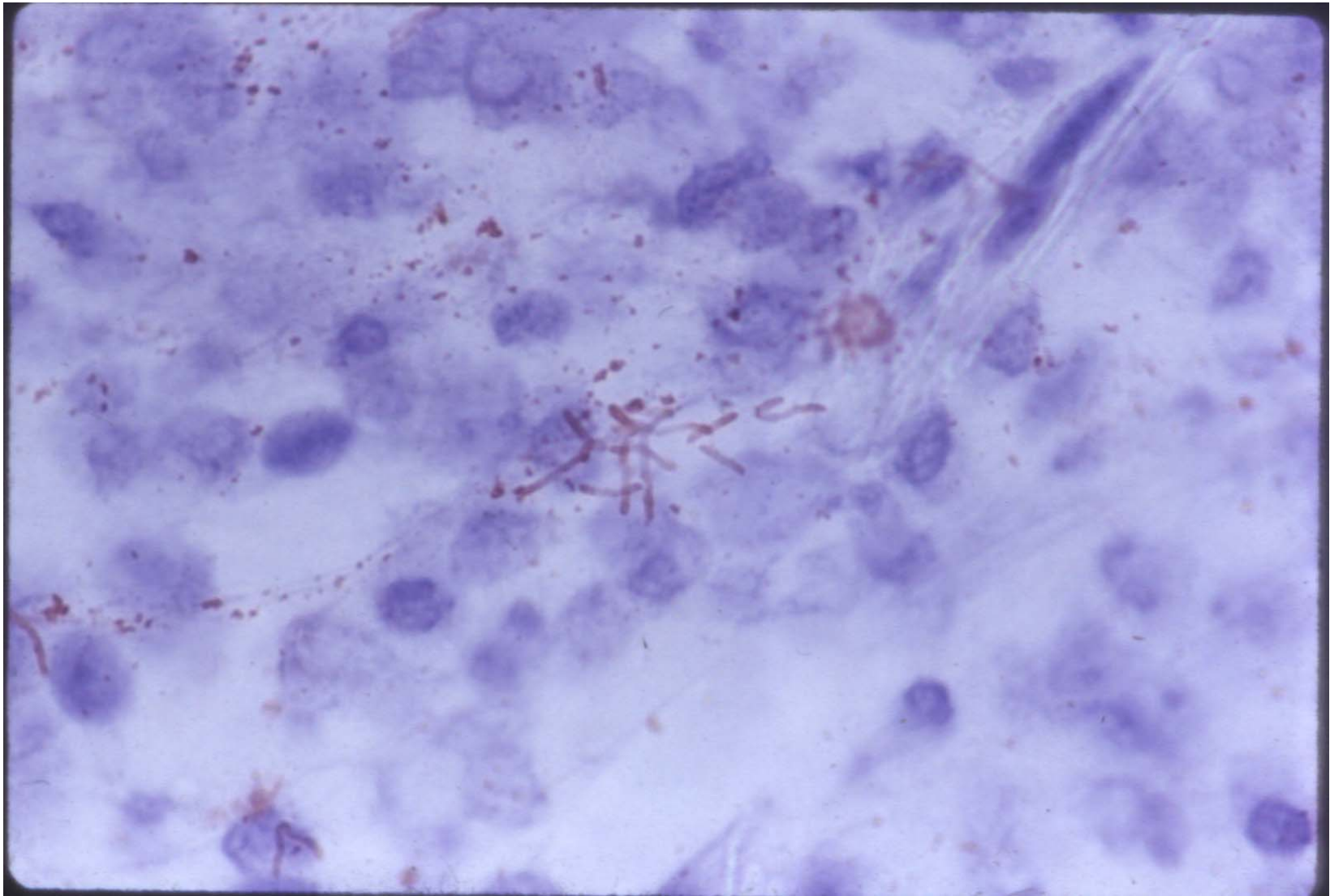
A

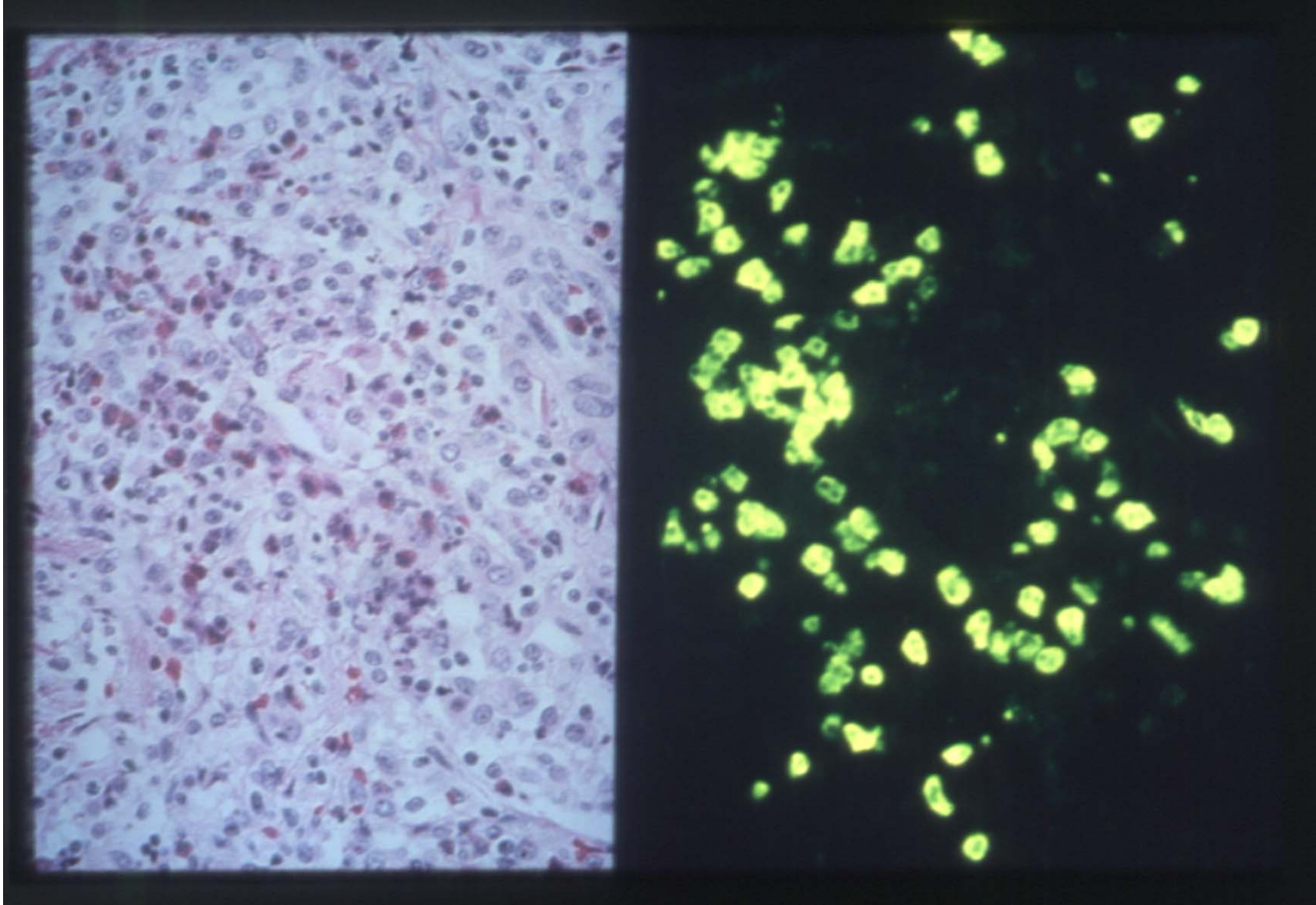


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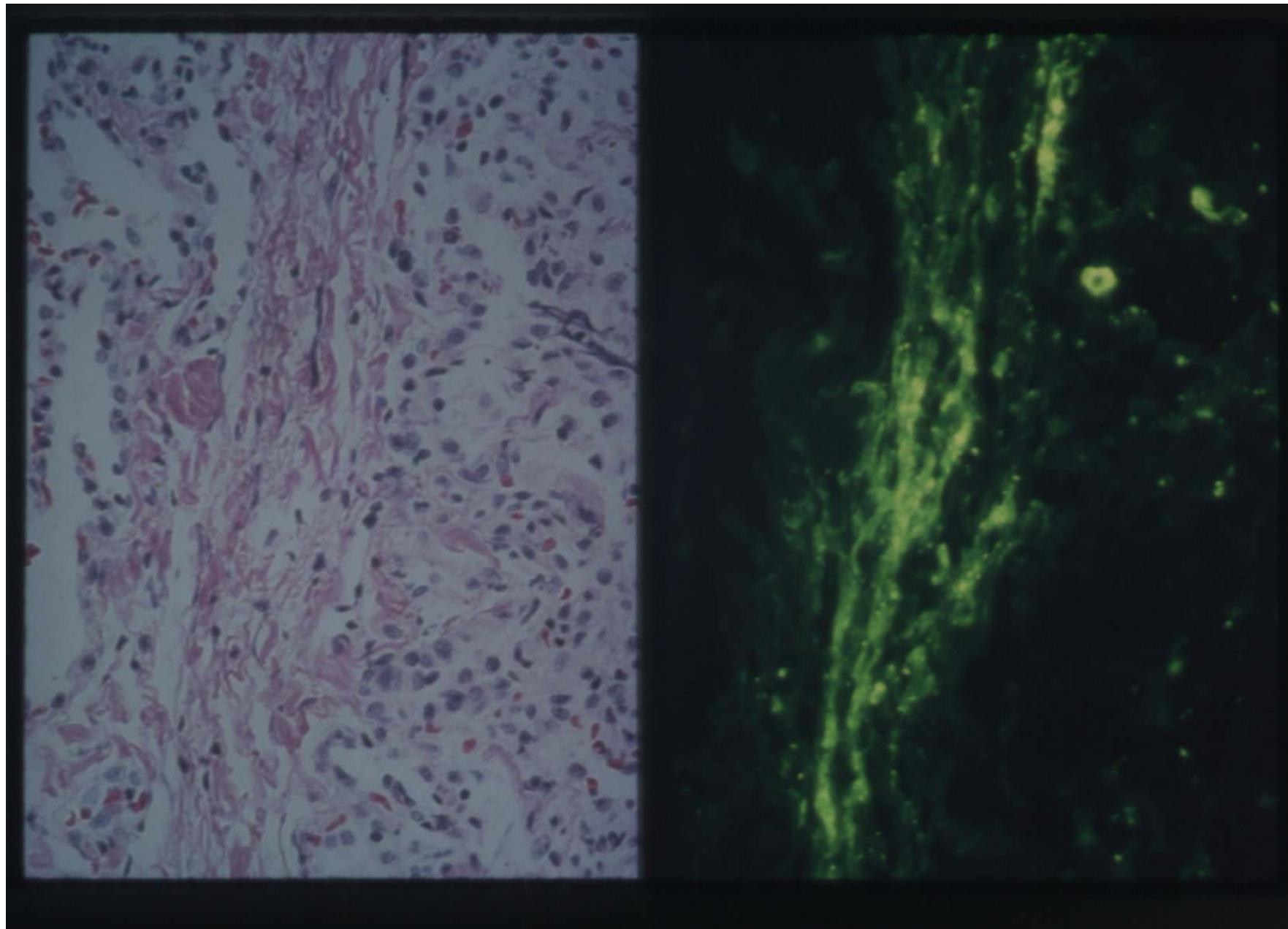
Figure 2.





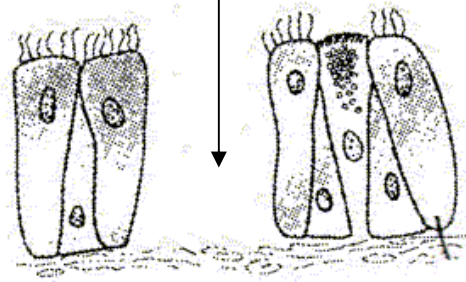






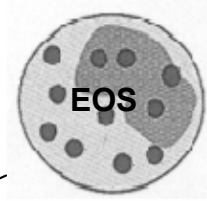
**Aspergillus Allergens + Proteases**

Activation, damage, leakage

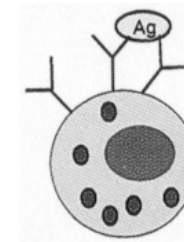


**Lung Damage**

ECP, MBP, EDN, EPO



Recruitment  
Activation

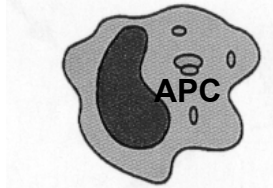


**Mast cell**  
FcεR1  
IgE priming

**Allergic Inflammation**

↑IL-8, MCP-1, IL-6  
↑Mucus  
↑TGF-β, ↓EGFR  
↑Myofibroblast activation  
**Airway remodeling**

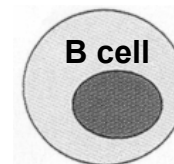
**HLA-DR2+/DR5+**



↑IL4 Activity  
↑IL-4Rα SNP

↑IL-5

↑IgE production

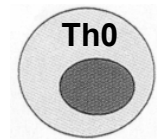


↑CD23, ↑CD86

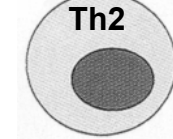
DC, Epi, SMC

CD40 CD86/CD80

CD40L CD28



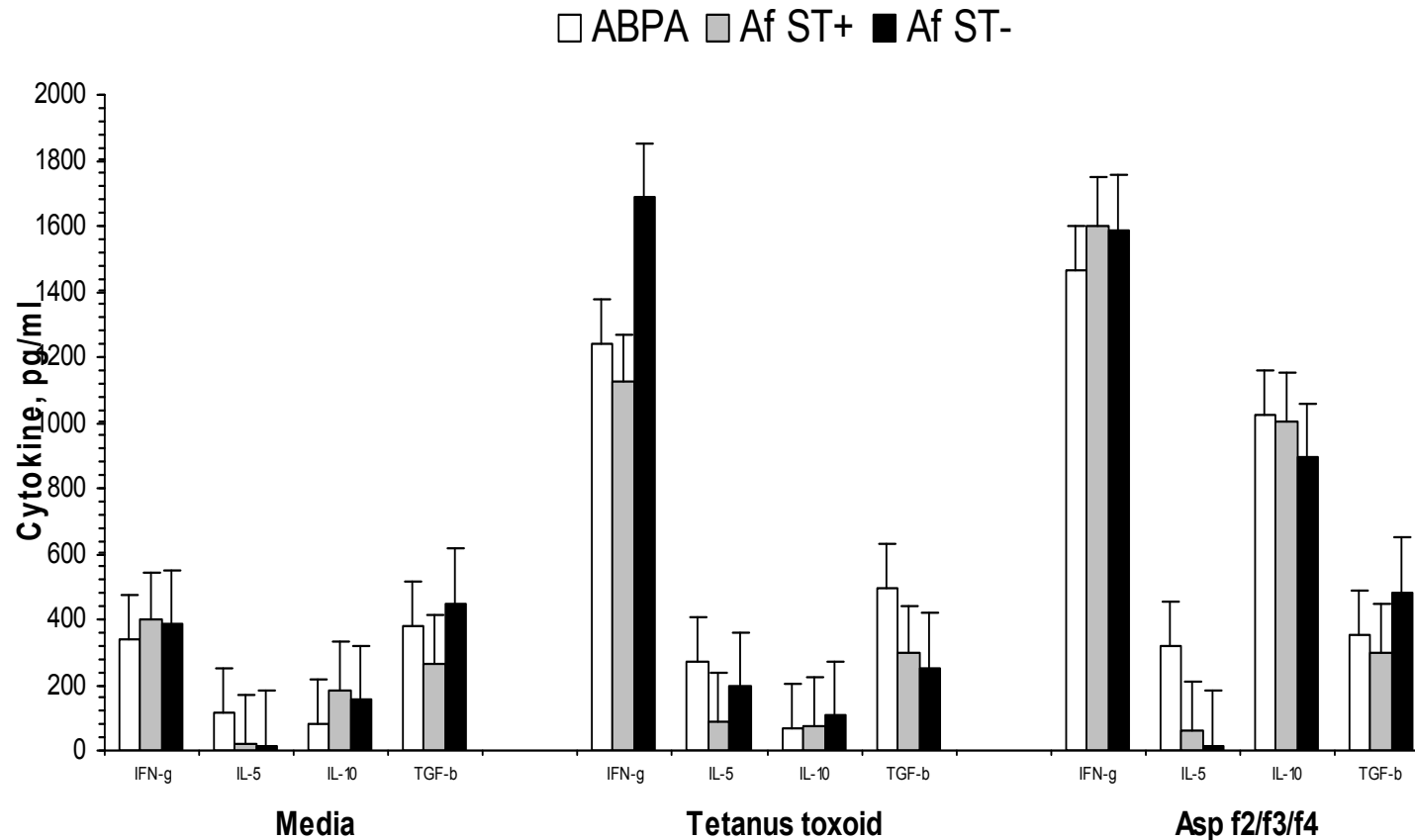
IL-4



↑IL-10

↓IL-12

# Tetanus and Asp f2/f3/f4 Stimulated Cytokine Synthesis



Cytokine synthesis by *tetanus toxoid* and *Aspergillus* stimulated PBMC (*Th1/Th2* cytokine synthesis). Following Asp f2/3/4 stimulation, ABPA CF had significantly increased IL-5 synthesis compared to Af ST+ and Af ST- CF patients. IL-10 synthesis was elevated in ABPA, Af ST+ and Af ST- CF patients in Asp f2/f3/f4 stimulated cultures but not in tetanus toxoid stimulated cultures; TGF-b synthesis was comparable in all three patient groups. Data presented as Mean  $\pm$  SE.

TABLE 2. HLA-DR ALLELE FREQUENCY IN ASTHMATIC AND CF PATIENTS WITH ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

<i>Study</i>	<i>ABPA (41)</i>	<i>Non-ABPA (84)</i>	<i>p</i>
HLA-DR2	48.8	21.4	0.001
HLA-DR4	12.2	16.7	
HLA-DR5	26.8	16.7	
HLA-DR7	22.0	26.2	
HLA-DR2/DR5	70.7	35.7	0.005
HLA-DR2/DR5/DR4/DR7	82.9	63.1	

Data presented as percentage (%) of patients.

*p* value by  $\chi^2$  comparing ABPA versus non-ABPA.

TABLE 3. IL-4RA, IL-13, AND IL-10 -1082 POLYMORPHISMS  
IN ASTHMATIC AND CF PATIENTS WITH ALLERGIC  
BRONCHOPULMONARY ASPERGILLOSIS

<i>Study</i>	<i>ABPA (41)</i>	<i>Non-ABPA (84)</i>	<i>p</i>
IL-4RA SNPs			
ile75val	80.5 (0.610)	63.1 (0.440)	0.05
ile/ile	19.5	36.9	0.05
ile/val	39.0	38.1	
val/val	41.5	25.0	0.06
asn98thr	4.9	6.0	
glu400ala	12.2	26.2	
cys431arg	4.9	9.5	
ser503pro	19.5	33.3	
gln576arg	19.5	45.2	
ile75val + cytoplasmic	24.4	33.3	
75val + 100gln	31.0	29.6	
IL-13 arg110gln	36.7 (0.250)	38.2 (0.224)	
arg/arg	63.3	61.8	
arg/gln	36.7	38.2	
gln/gln	13.3	6.6	
IL-10 -1082G/A			
G	90.0 (0.683)	77.0 (0.549)	
GG	46.7	32.8	
GA	43.3	45.9	
AA	10.0	21.3	

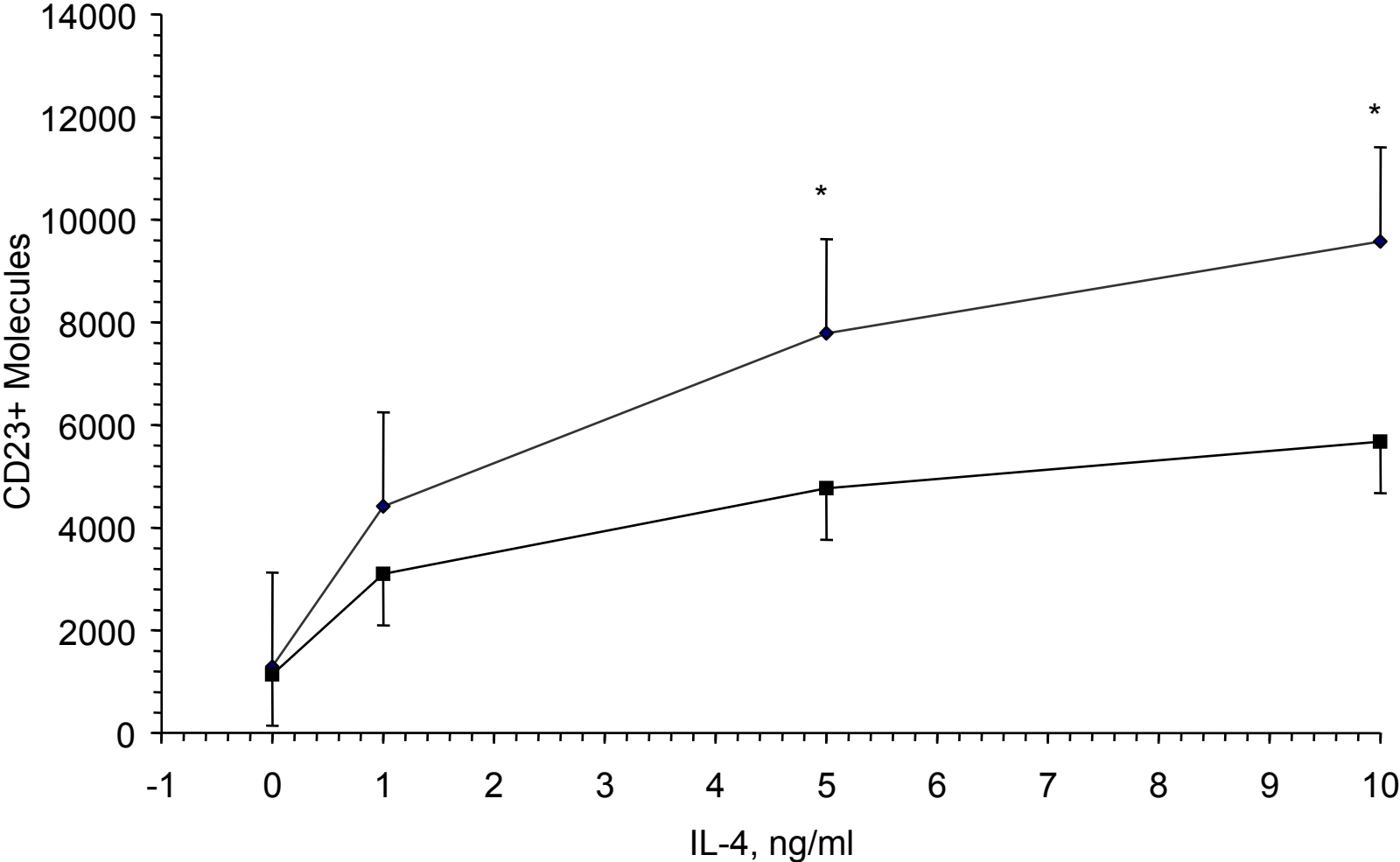
Data presented as percentage (%) of patients. Numbers in parentheses indicate allele frequency. *P* value by  $\chi^2$  comparing ABPA versus non-ABPA.

IL-4R $\alpha$ , IL-4 receptor  $\alpha$ -chain; SNP single-nucleotide polymorphisms.

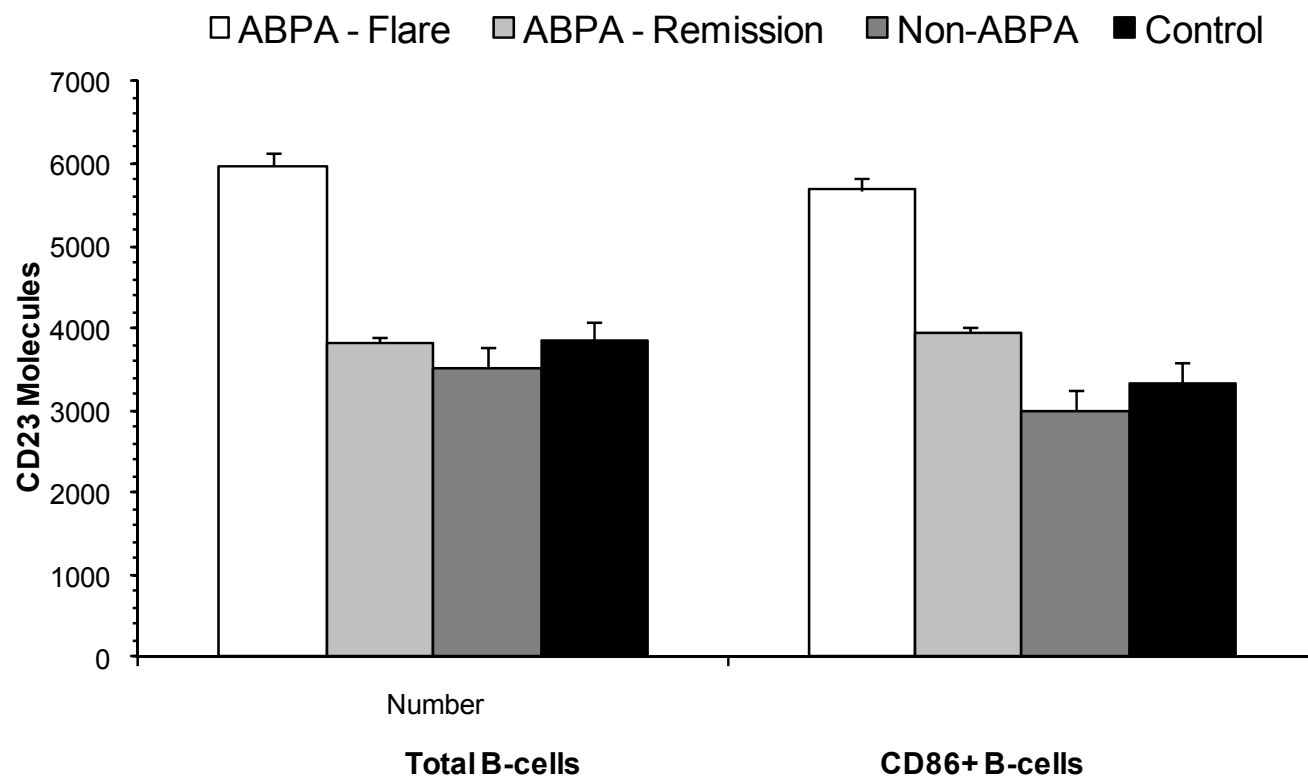
# IL-4 Stimulated CD23+ Molecules per B-cell

◆ ABPA (36)

■ Non-ABPA (51)



# CD23 Molecules per B-cell in ABPA Flare vs Remission



The number of CD23 molecules in ABPA patients during flares and remission. ABPA CF patients during acute flares had significantly increased CD23 molecules on total B-cells and CD86+ B-cells compared to ABPA during remission. In addition, the number of CD23 molecules was significantly increased compared to non-ABPA patients and controls. Data presented as Mean  $\pm$  SE.

TABLE 4. ODDS RATIOS OF HLA-DR, IL-4RA, AND IL-10 -1082 POLYMORPHISMS IN ASTHMATIC AND CF PATIENTS FOR THE DEVELOPMENT OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

<i>Study</i>	<i>ABPA (41)</i>	<i>Non-ABPA (84)</i>	<i>p</i>	<i>OR (95% CI)</i>
HLA-DR2/DR5	70.7	35.7	0.005	4.4 (1.9–9.8)
IL-4RA 75val	80.5	63.1	0.05	2.4 (1.0–5.9)
HLA-DR2/5 + IL-4RA 75val	61.0	19.0	0.0001	6.6 (2.9–15.2)
IL-4RA 75val + IL-10 -1082G	76.7	54.7	0.04	2.7 (1.0–7.2)
HLA-DR2/5 + IL-4RA 75val + IL-10 -1082G	50.0	11.1	0.0001	8.0 (3.0–21.7)

Data presented as percentage (%) of patients. *P* value by  $\chi^2$  comparing ABPA versus non-ABPA. IL-4RA, IL-4 receptor  $\alpha$ -chain.



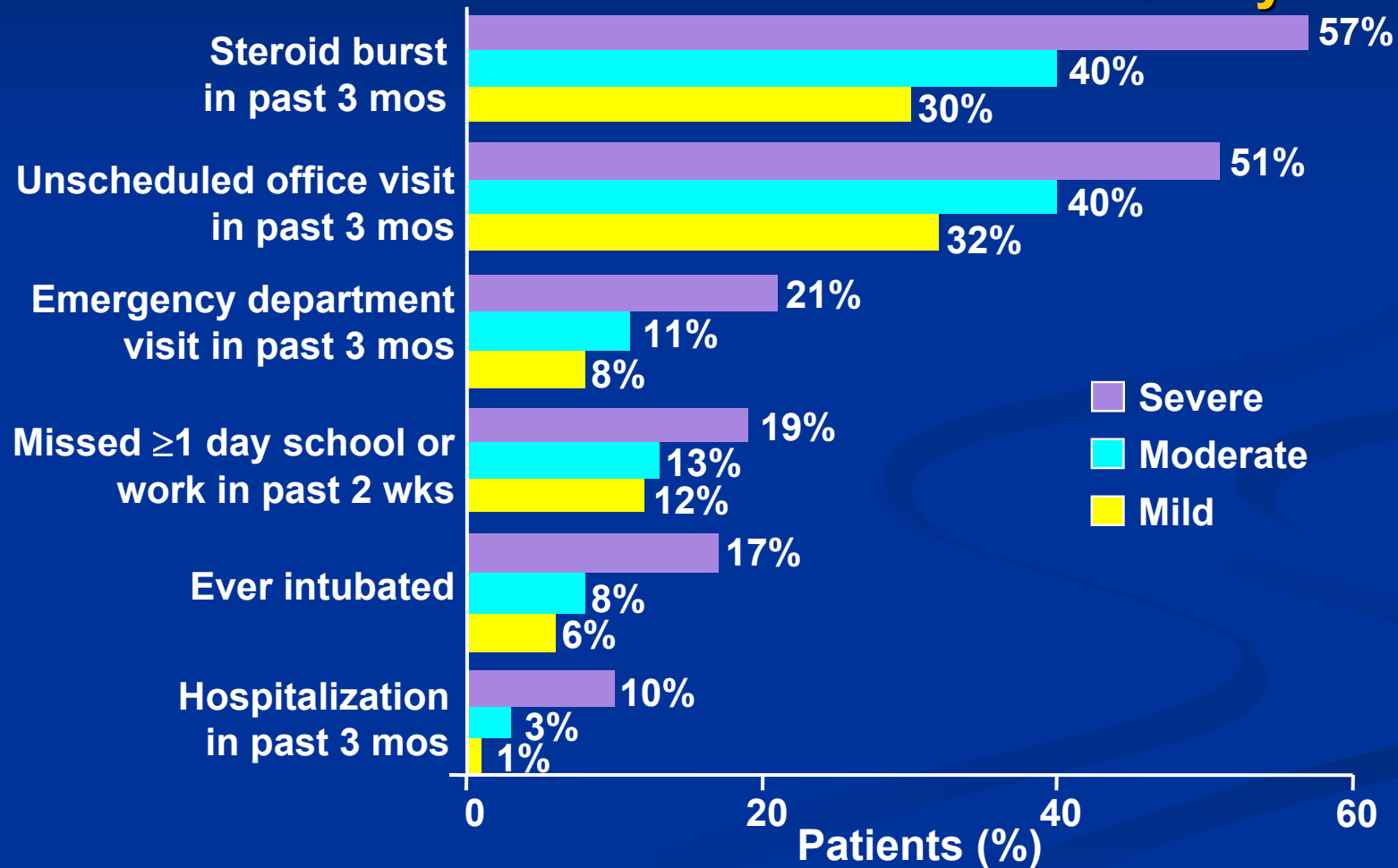
# Genetics of *Alternaria*-Sensitive Moderate-Severe Asthma in Children

- We hypothesize that in *Alternaria* sensitive patients with moderate-severe asthma, a similar pathogenesis as is seen in ABPA might occur

# Healthcare utilization and missed work/school days by asthma severity

Dolan CM, Ann Allergy Asthma Immunol 92:32-39, 2004

## Data From the TENOR Study



TENOR = The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens

# Annual per-patient direct and indirect costs of asthma in adults

Cisternas MG, J Allergy Clin Immunol 111:1212-1218, 2003

Asthma Severity	Meds (%)	Out-Patient Care (%)	Hospital Use (%)	Other Medical* (%)	Total Direct Costs (\$)	Indirect Costs† (%)	Total Costs (\$)
Mild	47	7	4	5	1681	22	2646
Moderate	39	7	5	4	2473	33	4530
Severe	19	7	17	8	6354	46	12,813

N=401; adults with asthma ≈18 to 50 years of age.

\*Emergency department and outpatient medical procedures.

†Estimated cost of lost work productivity.

# Sensitisation to airborne molds and severity of asthma

Zureik M, BMJ 325:1-7, 2002

- European Community Respiratory Health Survey
  - 30 centers
  - 1132 adults 20-44 years old
- *A. alternata* or *C. herbarum* sensitivity associated with increased severity of asthma vs mild asthma
  - Severe asthma: OR 2.34 (1.56-3.52 95% CI)
  - Moderate asthma: OR 1.56 (1.05-2.32 95% CI)
- *D. pteronyssinus* also associated with increased severity of asthma vs mild asthma
  - Severe asthma: OR 1.85 (1.36-2.51 95% CI)
  - Moderate asthma: OR 1.22 (0.93-1.60 95% CI)
- No association with pollens or cat sensitivity

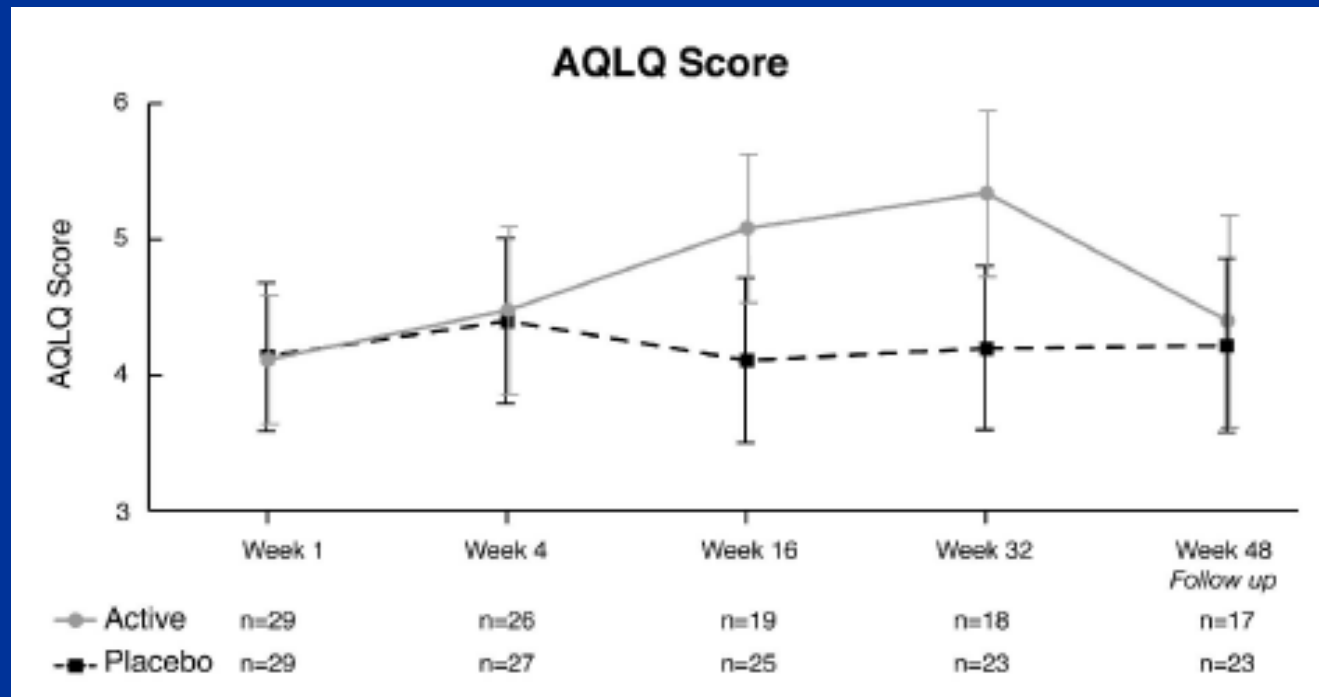
# Randomized controlled trial of oral antifungal treatment for severe asthma with fungal sensitisation (SAFS), the FAST study

- Itraconazole-placebo controlled 32 week trial in fungus-sensitive severe asthma
- Patients
  - 49.2 yrs
  - 48% male
  - Sensitivities
    - *Aspergillus* – 66%
    - *Cladosporium* – 52%
    - *Alternaria* – 34%
    - *Penicillium* – 48%
    - *Candida* – 66%
    - *Trichophyton* – 31%
    - *Botrytis* – 28%

# Effect of Itraconazole in SAFS

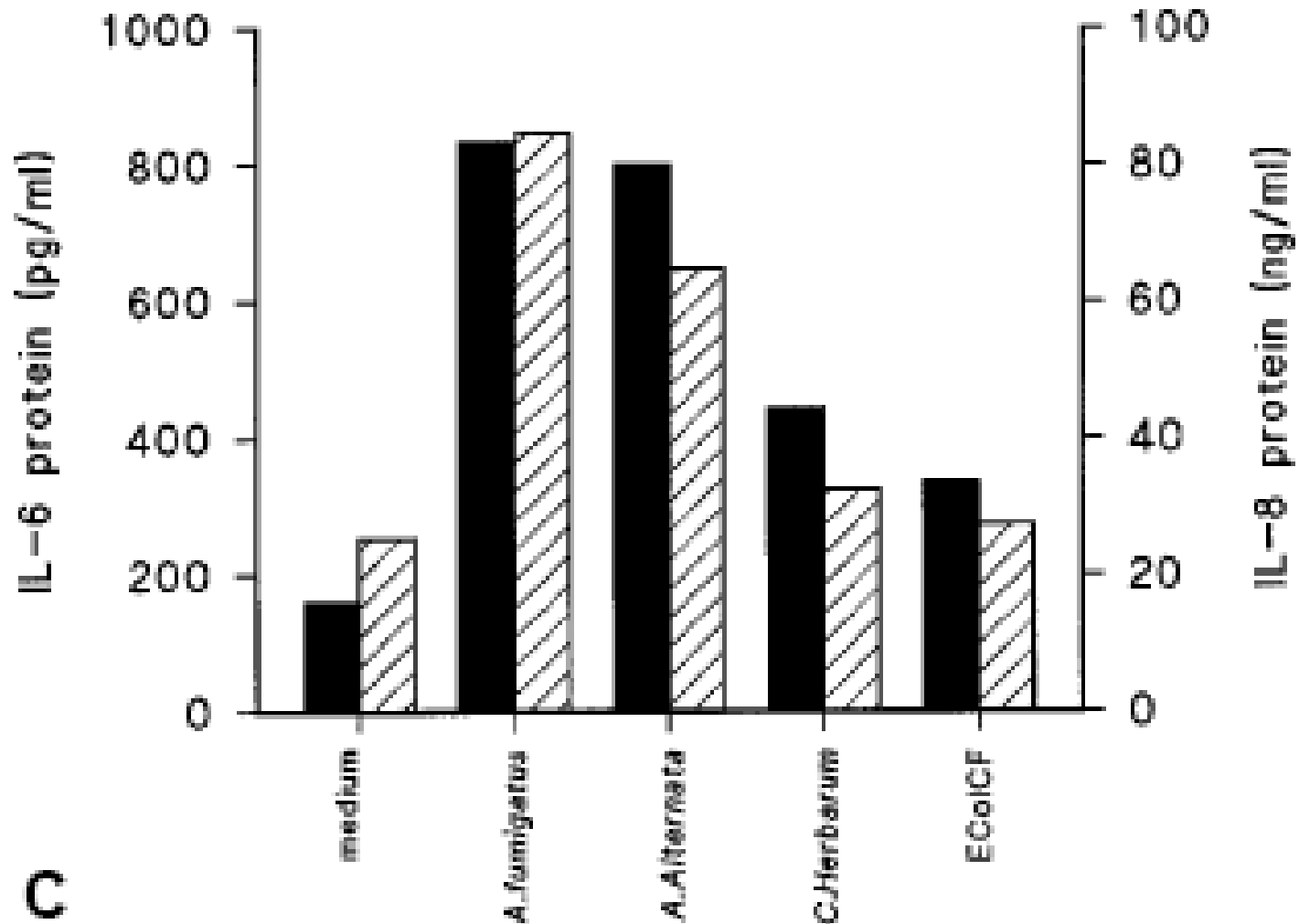
Denning DW et al

- AQLQ -  $\uparrow$  0.85
- IgE level -  $\downarrow$  27%; 187 IU/ml  $\rightarrow$  136 IU/ml
- FEV-1 – no change
- PF -  $\uparrow$  20.8 L/min



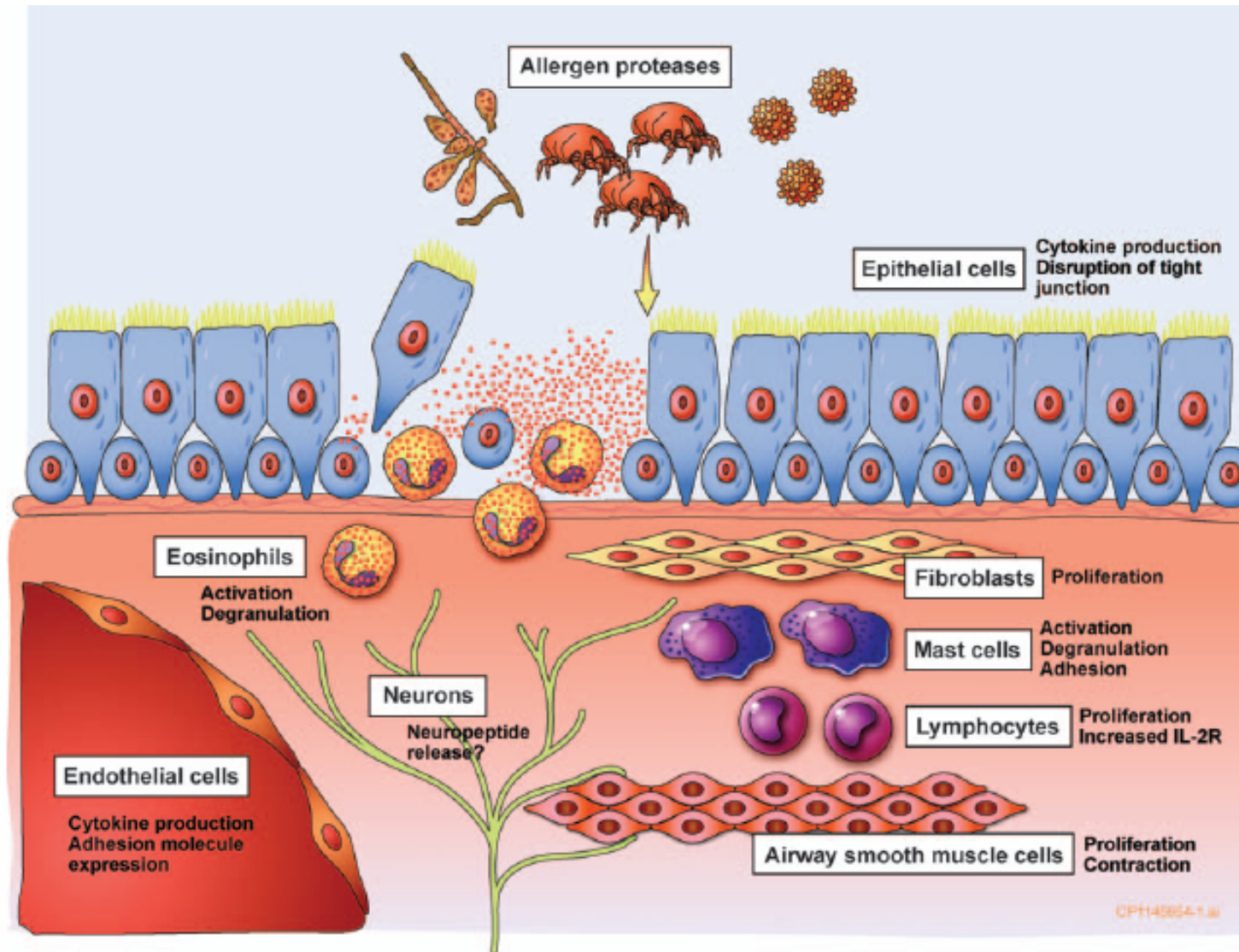
# Protease-dependent activation of epithelial cells by fungal allergens leads to morphologic changes and cytokine production

Kauffman HF, JACI 105:1185-1193, 2000



# The role of protease activation of inflammation in allergic respiratory disease

Reed CE, JACI 114:997-1008, 2004





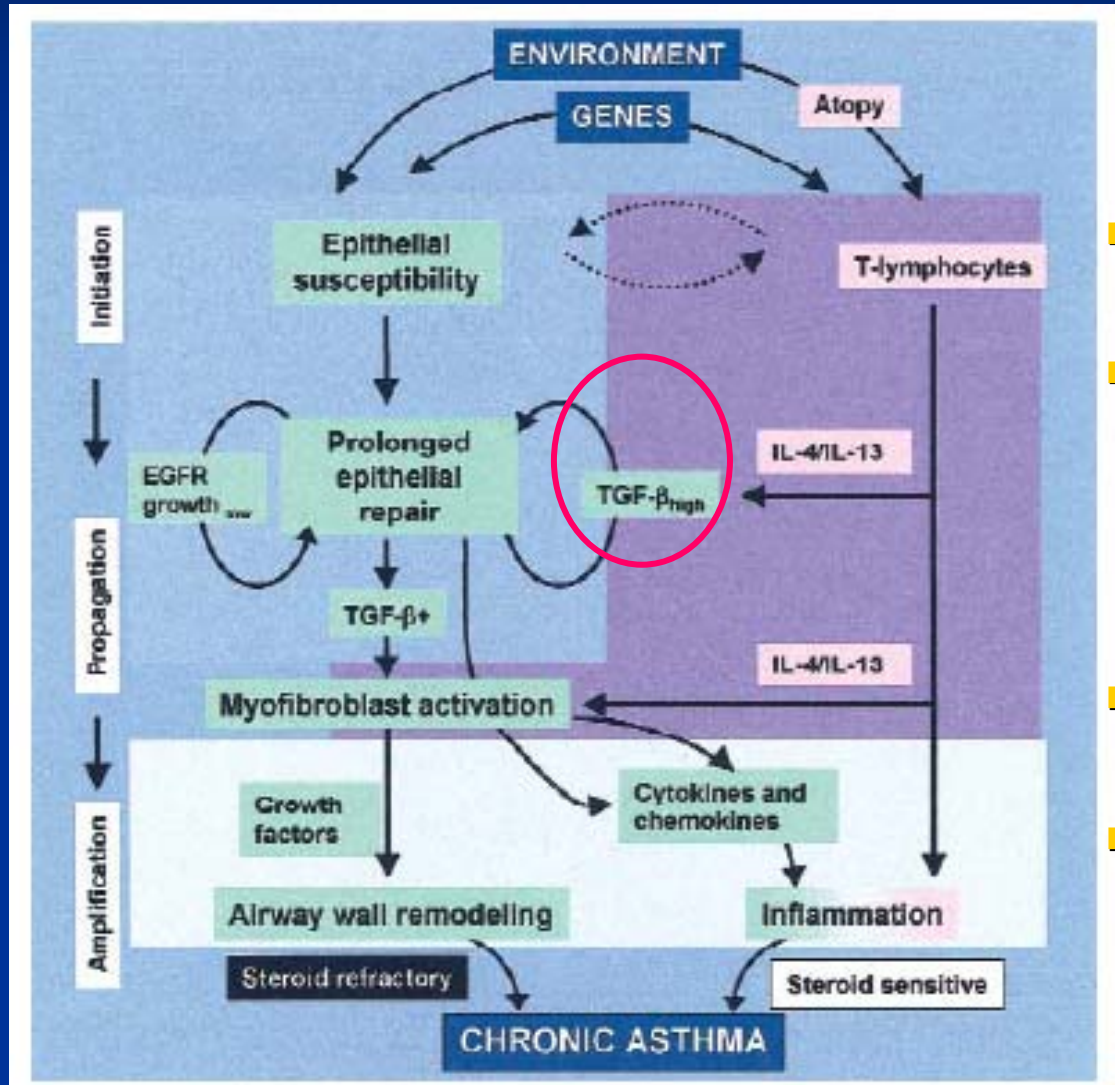
# Effects of PAR-2 Stimulation

Reed CE, JACI 114:997-1008, 2004

Airway epithelium	↑ expression in asthma ↑ methacholine response ↑ IL-6, IL-8, GM-CSF, eotaxin ↑ IgE production
Airway smooth muscle	↑ methacholine response
Lymphocytes	↑ specific IgE synthesis (OA)
Monocytes, dendritic cells	↑ GM-CSF ↓ IL-4 expression
Endothelial cells	↑ IL-6, IL-8, NF- $\kappa$ B
Fibroblasts	↑ IL-8, MCP-1

# Interaction between Th2 inflammation and the EMTU in asthma pathogenesis

Davies DD, JACI 111:215-225, 2003



## Asthmatic Bronchial Epithelia

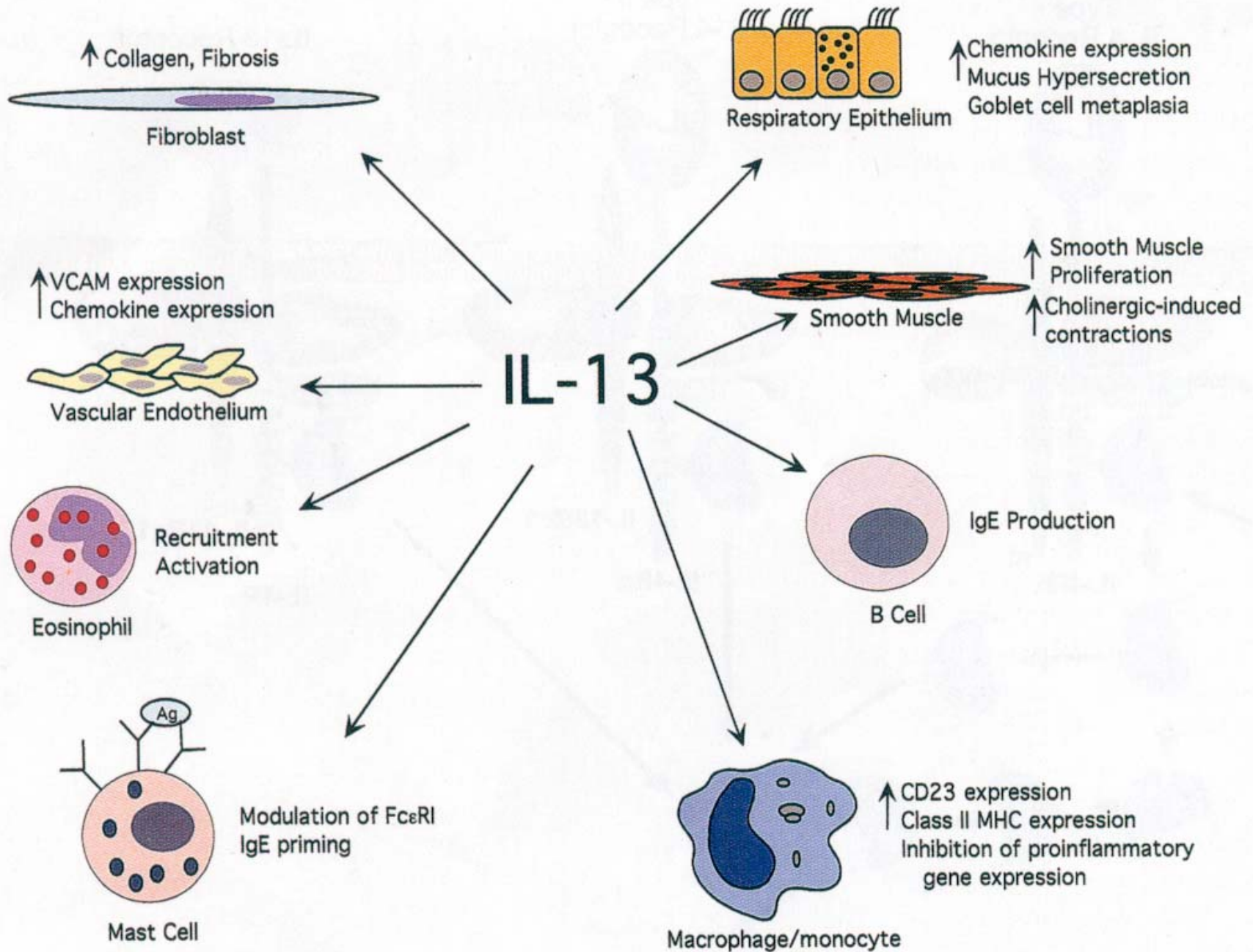
IL-13 stimulates TGF-β synthesis

IL-4, IL-13 stimulates STAT-6

- ↑ synthesis of GM-CSF, IL-8, TGF-α (↑ mucin gene expression), eotaxin

Der p enhances IL-4, IL-13 stimulation

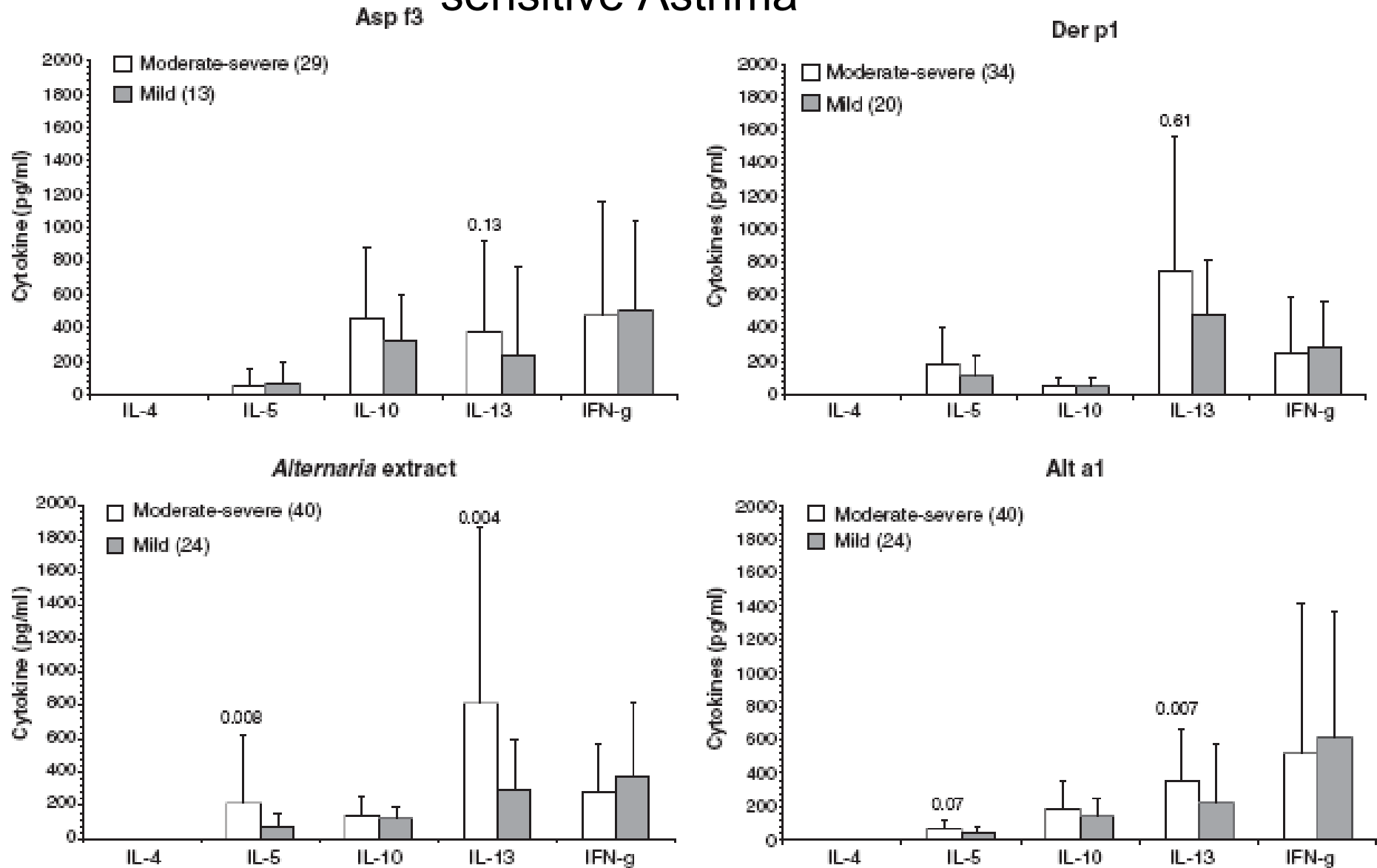
Der p stimulates IL-5, IL-13 secretion of BE explants



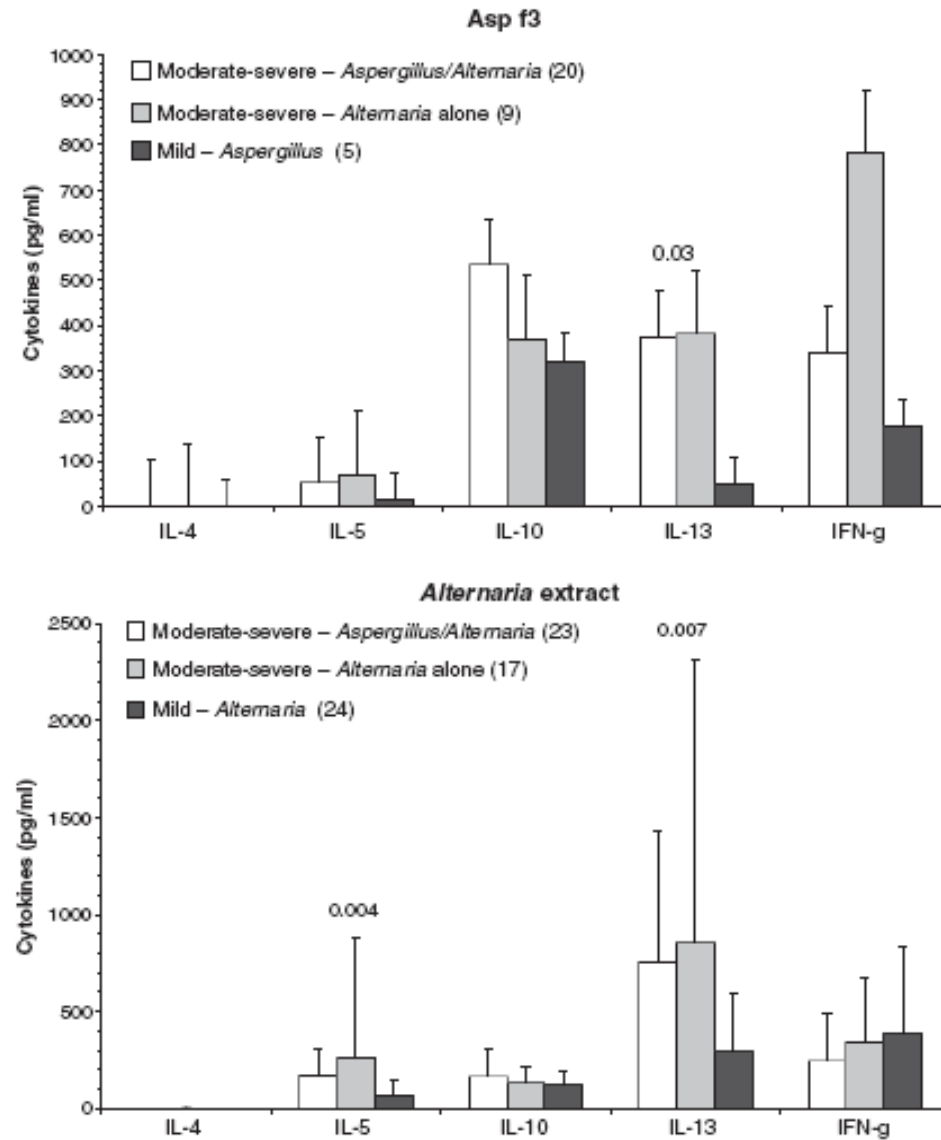
**Table 1** Demographics of children with moderate-severe asthma

Study	Moderate-severe (96)	Mild (90)	<i>P</i>
Age, years	11 ± 4	10 ± 4	
Sex, % male/female	58/42	59/41	
White/Black/Hispanic, %	33/67/0	66/30/4	<0.0001
Atopic dermatitis, %	36	28	
Medications, %#			
Omalizumab	28	0	<0.0001
ICS-H	41	4	<0.0001
ICS-M	46	24	0.003
ICS-L	12	52	<0.0001
LABA	87	36	<0.0001
LTRA	78	60	0.011
Pulmonary function*			
FVC	89 ± 15	100 ± 12	<0.0001
FEV-1	79 ± 16	95 ± 12	<0.0001
FEF-25–75	65 ± 22	90 ± 21	<0.0001
FEV-1/FVC	84 ± 14	93 ± 10	<0.0001
IgE, IU/ml*	353 ×/+ 4.16	100 ×/+ 5.28	<0.0001
Sensitivities, %#			
<i>Alternaria</i>	63	54	
<i>Cladosporium</i>	32	25	
<i>Helminthosporium</i>	24	22	
<i>Aspergillus</i>	30	15	0.01
Der p and/or Der f	52	33	0.01
Cat	42	29	
CR	28	14	0.03
Trees	61	57	
Grasses	46	44	
Weeds	56	43	

# *Alternaria*-stimulated Cytokine Synthesis in *Alternaria*-sensitive Asthma



# Asp f3 and *Alternaria*-stimulated Cytokine Synthesis in *Aspergillus/Alternaria*-sensitive and *Alternaria*-sensitive Asthma



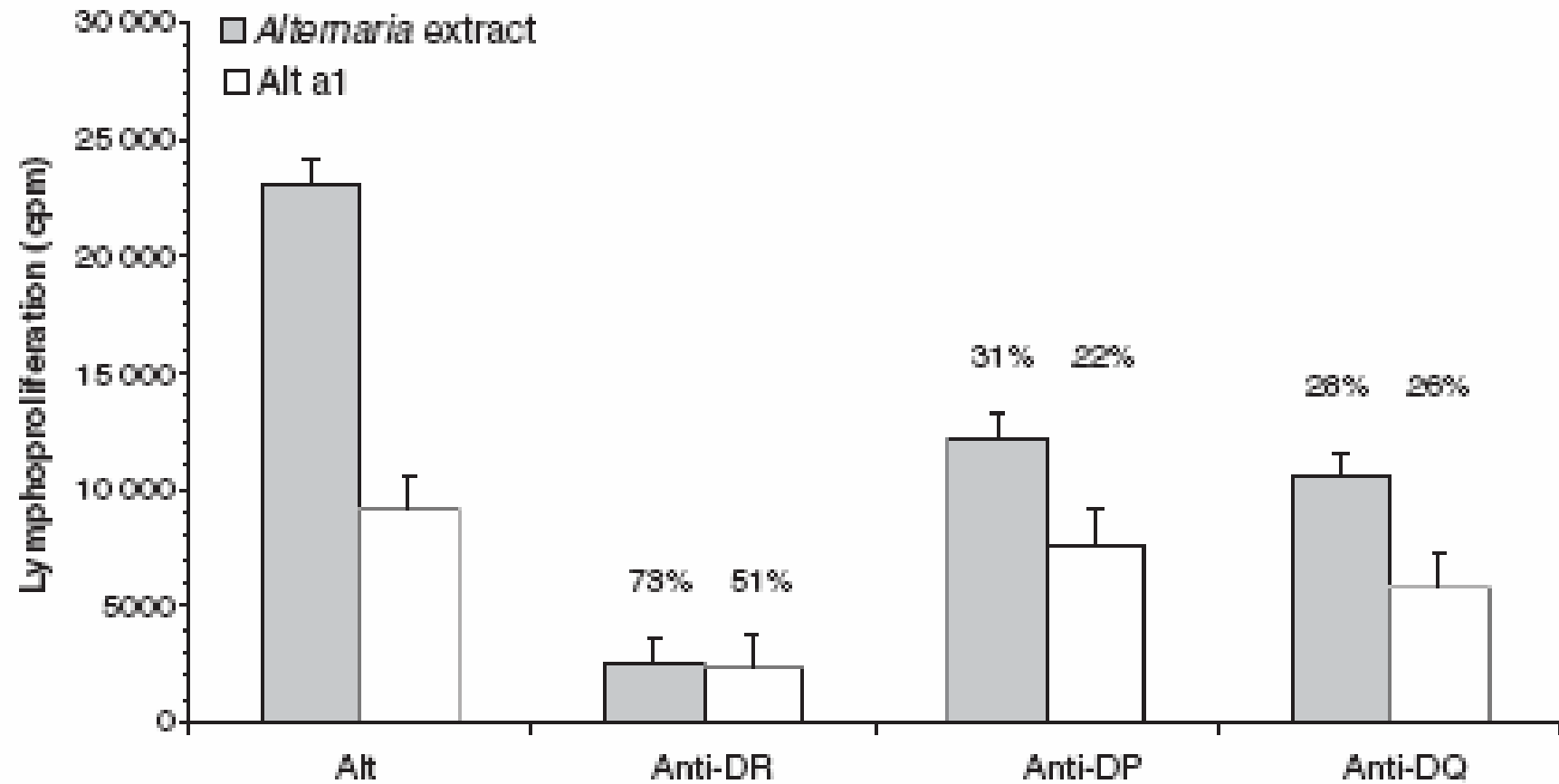
**Table 2** HLA-DR and HLA-DQ allele frequencies in children with mold-sensitive moderate-severe asthma

Study	M-S <i>Mold</i> -sensitive (68)	M-S Not <i>mold</i> -sensitive (28)	M <i>Mold</i> -sensitive (59)	M Not <i>mold</i> -sensitive (31)	<i>P</i> M-S <i>Mold</i> vs M <i>Mold</i>
HLA-DRB1					
*01	10 (0.051)	14 (0.071)	10 (0.059)	19 (0.097)	
*03	29 (0.154)	25 (0.125)	20 (0.102)	19 (0.097)	
*04	16 (0.088)	18 (0.089)	31 (0.161)	32 (0.161)	
*07	21 (0.110)	18 (0.089)	25 (0.136)	16 (0.097)	
*08	6 (0.029)	11 (0.054)	12 (0.068)	10 (0.048)	
*09	6 (0.029)	4 (0.018)	3 (0.017)	0 (0.000)	
*10	2 (0.007)	4 (0.018)	3 (0.017)	0 (0.000)	
*11	22 (0.118)	14 (0.089)	31 (0.169)	26 (0.129)	
*12	6 (0.029)	14 (0.071)	2 (0.008)	10 (0.048)	
*13	38 (0.199)	25 (0.125)	20 (0.110)	36 (0.177)	
*14	3 (0.015)	0 (0.000)	2 (0.008)	0 (0.000)	
*15	25 (0.147)	39 (0.214)	24 (0.136)	23 (0.113)	
*16	4 (0.022)	7 (0.036)	2 (0.008)	7 (0.032)	
HLA-DQB1					
*02	41 (0.243)	39 (0.196)	32 (0.178)	32 (0.177)	
*03	43 (0.243)	32 (0.179)	61 (0.381)	68 (0.355)	0.0084 (0.0133)
*04	10 (0.059)	11 (0.054)	10 (0.051)	7 (0.032)	
*05	31 (0.169)	39 (0.250)	22 (0.119)	32 (0.161)	
*06	44 (0.287)	54 (0.321)	49 (0.271)	48 (0.274)	

Data presented as percentage of patients with allele and in parentheses allele frequency.

Chi-squared test for independence was used for multiple comparisons of HLA-DR and HLA-DQ.

# Inhibition of *Alternaria* Stimulation by Blocking anti-HLA mAbs





# *Alternaria*-stimulated Cytokine Synthesis in HLA-DQ3+ and -DQ3- *Alternaria*-Sensitive Asthma

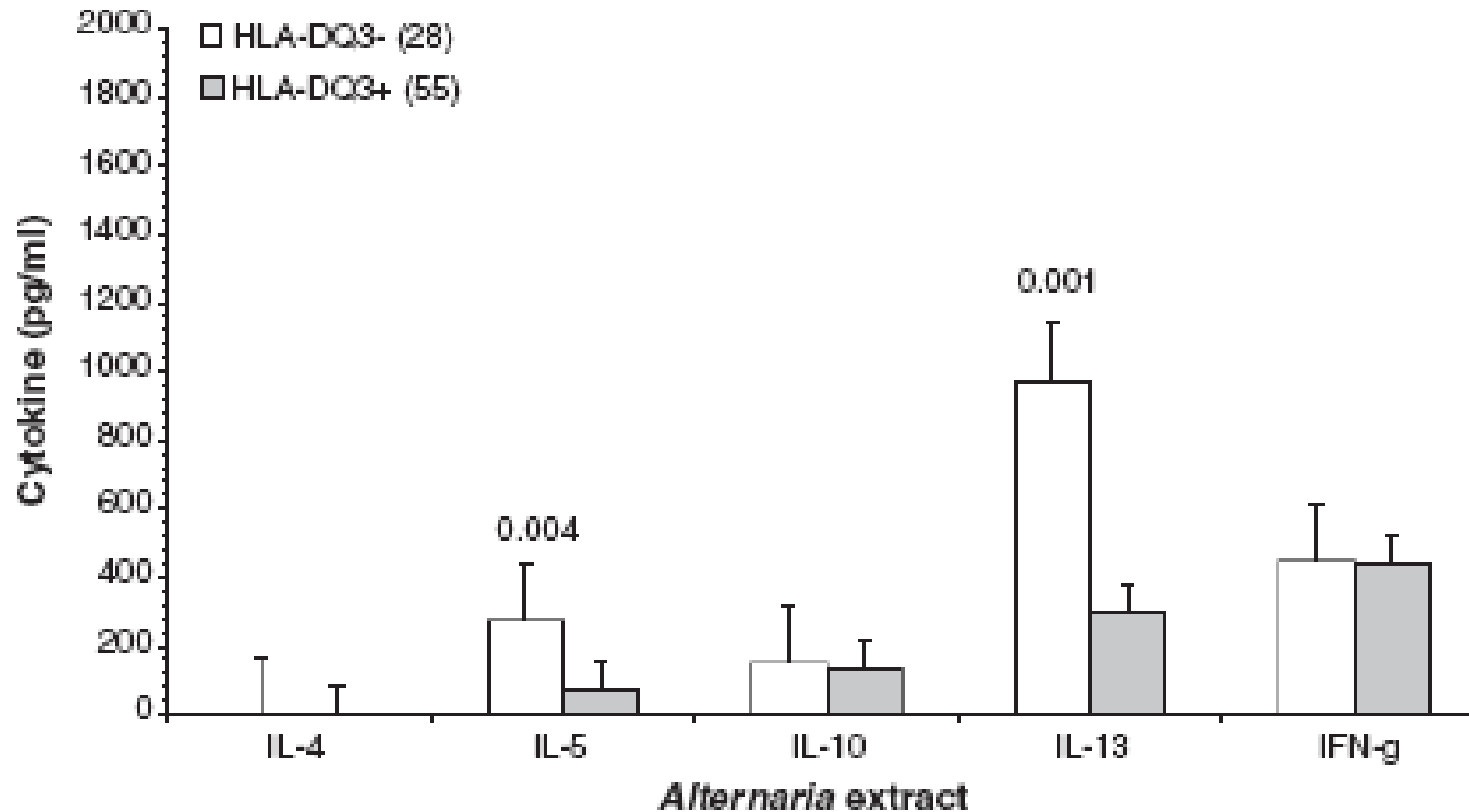
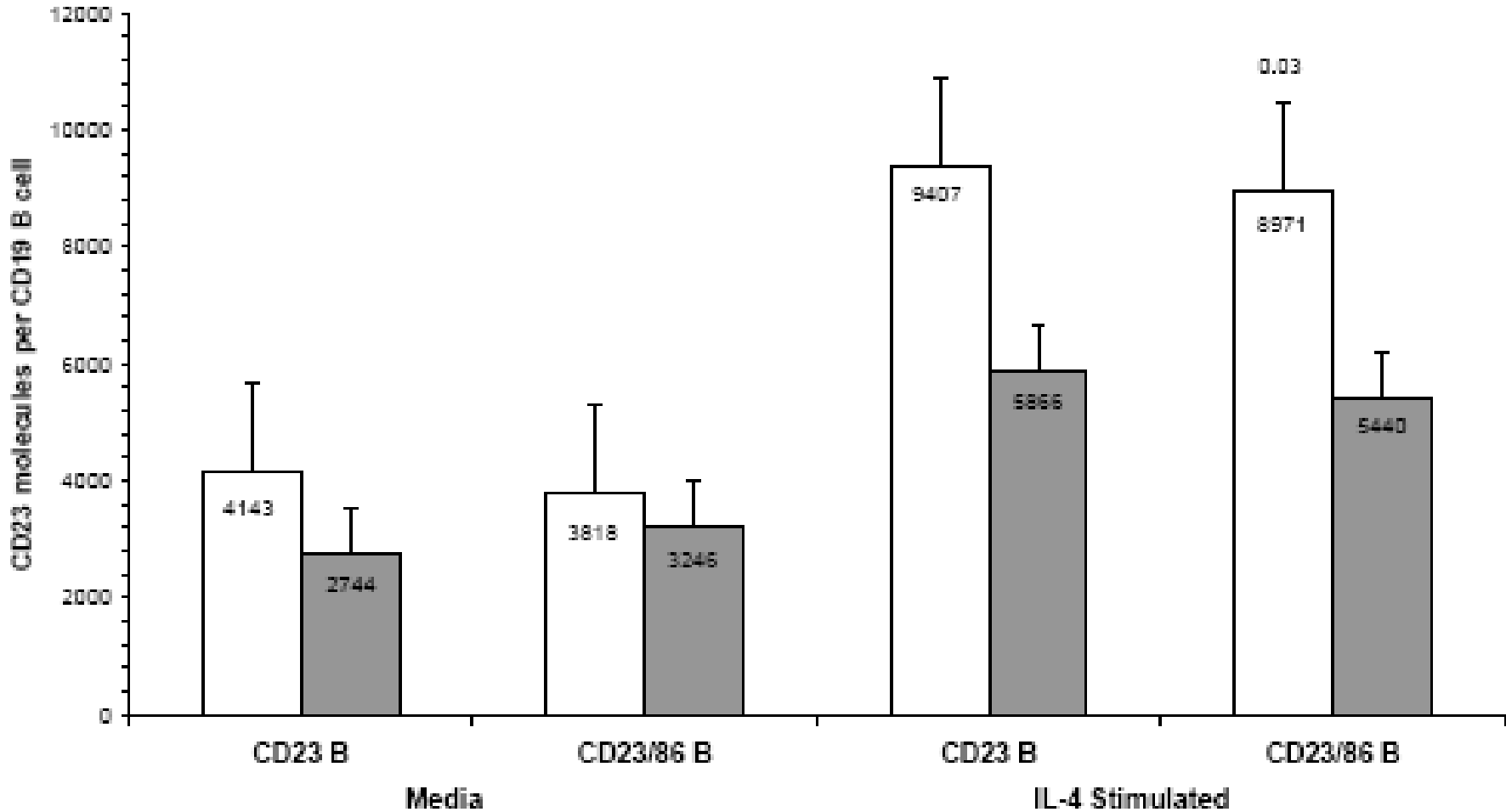


Table 2. IL-4RA and IL-13 polymorphisms in children with *Alternaria*-sensitive moderate-severe asthma compared to *Alternaria*-sensitive mild asthma.

Study	Moderate-Severe (60)	Mild (49)	<i>P</i>
<b>IL-4RA SNPs</b>			
ile75val	83 (0.627)	57 (0.388)	0.005 (0.012)
glu400ala	61 (0.390)	49 (0.265)	
cys431arg	15 (0.102)	22 (0.112)	
ser503pro	53 (0.347)	37 (0.214)	
gln576arg	75 (0.534)	59 (0.406)	
<b>IL-13 SNP</b>			
arg110gln	31 (0.178)	37 (0.204)	0.012
75val/576arg	63	38	
75val/110gln	31	17	
75val/576arg/110gln	22	8	0.07

# Up-Regulation of CD23 by IL-4 in *Alternaria*-Sensitive Asthma

□ Severe ■ Mild



# IL-4R alpha mutations are associated with asthma exacerbations and mast cell/IgE expression

Wenzel SE, Am J Respir Crit Care Med 175:570-576, 2007

- Severe Asthma Research Program (SARP)
- Criteria
  - Major ↑
    - Requirement of high-dose ICS
    - Oral corticosteroids  $\geq 50\%$  of year
  - Minor ↑
    - Use of LABA, LTRA, theophylline
    - SABA use on daily or near daily basis
    - FEV-1  $< 80\%$ , PF variability  $> 20\%$
    - $\geq 1$  ED visit(s) per year
    - $\geq 3$  oral corticosteroid bursts per year
    - Deterioration with  $\leq 25\%$  reduction of ICS dose
    - Near-fatal asthma event in the past

# IL-4R alpha mutations are associated with asthma exacerbations and mast cell/IgE expression

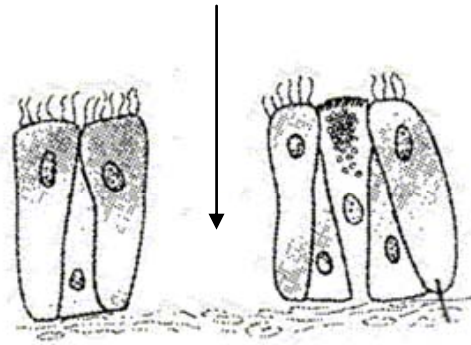
Wenzel SE, Am J Respir Crit Care Med 175:570-576, 2007

- Severe asthma
  - European Network for the Understanding of Mechanisms of Severe Asthma (ENFUMOSA)
  - Severe Asthma Research Program (SARP)
- IL-4RAs glu400ala (E375A) and gln576arg (Q551R) associated with more severe asthma exacerbations and lower lung function
- IL-4RA glu400ala (E375A) associated with higher bronchial tissue mast cells and higher levels of IgE bound to mast cells

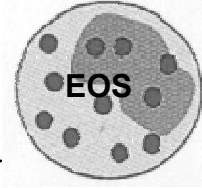
# SUMMARY

## ***AL*ternaria Allergens + Proteases**

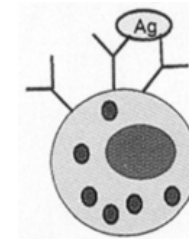
Activation, damage, leakage  
PAR-2



**Lung Damage**  
ECP, MBP, EDN, EPO



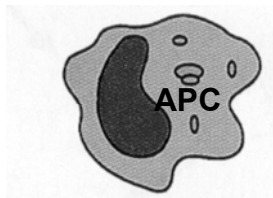
Recruitment  
Activation



**Mast cell**  
FcεR1  
IgE priming

## **Allergic Inflammation**

↑IL-8, MCP-1, IL-6  
↑ RANTES  
↑TGF-β, ↓EGFR  
↑Myofibroblast activation  
**Airway remodeling**

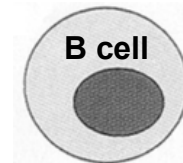


**HLA-DR restricted  
HLA-DQ3 protective**

## ↑IL4/IL-13 Activity

IL-4RA SNP  
IL-13 SNP

↑ IL-5

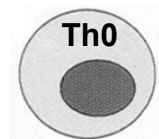


↑ IgE production

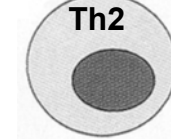
↑ CD23, ↑ CD86

CD40 CD86/CD80

CD40L CD28



IL-4



↑ TSLP, IL-10

↓IL-12

**Thank You**  
**Questions**