Microbiome and Allergic Airways Disease

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Microbiome

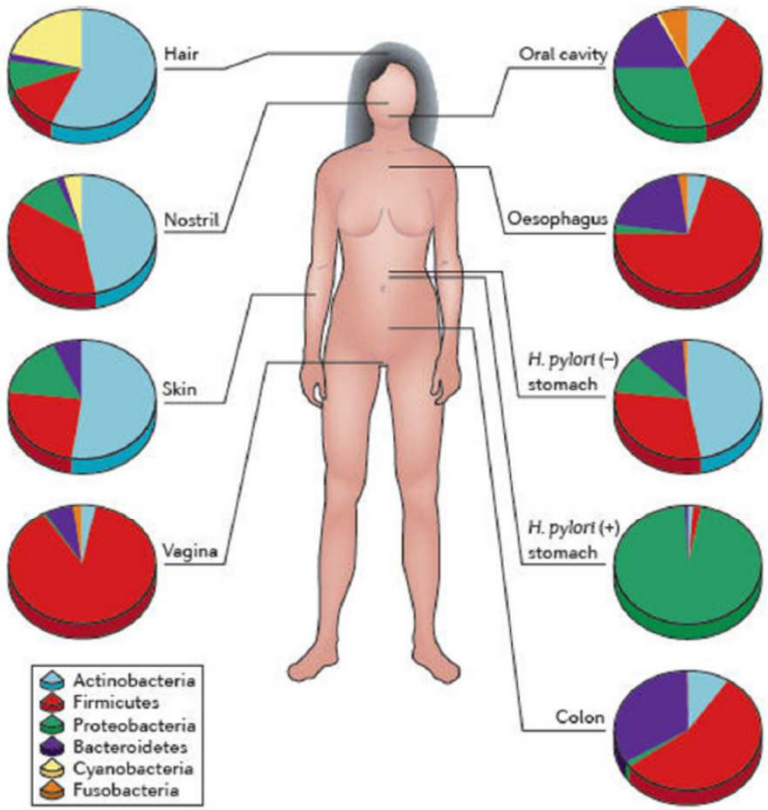

Figure 1. Compositional differences in the microbiome by anatomic site

High-throughput sequencing has revealed substantial intra-individual microbiome variation at different anatomical sites, and inter-individually for the same anatomical sites [4,5,25,52,89,93]. However, higher level (e.g. phylum) taxonomic features display temporal (longitudinal) stability in individuals at specific anatomical sites. Such site-specific differences as well as observed conservation between human hosts provide an important framework to determine the biological and pathological significance of a particular microbiome composition. The figure indicates percentages of sequences at the taxonomic phylum level from selected references. Certain features, such as the presence or absence of

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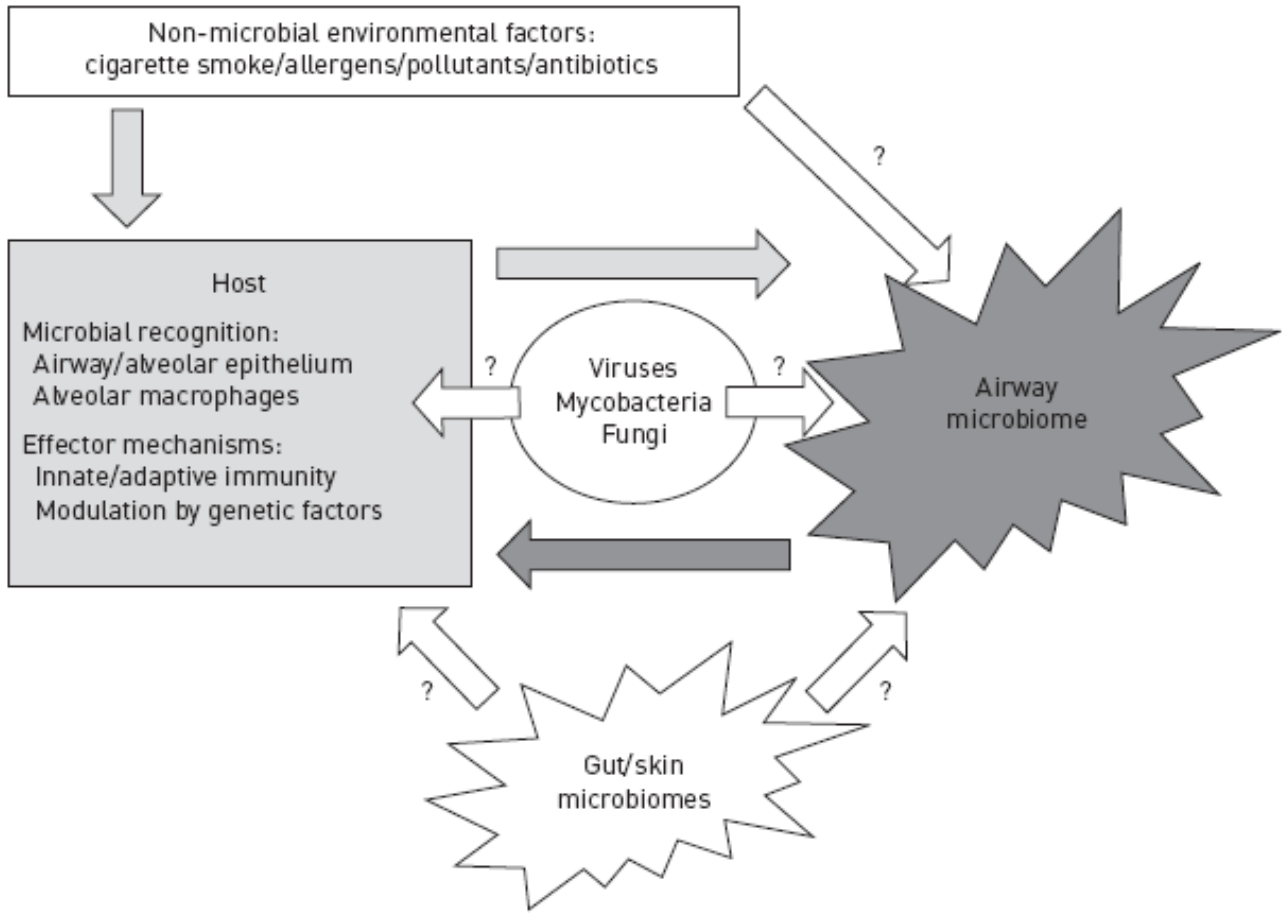
FIGURE 1 Schematic representation of host–microbe interactions in airways. Bacterial airway microbiome is composed of multiple and interacting bacterial communities. Bacteria may interact with other microbes (*e.g.* mycobacteria, viruses and fungi) and with the host, modulating immune responses. These interactions can be modulated by the effects of environmental factors on the host and/or the microbiome. The role of other bacterial communities, including gut and skin microbiome, in modulating airway diseases is also emerging.



Clémence Martin et al ERJ 2015,

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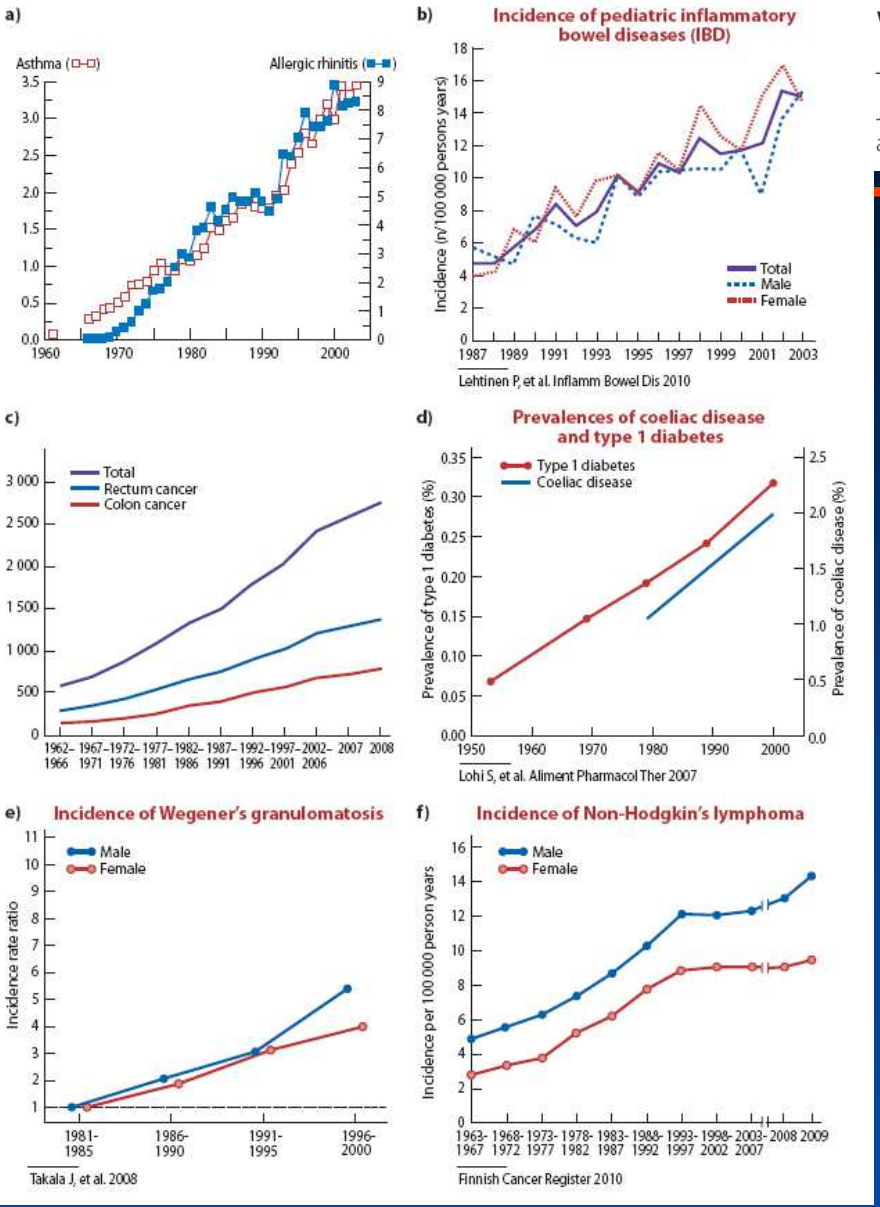
Examples of association of human conditions with particular microbiota characteristics

Disease	Relevant finding	Reference
Psoriasis	Increased ratio of Firmicutes to Actinobacteria	88
Reflux esophagitis	Esophageal microbiota dominated by gram-negative anaerobes Gastric microbiota with low or absent *H. pylori*	75,134
Obesity	Reduced ratio of Bacteroidetes to Firmicutes	17,31
Childhood-onset asthma	Absent gastric *Helicobacter. pylori* (especially cytotoxin-associated gene (*cagA*) genotype)	96,135
IBD (colitis)	Increased *Enterobacteriaceae*	113
Functional bowel diseases	Increased *Veillonella* and *Lactobacillus*	136
Colorectal carcinoma	Increased *Fusobacterium spp.*	101,102
Cardiovascular disease	Gut microbiota-dependent metabolism of phosphatidylcholine	137

The biodiversity hypothesis and allergic disease: world allergy organization position statement

Tari Haahtela[1*], Stephen Holgate[2], Ruby Pawankar[3], Cezmi A Akdis[4], Suwat Benjaponpitak[5], Luis Caraballo[6], Jeffrey Demain[7], Jay Portnoy[8], Leena von Hertzen[1], and WAO Special Committee on Climate Change and Biodiversity



WAO journal
WORLD ALLERGY ORGANIZATION

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Microbial diversity and asthma

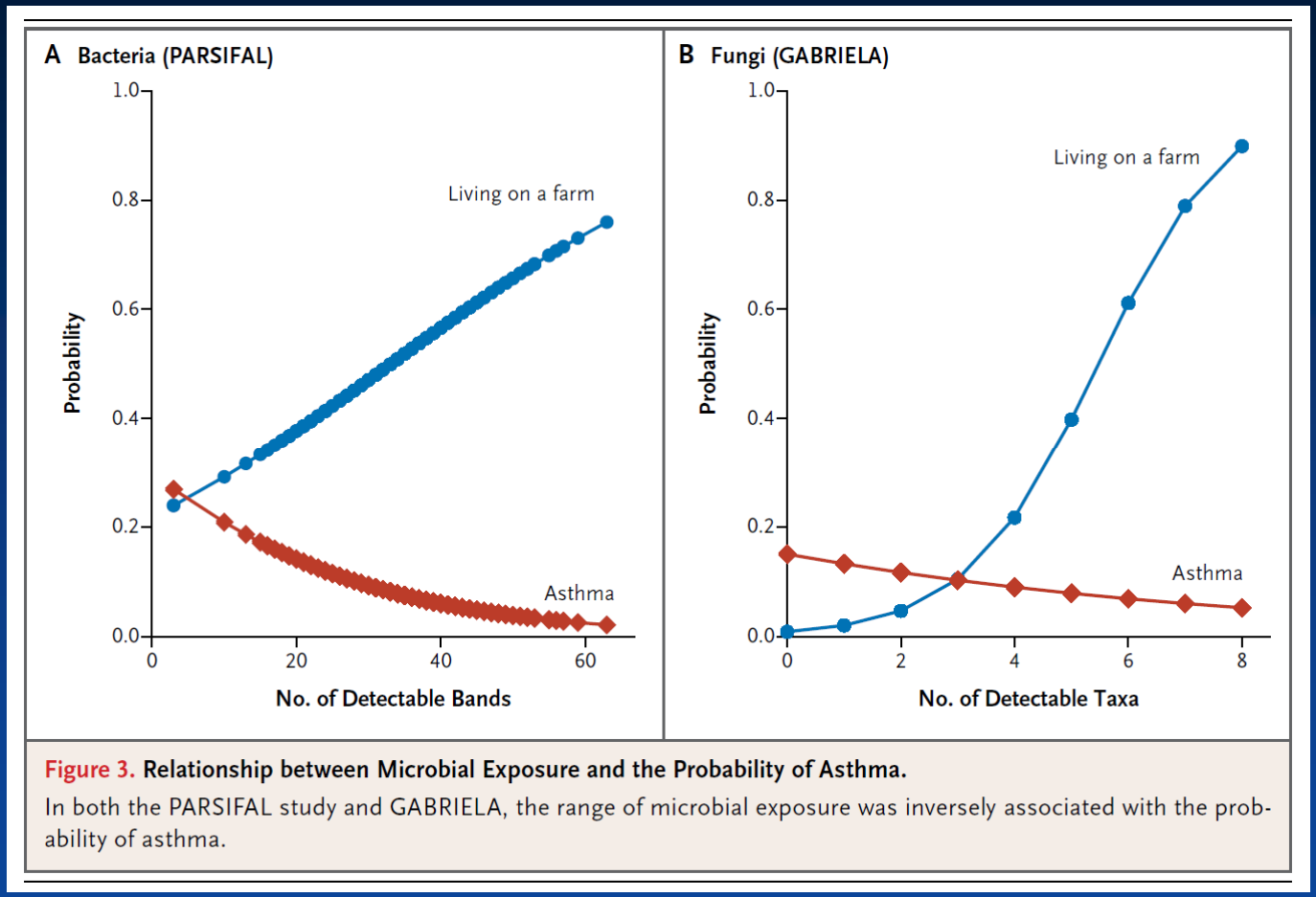

Figure 3. Relationship between Microbial Exposure and the Probability of Asthma.

In both the PARSIFAL study and GABRIELA, the range of microbial exposure was inversely associated with the probability of asthma.

Ege M et al. NEJM 2011:364:701-9

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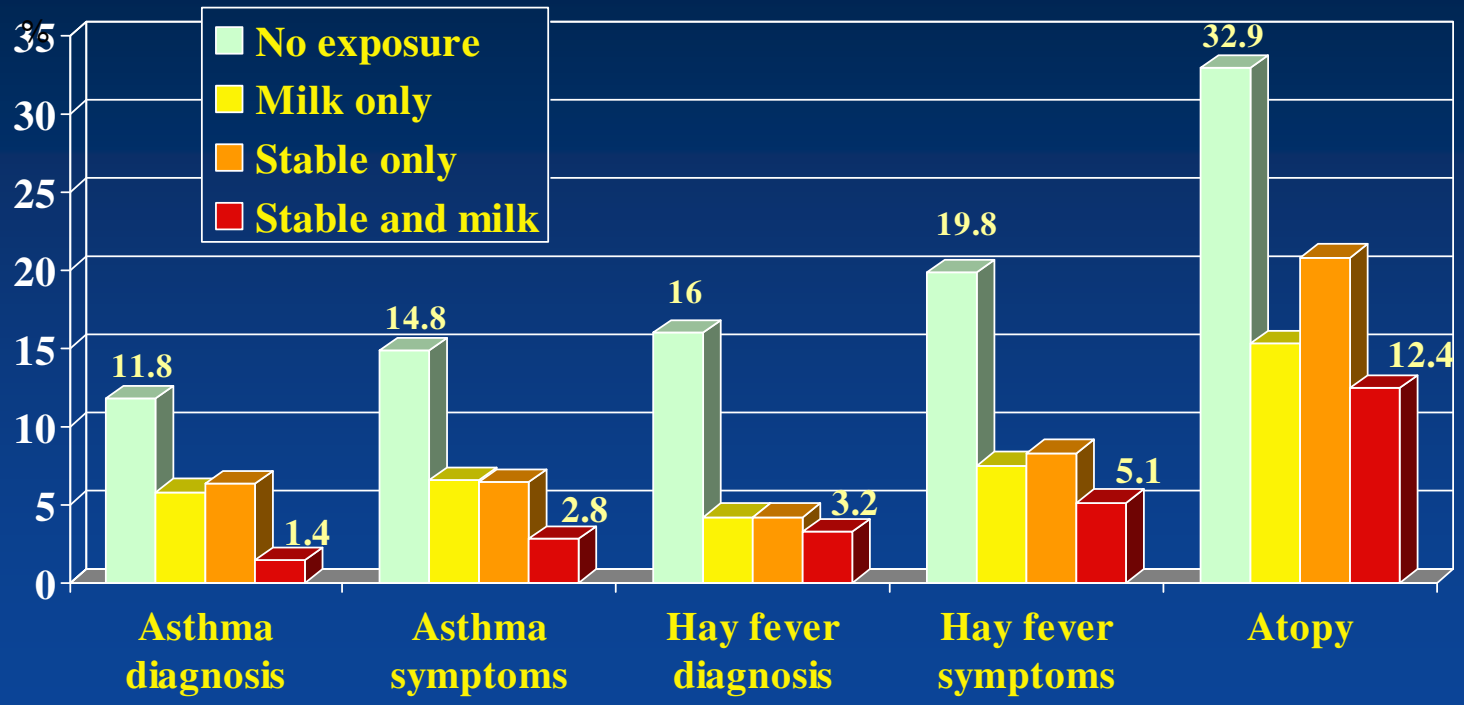
Exposure to stables (livestock farming) and/or farm milk in the 1st year of life



Farm vs non-farm children: 4 to 10 folds difference in asthma prevalence

ALEX-Study Lancet 2001

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Rural vs urban Beijing: Asthma symptoms past 12 months (video questionnaire)

	Urban 3531	Rural 3546	P
Wheeze attack	109 (3.1%)	11 (0.8%)	<0.001
Exercise induced wheeze	218 (6.2%)	51 (1.4%)	<0.001
Severe wheezing attack	58 (1.6%)	4 (0.1%)	<0.001

Ma Y et al. *Pediatr Pulmonol* 2009 ;44:793-9.

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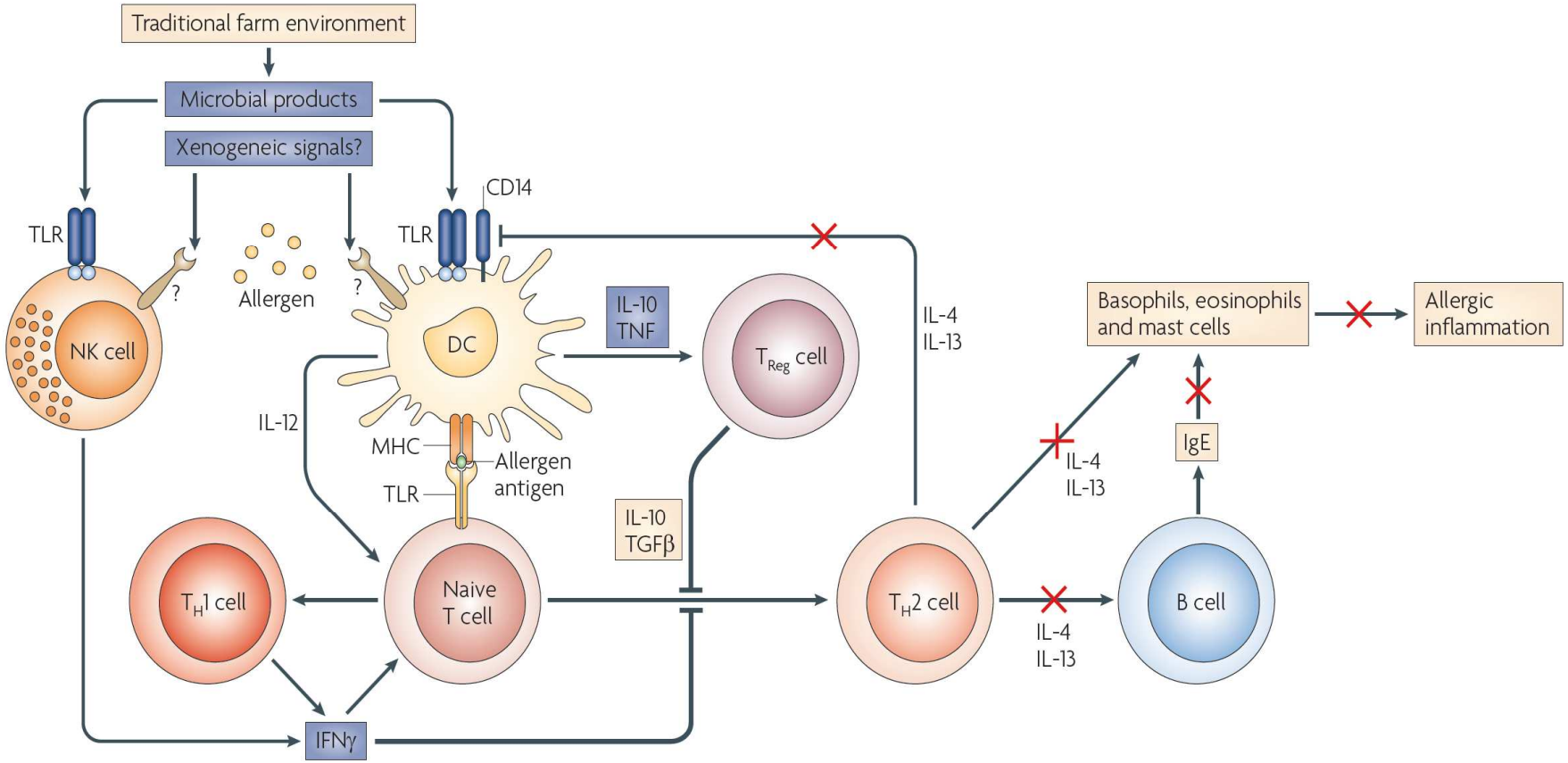
Possible protective mechanisms of microbial exposure in rural/farm environment



Von Mutius and Vercelli. Nature Reviews 2010

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OPEN ACCESS Freely available online

PLoS one

Disordered Microbial Communities in Asthmatic Airways

Markus Hilty[1], Conor Burke[2], Helder Pedro[3,4], Paul Cardenas[1], Andy Bush[1], Cara Bossley[1], Jane Davies[1], Aaron Ervine[2], Len Poulter[2], Lior Pachter[4], Miriam F. Moffatt[1], William O. C. Cookson[1]*

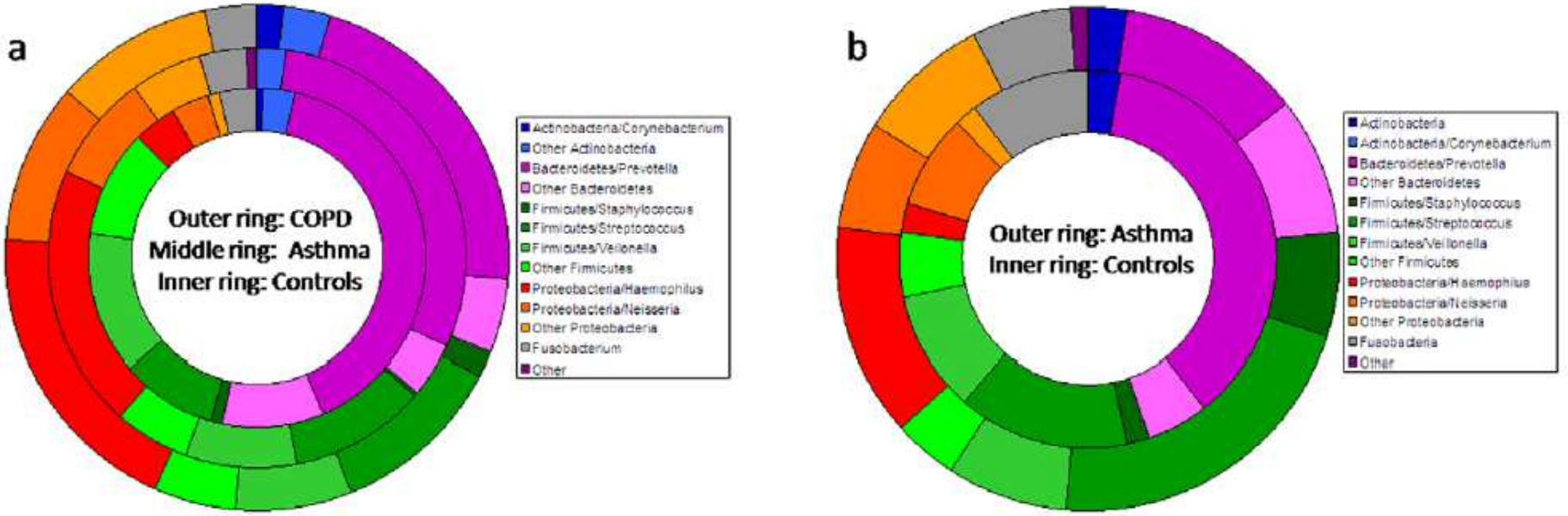

Figure 4. Distribution of common phyla and genera in diseased and normal bronchi. a) Distribution of the phyla from sheathed bronchoscopic brushings of the LUL for patients with asthma and COPD and normal subjects, subdivided into the seven most frequent genera (*Crovnebacterium, Prevotella, Staphylococcus, Streptococcus, Veilonella, Haemophilus and Neisseria*). b) Distribution of the phyla from bronhco-alveolar lavage (BAL) in children with difficult asthma and controls.

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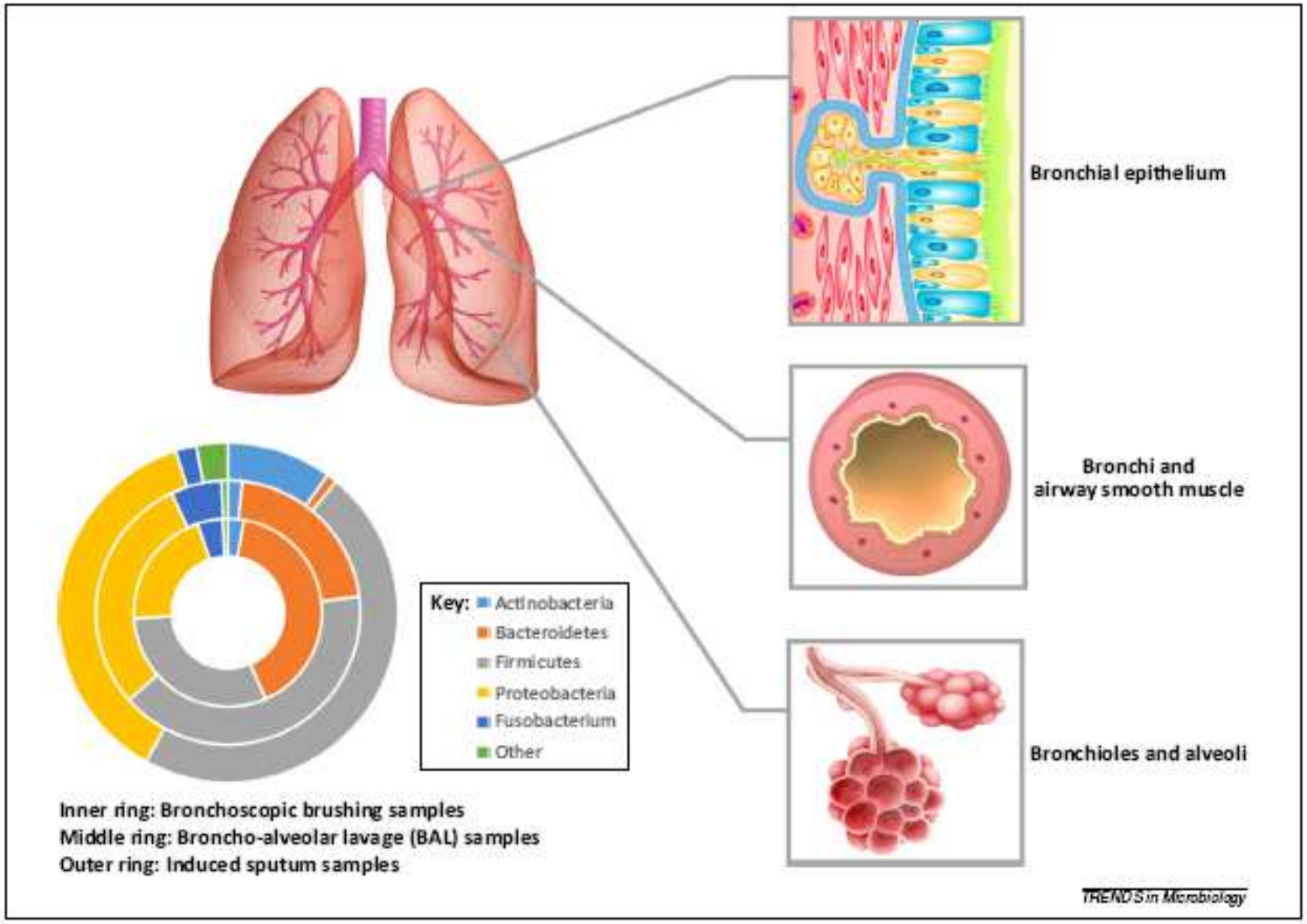
Microbioata in asthmatic airways



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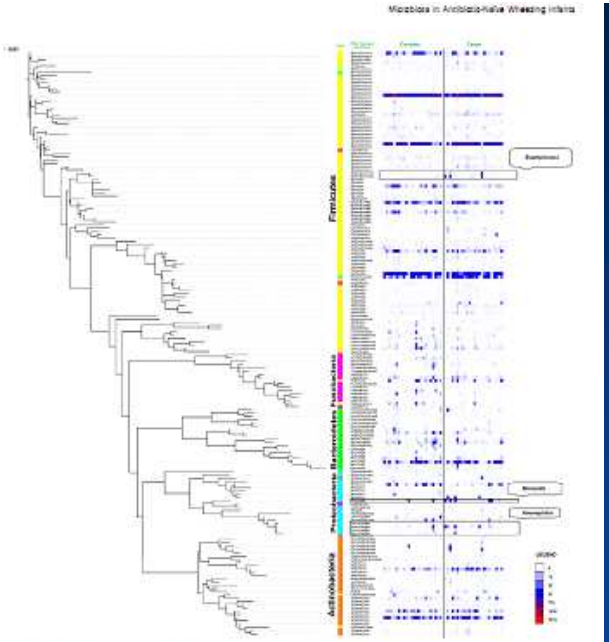
Upper Airways Microbiota in Antibiotic-Naïve Wheezing and Healthy Infants from the Tropics of Rural Ecuador

Paul Andres Cardenas[1,2,3], Philip J. Cooper[2,4,5], Michael J. Cox[1], Martha Chico[2], Carlos Arias[2], Miriam F. Moffatt[1☯], William Osmond Cookson[1*☯]



Results: We obtained 76,627 high quality sequences classified into 182 operational taxonomic units (OTUs). Firmicutes was the most common and diverse phylum (71.22% of sequences) with *Streptococcus* being the most common genus (49.72%). Known pathogens were found significantly more often in cases of infantile wheeze compared to controls, exemplified by *Haemophilus* spp. (OR = 2.12, 95% Confidence Interval (CI) 1.82–2.47; $P = 5.46 \times 10^{-23}$) and *Staphylococcus* spp. (OR = 124.1, 95%CI 59.0–261.2; $P = 1.87 \times 10^{-241}$). Other OTUs were less common in cases than controls, notably *Veillonella* spp. (OR = 0.59, 95%CI = 0.56–0.62; $P = 8.06 \times 10^{-86}$).

Discussion: The airway microbiota appeared to contain many more Streptococci than found in Western Europe and the USA. Comparisons between healthy and wheezing infants revealed a significant difference in several bacterial phylotypes that were not confounded by antibiotics or use of inhaled steroids. The increased prevalence of pathogens such as *Haemophilus* and *Staphylococcus* spp. in cases may contribute to wheezing illnesses in this age group.

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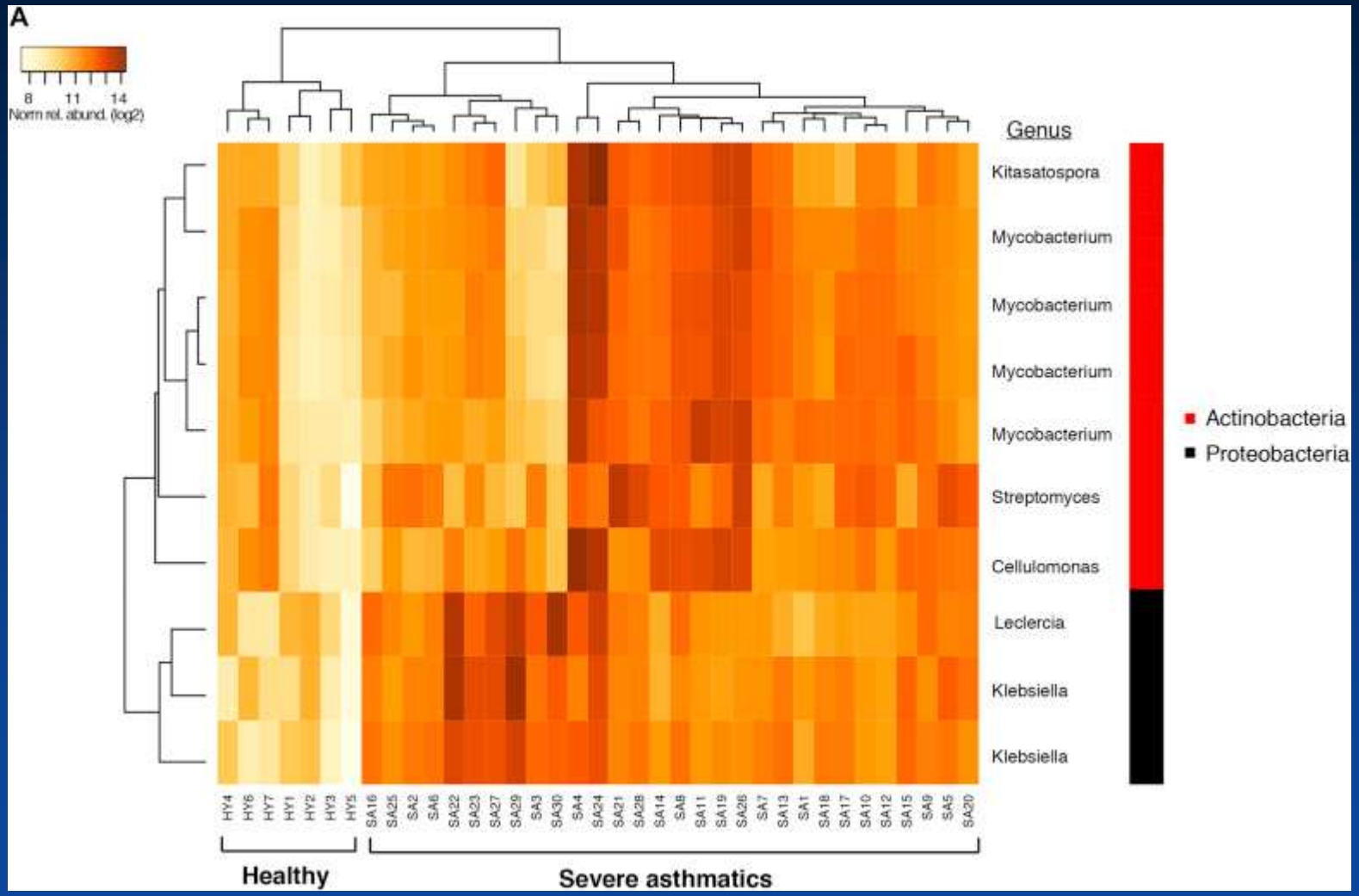
The airway microbiome in patients with severe asthma: Associations with disease features and severity



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The Effects of Airway Microbiome on Corticosteroid Responsiveness in Asthma.

Of the 39 asthmatics, 29 were CR, 10 were CS. BAL microbiome from CR and CS asthmatics did not differ in richness, evenness, diversity and community composition at the phylum level, but did differ at the genera level, with distinct genera expansions in 14 CR asthmatics.

Preincubation of asthmatic airway macrophages with Haemophilus parainfluenzae, a uniquely expanded potential pathogen found only in CR asthma airways, resulted in p38 MAPK activation, increased IL-8, MKP-1 mRNA ($p<0.01$) expression and inhibition of corticosteroid responses . This was not observed after exposure to commensal bacterium Prevotella melaninogenica. Inhibition of transforming growth factor beta associated kinase-1 (TAK1), upstream activator of MAPK, but not p38 MAPK restored cellular sensitivity to corticosteroids.

A subset of asthmatics demonstrates airway expansion of specific gram-negative bacteria, which trigger TAK1/MAPK activation and induce corticosteroid resistance. TAK1 inhibition restored cellular sensitivity to corticosteroids.

Goleva E

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Airway microbiome (infant nasopharyngeal) impacts severity of lower respiratory infection and risk of asthma development.

The nasopharynx microbiome during the critical first year of life in a prospective cohort of 234 children, showed initial colonized with Staphylococcus or Corynebacterium before stable colonization with Alloiococcus or Moraxella.

Transient incursions of Streptococcus, Moraxella, or Haemophilus marked virus-associated ARIs.

Early asymptomatic colonization with Streptococcus was a strong asthma predictor, and antibiotic usage disrupted asymptomatic colonization patterns.

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Gut microbiome: Early infancy microbial and metabolic alterations affect risk of childhood asthma

Gut microbiota of 319 subjects enrolled in the Canadian Healthy Infant Longitudinal Development (CHILD) Study showed that infants at risk of asthma exhibited transient gut microbial dysbiosis during the first 100 days of life.

The relative abundance of Lachnospira, Veillonella, Faecalibacterium, and Rothia was significantly decreased in children at risk of asthma. This was accompanied by reduced levels of fecal acetate and dysregulation of enterohepatic metabolites.

Inoculation of germ-free mice with these four bacterial taxa ameliorated airway inflammation in their adult progeny

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Scenario in the Upper Airways

The nose is a primary defender against inhaled pathogens

Cilia and mucous lining trap inhaled microbes

Inflammation from viral infection and allergic reactions

Inhaled medicines and oral antibiotics

There is a delicate balance of microbes that are maintained to keep that environment healthy. Weakened immune systems can throw off that balance and allow the wrong microbes to grow out of control.

http://commons.wikimedia.org/wiki/File:Human-nose.jpg

Summer 2012 Workshop in Biology and Multimedia for High School Teachers

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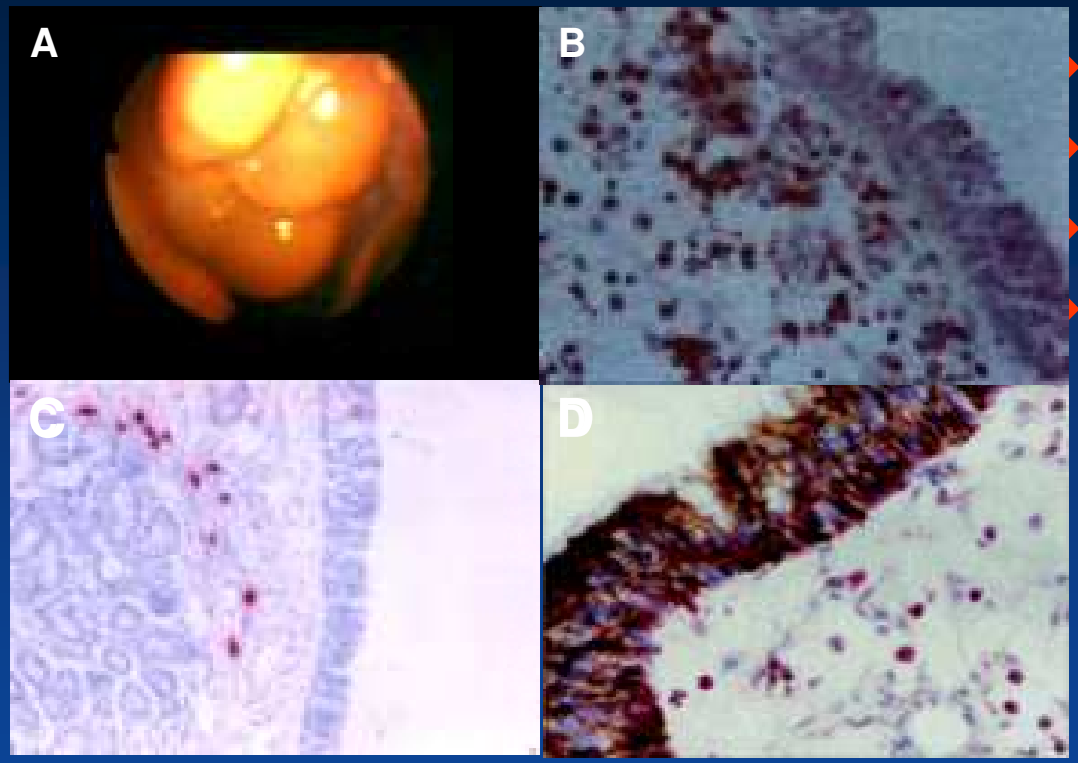
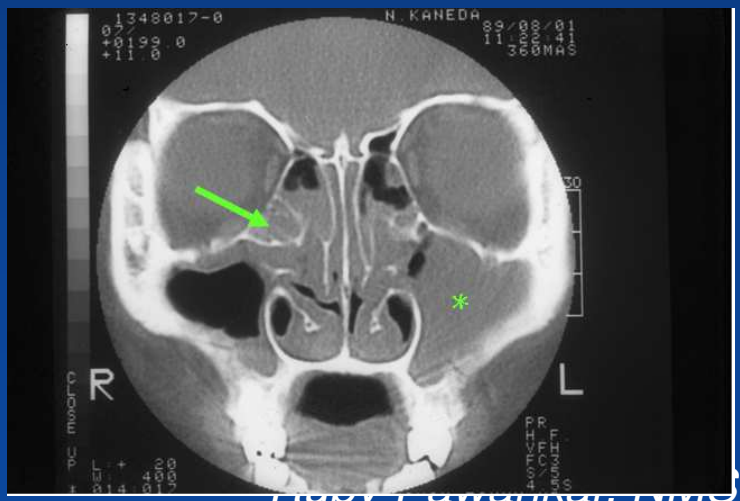
Chronic Rhinosinusitis with Nasal poylps



Hamilos DL et al. Clin Exp Allergy 1998.

- A. Nasal polyps.
- B. Eosinophil infiltration.
- C. Increased IL-5^{+} Th2 cells.
- D. Production of RANTES and eotaxin by epithelial and inflammatory cells.



MICROBIOME

Sinus Microbiome Diversity Depletion and *Corynebacterium tuberculostearicum* Enrichment Mediates Rhinosinusitis

Nicole A. Abreu,[1,2*†] Nabeetha A. Nagalingam,[2*] Yuanlin Song,[3‡] Frederick C. Roediger,[4] Steven D. Pletcher,[4] Andrew N. Goldberg,[4] Susan V. Lynch[2§]

Persistent mucosal inflammation and microbial infection are characteristics of chronic rhinosinusitis (CRS). Mucosal microbiota dysbiosis is found in other chronic inflammatory diseases; however, the relationship between sinus microbiota composition and CRS is unknown. Using comparative microbiome profiling of a cohort of CRS patients and healthy subjects, we demonstrate that the sinus microbiota of CRS patients exhibits significantly reduced bacterial diversity compared with that of healthy controls. In our cohort of CRS patients, multiple, phylogenetically distinct lactic acid bacteria were depleted concomitant with an increase in the relative abundance of a single species, *Corynebacterium tuberculostearicum*. We recapitulated the conditions observed in our human cohort in a murine model and confirmed the pathogenic potential of *C. tuberculostearicum* and the critical necessity for a replete mucosal microbiota to protect against this species. Moreover, *Lactobacillus sakei*, which was identified from our comparative microbiome analyses as a potentially protective species, defended against *C. tuberculostearicum* sinus infection, even in the context of a depleted sinus bacterial community. These studies demonstrate that sinus mucosal health is highly dependent on the composition of the resident microbiota as well as identify both a new sinopathogen and a strong bacterial candidate for therapeutic intervention.

Sci Transl Med **4**, 151ra124 (2012)

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Decreased diversity of nasal microbiota and their secreted extracellular vesicles in patients with CRS : a metagenomic analysis

- Nasal lavage (NAL) fluid samples were obtained from **5 patients** with CRS with polyposis, **3 patients with CRS** without polyposis, and t3 non-CRS controls.

- After preparation of bacteria and EV from samples using differential centrifugation, genomic DNA was extracted and 16S-rDNAamplicons were subjected to high-throughput pyrosequencing on a Roche 454 GS-FLX platform.

E.-B. Choi et al, Allergy 2014; 69:217

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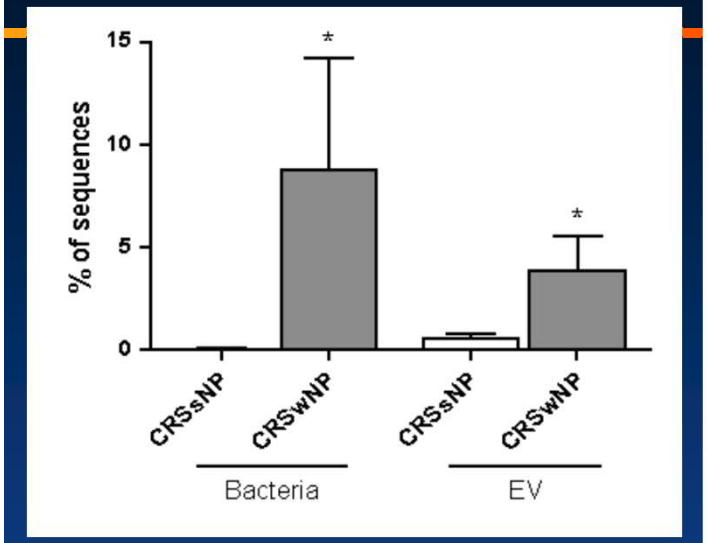

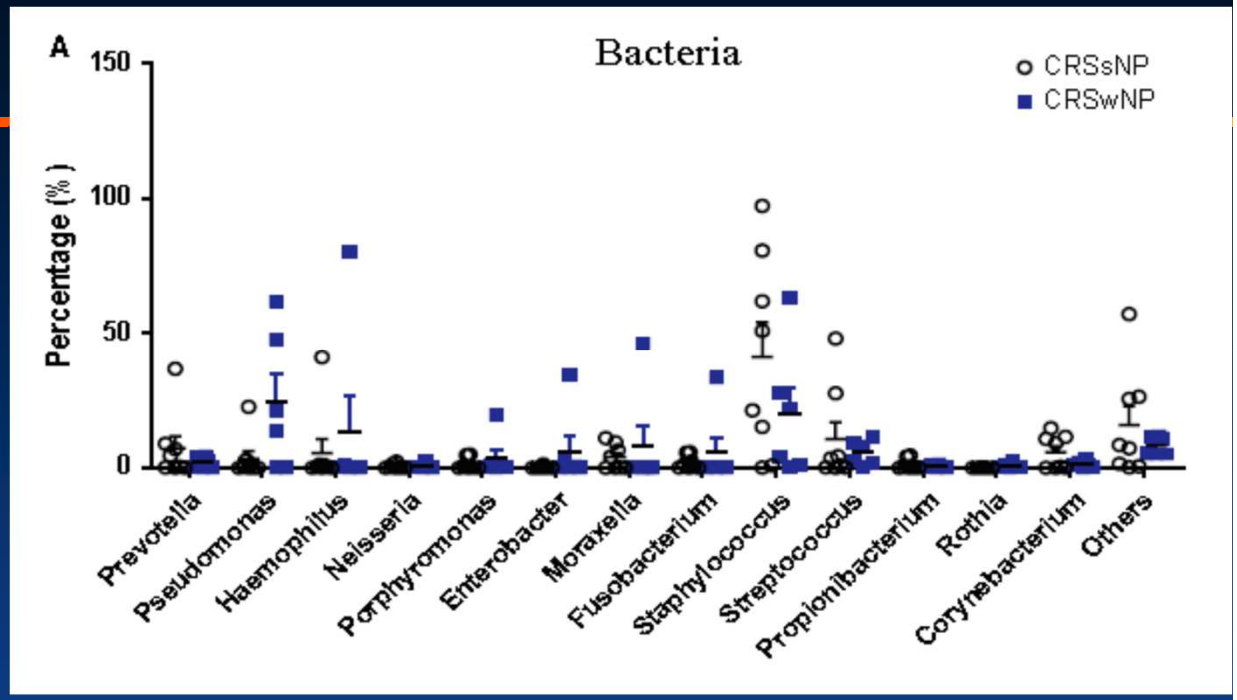

Compositional differences in bacteria and their secreted extracellular vesicles (EV) between chronic rhinosinusitis (CRS) with and without polyps.

a) Relative abundance of Staphylococcus aureus and its extracellular vesicles in CRSwNP and CRSsNP. *P < 0.05

b) Compositions of major bacterial genera in nasal lavage (NAL) fluids from patients having CRSwNP and CRSsNP.

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Results

Samples from patients with CRS had greater bacterial abundance and lower diversity, compared with non-CRS.

At each phylogenetic level, Bacteroidetes decreased while Proteobacteria increased in the CRS group at the phylum level.

At the genus level, Prevotella spp. decreased in the CRS group, while Staphylococcus spp. increased from both bacteria and EV. S. aureus and its secreting EV compositions were higher in samples from CRS with polyps compared with CRSsNP.

Conclusions: patients with CRS have altered nasal microbiota and decreased diversity in bacterial compositions as well as increased S. aureus abundance in those patients with polyps.

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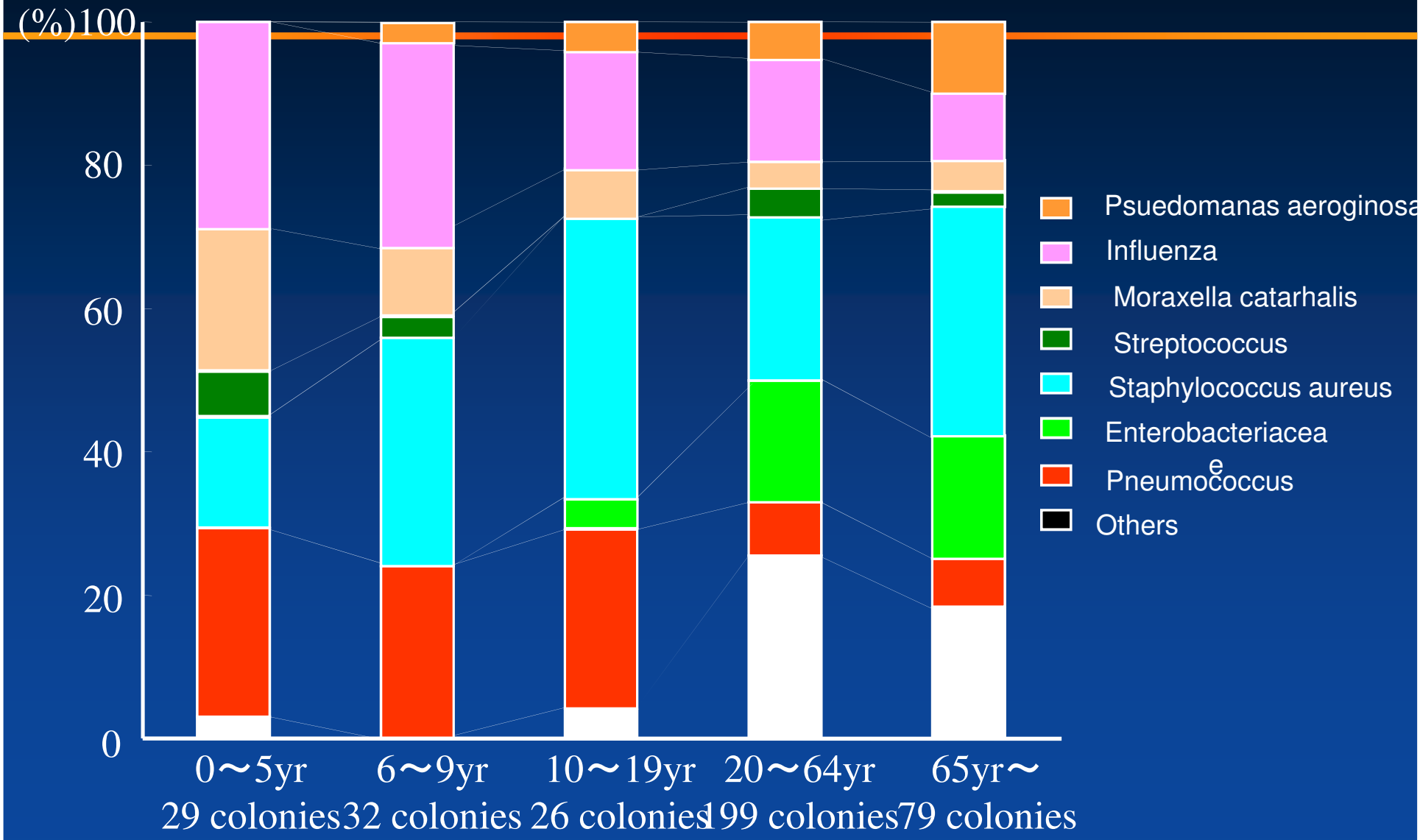
Age-based bacteriology of Chronic Rhinosinusitis in Japan



Baba S et al. Japan ORL infection symposium proceedings 18(1), 48, 2000,

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Inflammatory paterns IgE and microbiome

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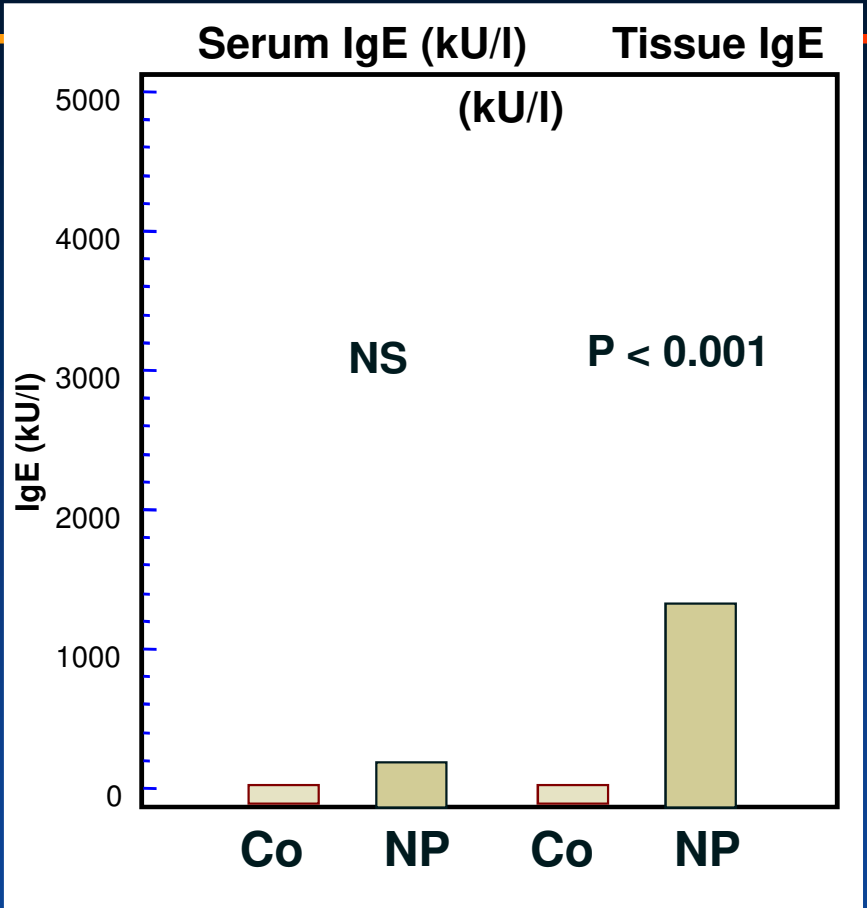
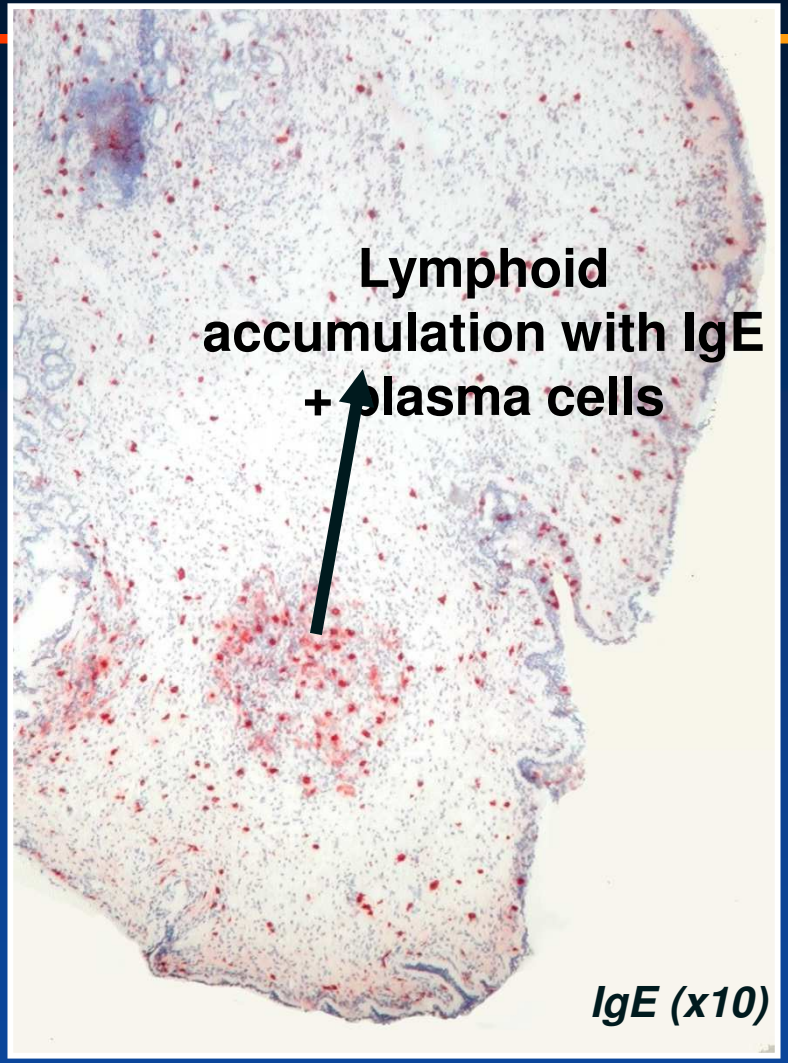
Local IgE production in Nasal Polyposis



Control (Co) : n = 24
Nasal polyp (NP) : n = 43



Bachert et al

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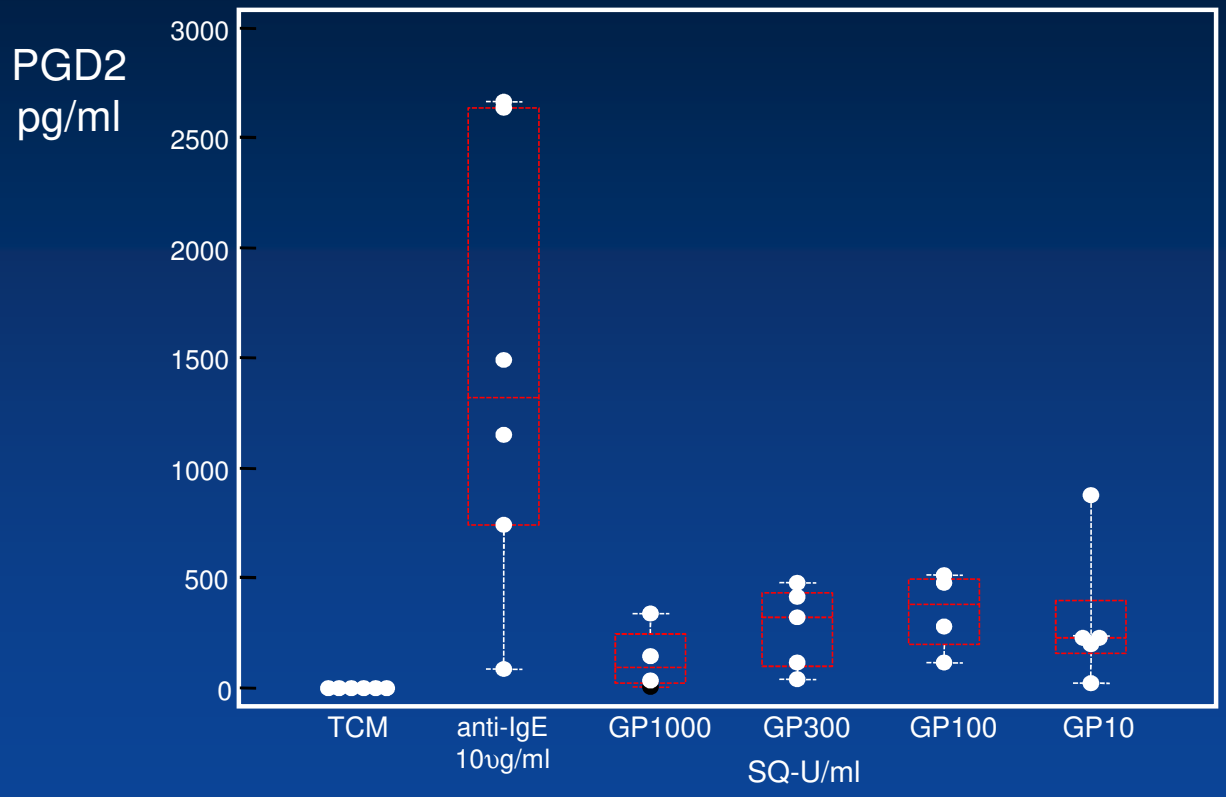
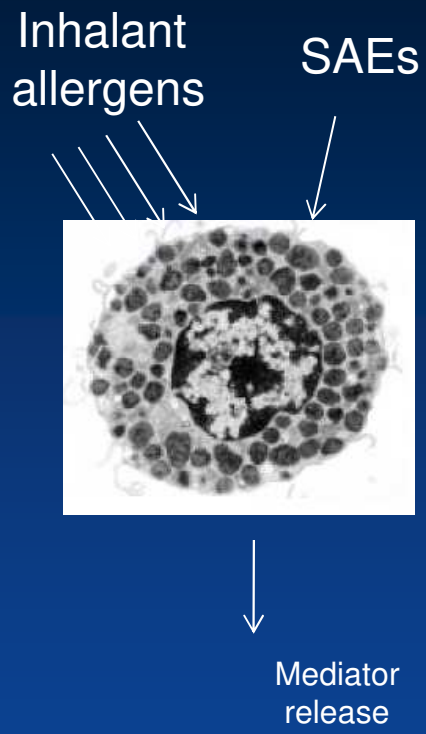
Nasal polyp patients with specific IgE to grass allergen in tissue, but not in serum react to allergen





Mucosal tissue polyclonal IgE is functional in response to allergen and SEB
Nan Zhang, G Holtappels, P Gevaert, J Patou, B Dhaliwal, H Gould, C Bachert. Allergy 2010

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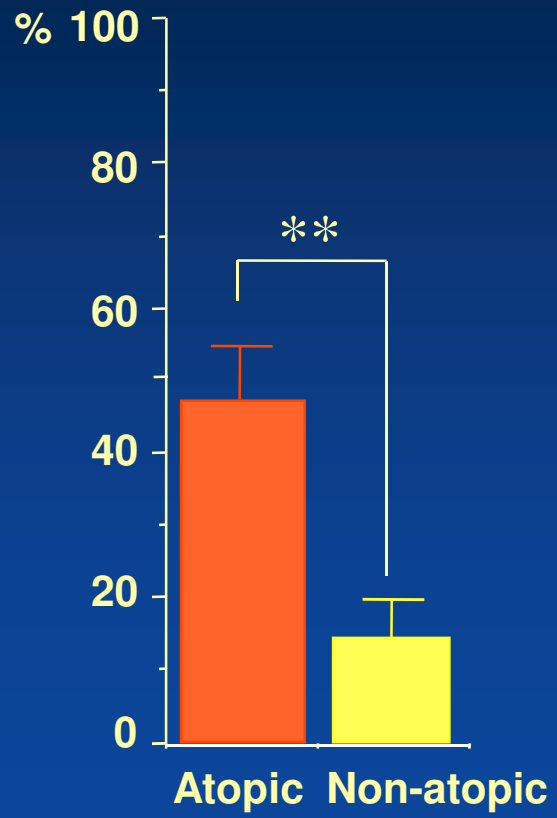
High Fc epsilon RI expression in nasal polyps of atopics



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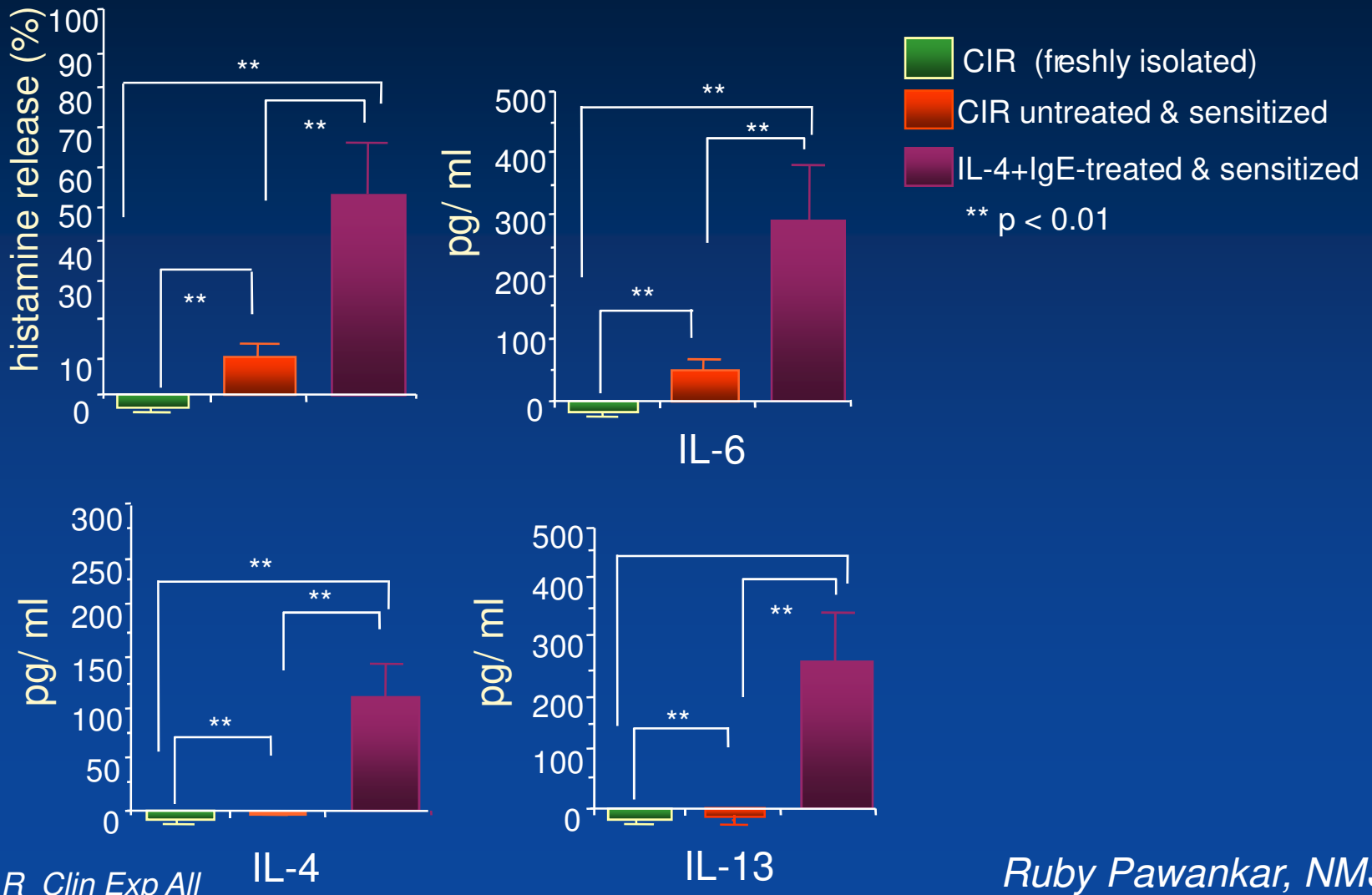
IL-4 + IgE enhance mediator release from nasal mast cells



Pawankar R Clin Exp All

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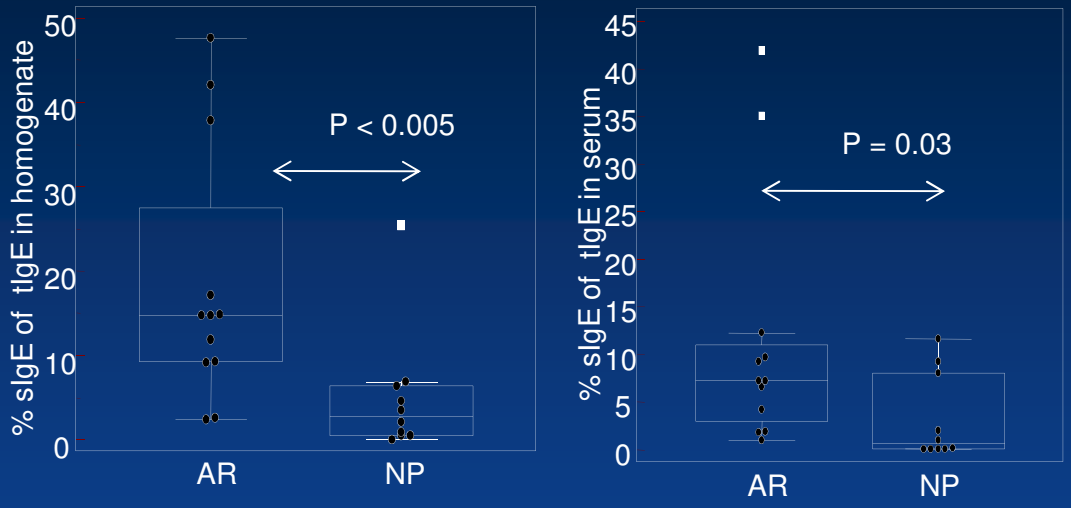
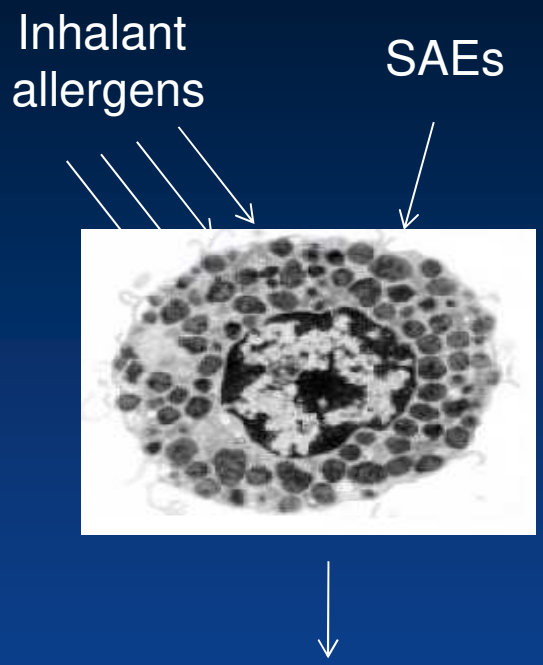
Chronic mast cell activation through polyclonal IgE





Mucosal tissue polyclonal IgE is functional in response to allergen and SEB

Nan Zhang, G Holtappels, P Gevaert, J Patou, B Dhaliwal, H Gould, C Bachert. Allergy 2010

Compared to AR subjects, the percentage of specific IgE abs to GP and HDM out of the total IgE values was significantly lower **a,** in nasal polyp tissue with 2.8% (0.5%-6.4%; p<0.005) and **b**, in serum with 0.6% (0.1 – 8.1%, p=0.03) vs.14.7% (9.3 - 27.5%) and 7.2% (3.1 – 11%), respectively.

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Correlation between ECP and polyp IgE / IgG antibodies

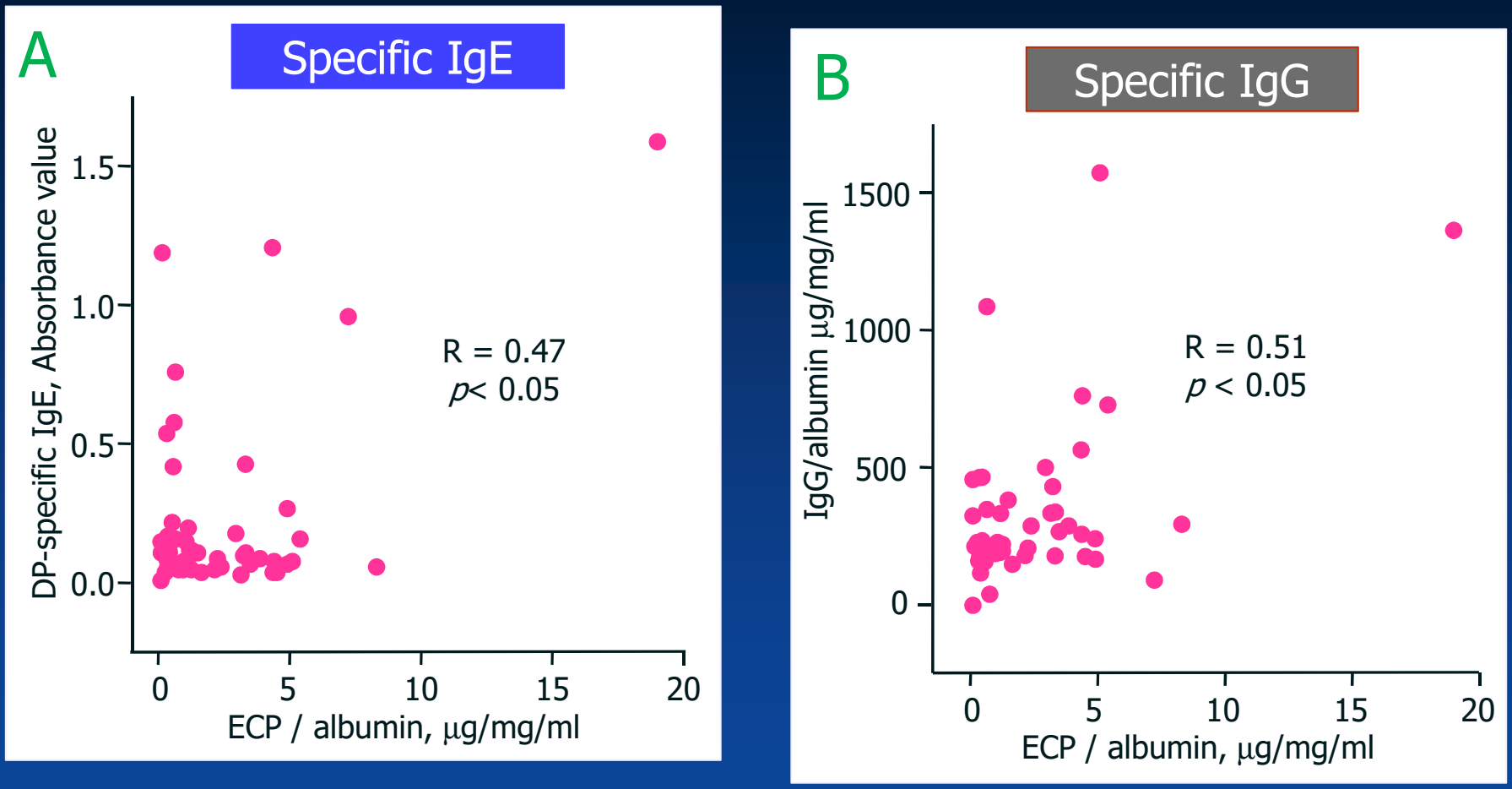

Fig. Correlation between ECP level and *D.pteronyssinus* (DP)-specific IgE antibody (A) or IgG (B) in nasal polyp homogenate.

Suh K et al. J Kor Med Sci 2002;17:375

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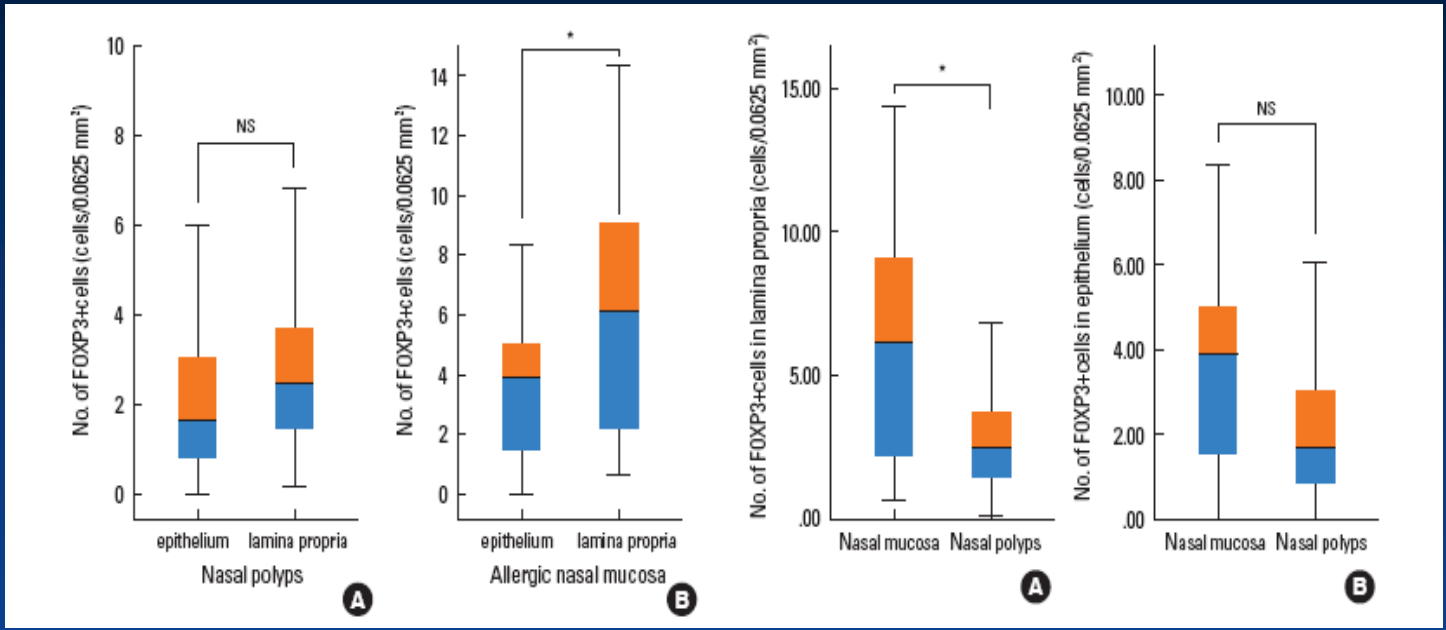
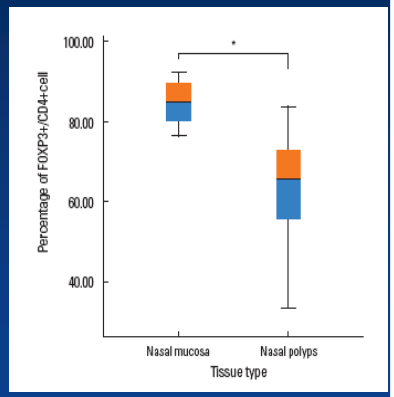
Low numbers of T-regulatory cells in nasal polyps





Kannika R, Pawankar R et al

Allergy Asthma Immunol Res. 2011

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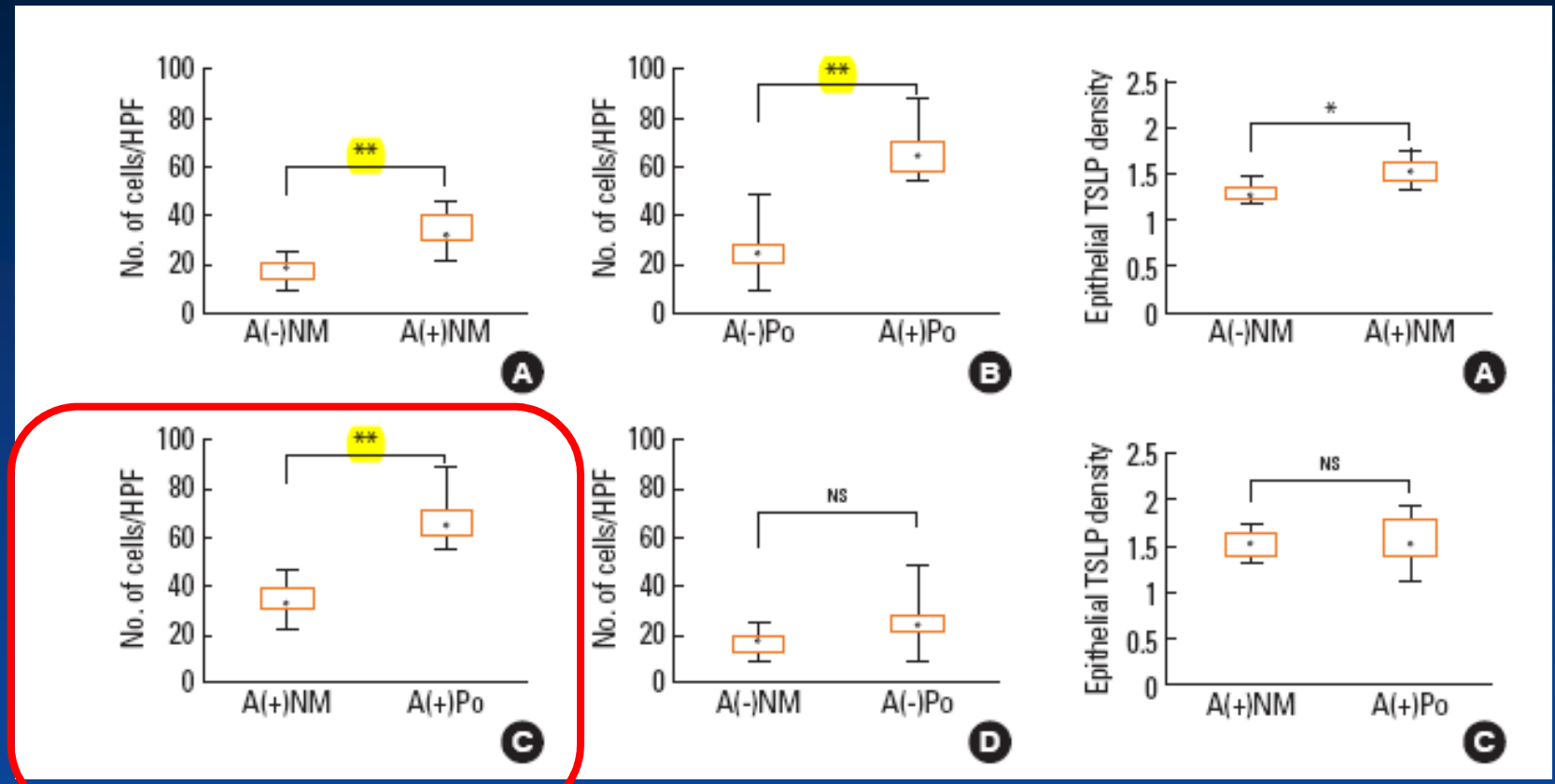
Fig. 4. The number of thymic stromal lymphopoietin (TSLP) positive cells in the nasal mucosa and nasal polyps. [illegible]



Kimura S, Pawankar R et al. AAIR, 2011

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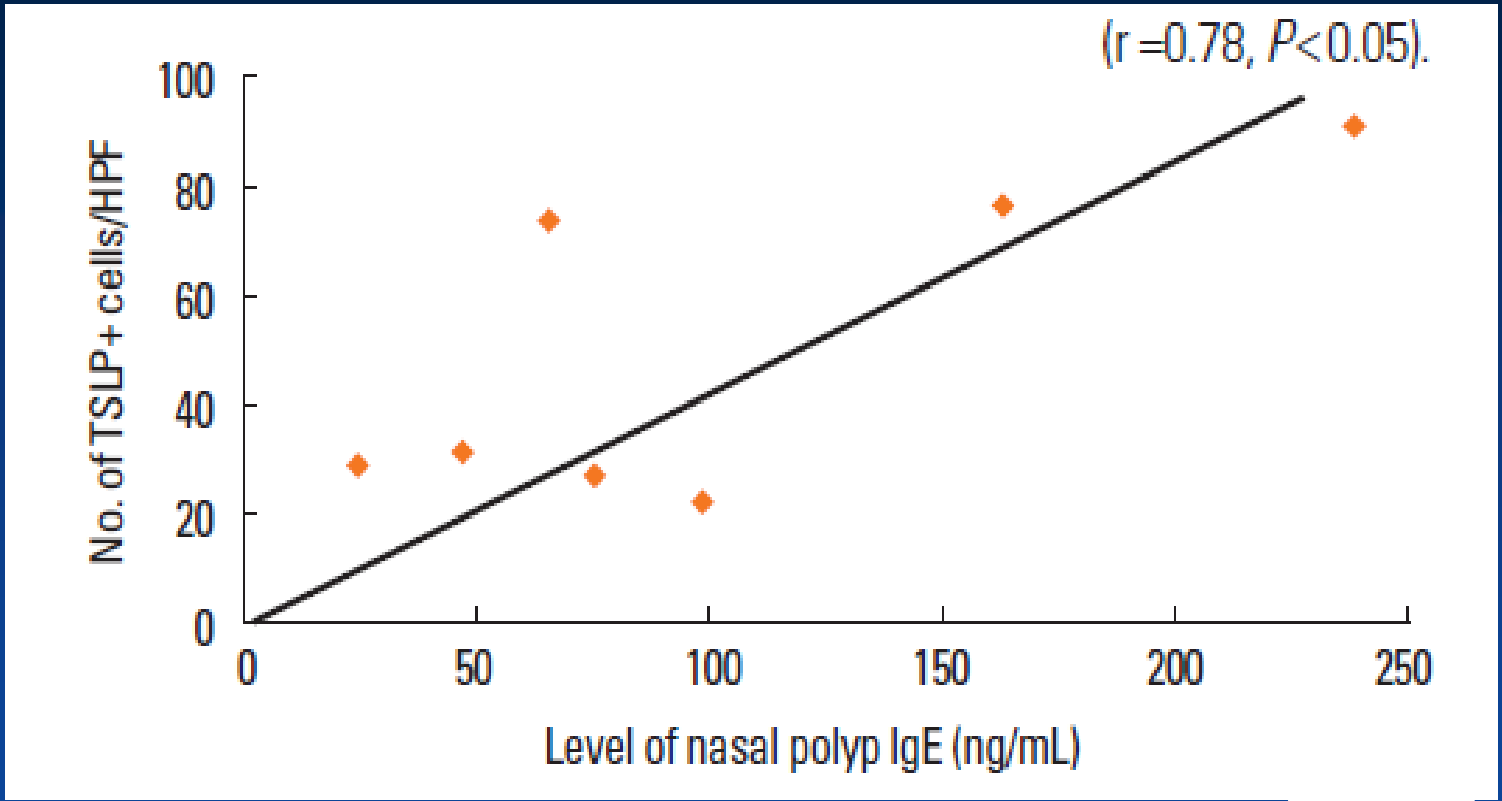
Correlation between TSLP and IgE in NP



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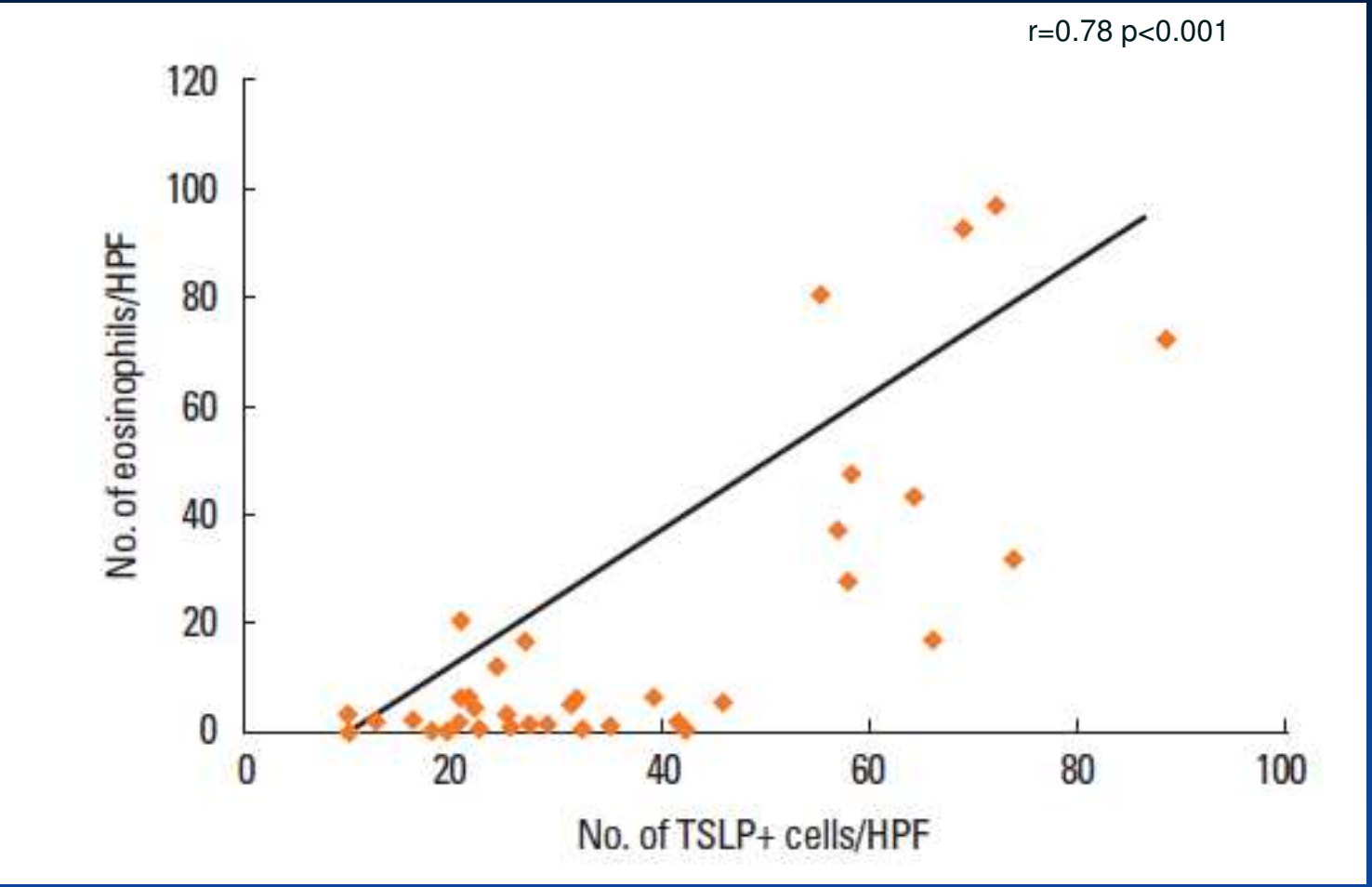
Correlation between TSLP and eosinophils in NP



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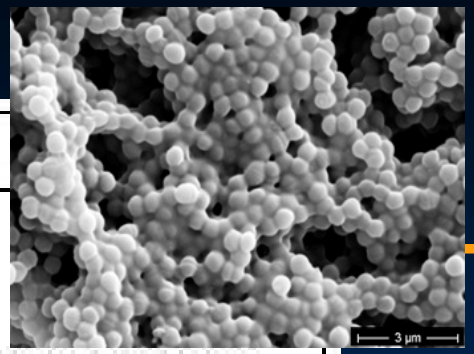

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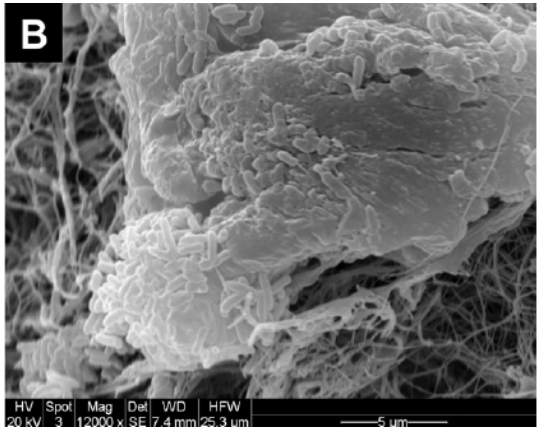
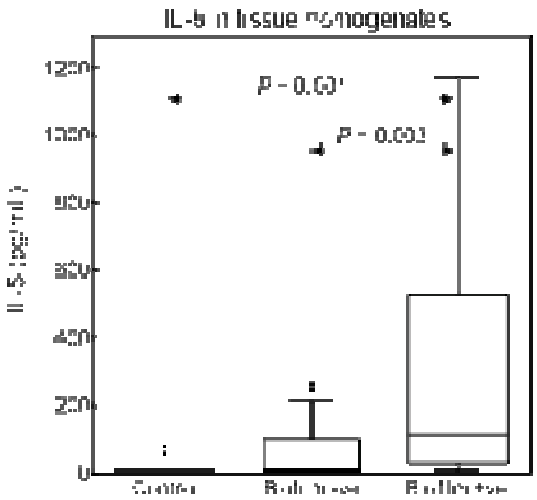
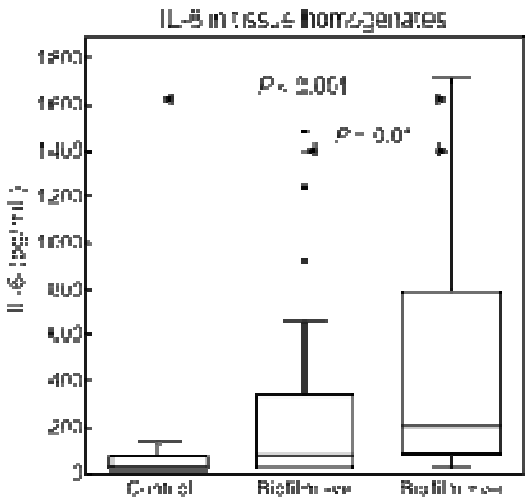
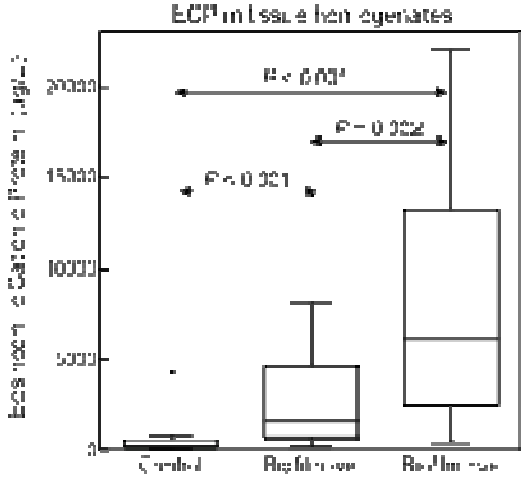








Adaptive immune responses in *Staphylococcus aureus* biofilm associated chronic rhinosinusitis

A Foreman; G Holtappels; AJ Psaltis, J Jervis-Bardy; J Field PJ Wormald; C Bachert. Allergy 2011

Allergy

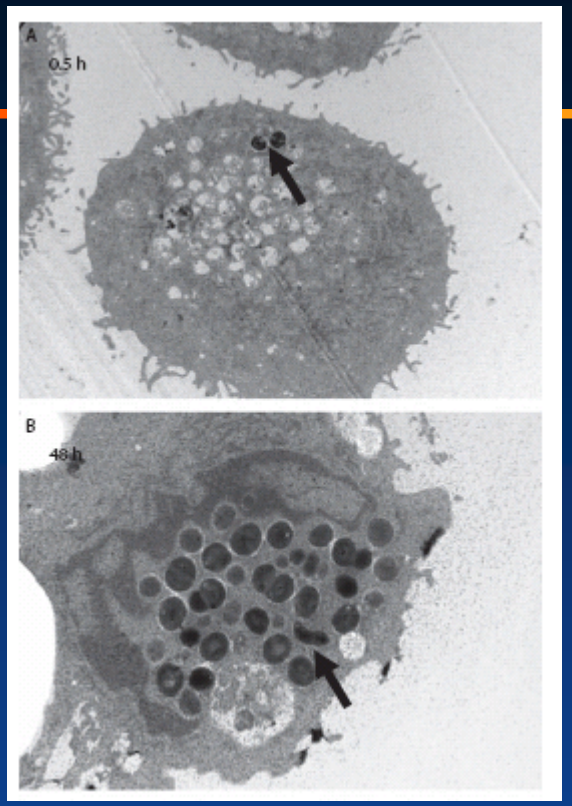
ORIGINAL ARTICLE AIRWAY DISEASES

Staphylococcus aureus invades the epithelium in nasal polyposis and induces IL-6 in nasal epithelial cells *in vitro*

F. Sachse[1], K. Becker[2], C. von Eiff[2]*, D. Metze[3] & C. Rudack[1]

[1]Department of Otorhinolaryngology; [2]Institute of Medical Microbiology; [3]Department of Dermatology, University Hospital Münster, Münster, Germany

Results: Peptide nucleic acid-fluorescence *in situ* hybridization positive bacterial cells were significantly increased in the epithelium of CRSwNP (17/25) compared to CRSsNP (0/5) and TM (1/10). Good concordance of PNA-FISH results and *S. aureus* cultivation was found applying Cohen's κ for CRSwNP ($\kappa = 0.841$) and TM ($\kappa = 1.0$). Intracellular persistence assay with *S. aureus* strain Newman and its corresponding small-colony variant mutant strain III33 demonstrated intracellular survival and replication of *S. aureus* within NPECs. Both *S. aureus* strains significantly induced IL-6 but not IL-13 in infected NPECs and in NPECs challenged with corresponding staphylococcal supernatants.

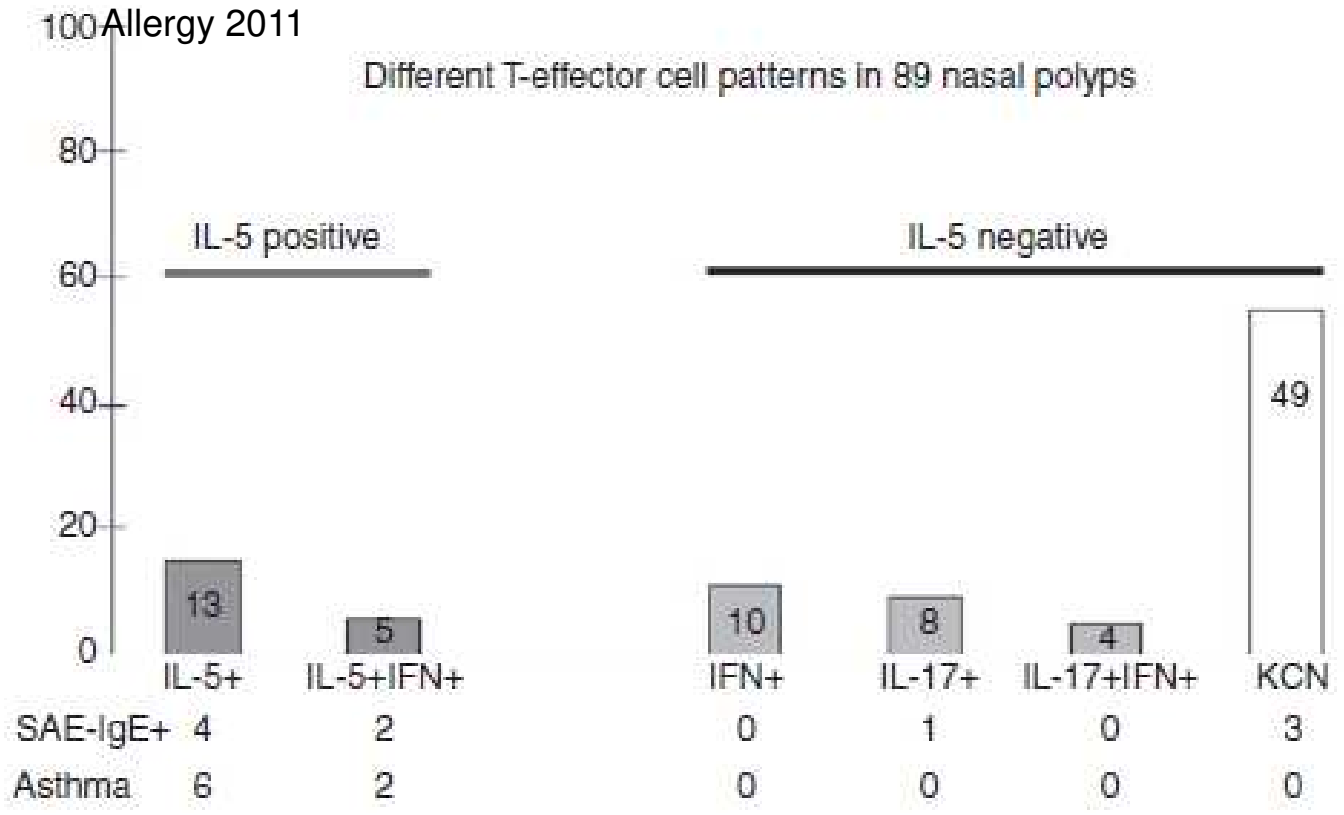


EM: Nasal polyp epithelial cells infected with S. aureus hemB mutant. Screenshots were taken at 0.5 and 24 h.

Ruby Pawankar, NMS

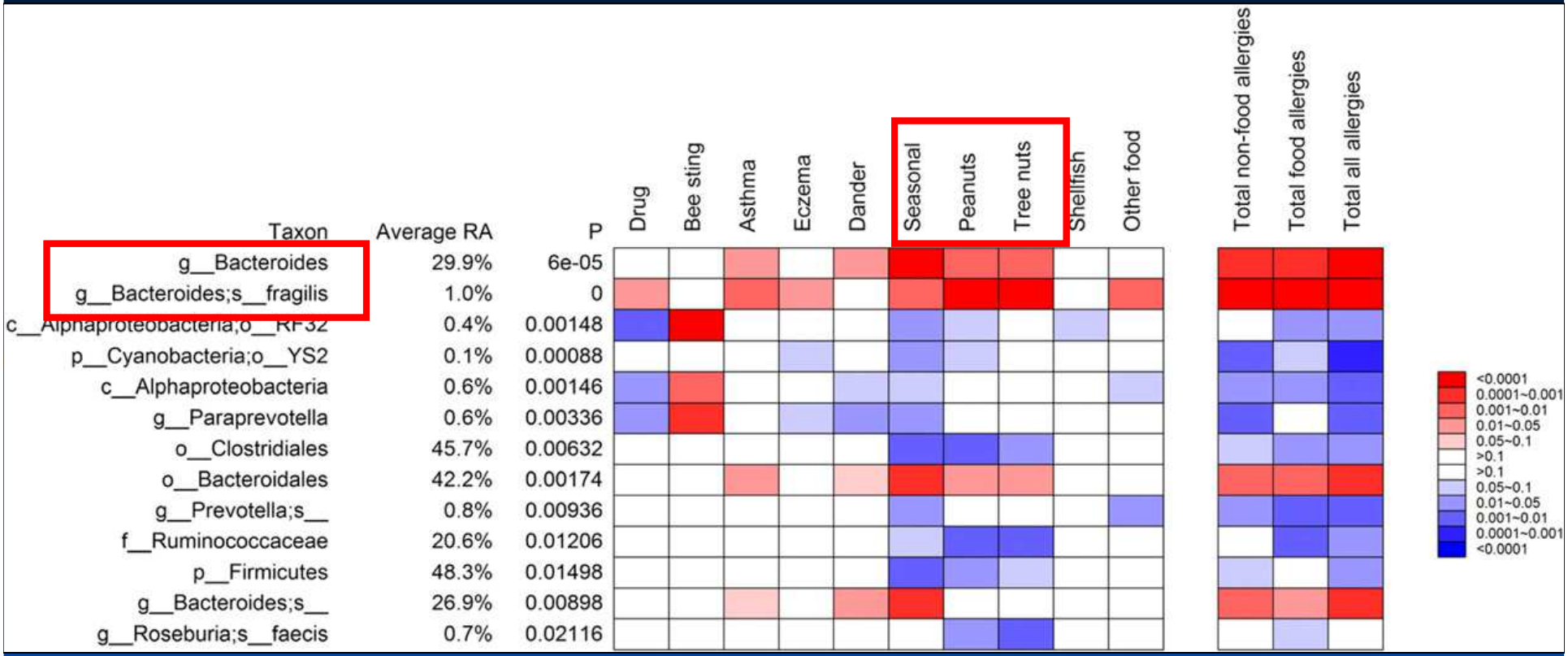
The association between bacterial colonization and inflammatory pattern in Chinese chronic rhinosinusitis patients with nasal polyps

L. Ba[1,2*], N. Zhang[3*], J. Meng[1], J. Zhang[4], P. Lin[4], P. Zhou[1], S. Liu[1] & C. Bachert[3]



Ruby Pawankar, NMS

Gut microbial population increases the risk for FA and respiratory allergy



Allergies associated with low diversity
Higher bacteroidales and lower Clostridales

Hua X et al. EBioMedicine. 2015;3:172-9

What can we do:
Gut microbiome, diet and lung inflammation

Feeding mice with a diet high in fiber

↓

Increase in the relative abundance of *Bacteroidaceae* and *Bifidobacteriaceae*

↓

Systemic short chain fatty acids SCFA

↓

Tolerogenic DCs

↓

Decreased lung inflammation induced by HDM

Trompette A., et al. Nat. Med. 2014;20:159–166

Conclusion

Microbiota in early life can influence development of inflammatory diseases like asthma, CRS, other allergic diseases

The nature and mechanisms of growing biofilm is crucial for efficient prophylaxis and treatment of chronic biofilm infections.

Better understanding of the microbiome and alterations in allergic diseases, relation to disease severity in early life may guide early interventions

Early interventions to restore the dysbiosis may prevent the development of disease.

Ruby Pawankar, NMS