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Viruses and bacteria in Th2 biased allergic airway disease

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Abstract:

Allergic airway diseases are typically characterized by a type 2-biased inflammation. Multiple distinct viruses and bacteria have been detected in the airways. Recently, it has been confirmed that the microbiome of allergic individuals differs from those of healthy subjects, showing a close relationship with the type 2 response in allergic airway disease. In this paper, we summarize recent findings on the prevalence of viruses and bacteria in type 2-biased airway diseases and on mechanisms employed by viruses and bacteria in propagating type 2 responses. The understanding of the microbial composition and post-infectious immune programming is critical for the reconstruction of the normal microflora and immune status in allergic airway diseases.

Key words: asthma; allergy rhinitis; chronic rhinosinusitis; microbiome; Th2 response;

Introduction

The role of environmental and gastrointestinal pathogens in allergic airway inflammatory pathology (whether protective or provocative) has been widely studied. The hygiene hypothesis proposes that a low prevalence of childhood asthma is associated with exposure of infants to a microbe-rich environment (1, 2); a low diversity of gut microbiome in early infancy precedes asthma at the school age (3). However, the Copenhagen Prospective Studies on Asthma in Childhood recently suggested that individual susceptibility and inflammatory consequences after infection, rather than the specific microbial trigger, are associated with school-age asthma development(4). Therefore, understanding the microbial composition and post-infectious immune programming in type 2-biased airway diseases are very important for therapeutic interventions in the future. Allergic asthma, allergic rhinitis and chronic rhinosinusitis with nasal polyps (CRSwNP) are typical airway disorders that are characterized by a type 2-biased inflammation. In this review, we summarize the prevalence of viruses and bacteria in type 2-biased airway diseases, on the mechanisms employed by viruses and bacteria in propagating type 2 responses and the impact of T helper 2 (Th2) cytokines on the defense against infections.

The prevalence of viruses in the airways

The nasopharynx is regarded as a considerable reservoir for bacteria and viruses in our body. Viral infections starting from the nasopharynx in early life are reportedly associated with an increased risk of developing asthma later in life (**Table1**). Viral infections that affect allergic upper airway diseases can also lead to acute asthma exacerbations in the lower airways (5). To clarify the prevalence of viral communities in the development of asthma, nasopharyngeal samples were obtained for 16S rRNA gene deep sequencing analysis from infants at scheduled visits at 2, 6, and 12 months of age, or within 48 h after onset of an acute respiratory infection (ARI) during the first year of life in the extension of the Childhood Asthma Study (CAS) (6). The most common viruses found in the nasopharynx of healthy children during ARI were human rhinovirus (HRV) (40%) and human respiratory syncytial virus (HRSV) (11%) (6). Among those viruses, HRV-C rather than the other HRV subtypes or HRSV, which was detected in nasopharynx of healthy children during ARI accompanied by wheezing symptoms, was positively associated with later chronic wheezing among all children and particularly for those who were atopic by 2 years(6). In a recent study, HRSV and HRV induced lower respiratory tract infections in 412 pediatric patients up to the age of 3 years were associated with the development of wheezing and exacerbations(7). In addition, pediatric patients with HRV induced wheezing were more likely to develop subsequent recurrent wheezing and asthma during the following 3 years (7). Both HRV and HRSV are well known risk factors for subsequent wheezing or asthma development in infancy, but they also have specific susceptibilities according to their age. HRV infection carries a markedly higher risk for persistent wheezing in younger infancy (under 2 years of age) (8), while HRSV infections are related to the development of asthma in children at school age (9).

In hospitalized children aged between 3 to 16 years, HRV and HRSV were the most frequent viruses in nasopharynx secretion during asthma exacerbations (10). Similarly, a Japanese group also demonstrated that HRSV was related to wheezing episodes and was more frequent in patients with a history of asthma (11). Besides HRSV and HRV, also influenza viruses (IFV) were involved in asthma exacerbations in children particularly with atopic sensitizations, but not in non-atopic children (12). In atopic Turkish children with asthma and allergic rhinitis, significantly higher herpes simplex type 1 (HSV-1) IgG seropositivity was detected in the serum than in the matched non-atopic group(13), suggesting a possible relationship between pediatric allergic airway diseases and HSV-1 infections. In adults, higher incidences

of HRV A/B/C, HRSV A, and metapneumovirus infections were observed in nasopharyngeal swabs during asthma exacerbations (14).

The presence of picornavirus, HRSV, IFV, and parainfluenza viruses was significantly increased in CRS patients compared with that seen in controls (64% vs. 30%)(15). Particularly, HRV was the only virus that demonstrated a significant increase in infection rate in both nasal lavage and epithelial cells scrapped from nasal inferior turbinate of CRS patients vs. controls (15). High rates of this virus were also found in epithelial cells scrapped from the nasal middle meatus of Chinese CRSwNP patients (16). Concordantly, a high prevalence of HRV was also present in sinus tissues in Brazilian CRS patients, and there was a peak in detection during the autumn and winter seasons, when respiratory viruses are far more prevalent (17). Unfortunately, CRS was not further differentiated in that study. In contrast to the Th2-biased inflammatory characteristics of CRSwNP, CRSsNP often exhibits a Th1-cell inflammation pattern (18). Due to their different inflammatory patterns, it is necessary to characterize the prevalence of viruses in each subgroup of CRS in the future.

Allergic rhinitis is an allergic inflammation of the upper airways, and it is also a known risk factor for wheeze and asthma. In general, asthma occurs in 10–40% of patients with allergic rhinitis, whereas allergic rhinitis occurs in 20–80% of patients with asthma (19, 20). Interestingly, both the cross-sectional survey of Singaporean and the Pollution and Asthma Risk: an Infant Study birth cohort studies demonstrated that allergic rhinitis may start as early as in the first 2 years of life (21, 22). In early childhood, HRV was the most frequent virus detected in nasal swab samples of prolonged/recurrent rhinitis (23). Interestingly, HRV-positive infants with recurrent rhinitis had a higher rate of wheeze compared to HRV-negative infants (23). Similar to childhood allergic rhinitis, an abundance of respiratory viruses, especially HRV, was detected in nasal lavage samples of adult perennial allergic rhinitis patients, but respiratory viral infection was independently correlated with symptom scores(24).

The prevalence of bacteria in the airways

Bacterial community composition is a strong determinant of general health and disease, as well as a potential modulator of inflammatory processes contributing to airway diseases. Here bacteria which affect airway diseases are listed in **Table 2**. In infants, the nasopharynx microbiome is qualitatively dominated by six common genera: *Haemophilus*, *Streptococcus*, *Moraxella* (those three genera are common in ARI), *Staphylococcus*, *Alloiococcus* and *Corynebacterium* (more common in healthy samples) (6), which is consistent with previous studies in children (25, 26) and in adults (27). Similarly, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pyogenes* and *Staphylococcus aureus* (*S. aureus*) were also observed in nasopharynx swabs of Asian infants (28). However, the Asian infants had a higher colonization of *S. aureus* (88.9–100.0%) than in other studies at the first year of life, and 50% of *S. aureus* was methicillin-resistant *S. aureus* (28). Nevertheless, the composition of microbial communities seems unstable over time. Early colonization typically involved *Staphylococcus* or *Corynebacterium*, which later was replaced by *Moraxella* or *Alloiococcus* in healthy infants, indicating that the composition of microbiome undergoes dynamic changes (6). Whether dynamic change in the early microbiome is related with later allergic airway diseases requires more research. Clearly, neonatal colonization with *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumonia* had a strong correlation with subsequent recurrent wheeze and childhood asthma (6, 29, 30). Evidence was also available that *S. aureus* acted as a risk factor for asthma development in children and young adults, but not in older adults (31-33). It is evident that bacterial organisms act as important contributors to asthma exacerbations. A study of the bacterial composition of the nasopharynx in adult asthmatic patients indicated that *Streptococcus pneumoniae* infection was also associated with asthma exacerbations in clinical practice (14).

In CRS, inflammation development takes place in the middle meatus. A high prevalence of *Staphylococcus epidermidis* (96.4%), *S. aureus* (67.9%), and *Propionibacterium acnes* (92.9%) was found in middle meatus swab of healthy adults (34). Bacteria such as *Pseudomonas*, *Staphylococcus*, and *Streptococcus* were numerically increased in sinus tissues of CRS patients compared to healthy controls (35). Interestingly, a higher abundance of *Staphylococcus*, *Acinetobacter*, *Pseudomonas aeruginosa*, and *Ralstonia* species, and a lower level of *Prevotella*, *Fusobacterium* and *Campylobacter* species were detected in CRS patients with concomitant asthma than in CRS patients without asthma (35, 36).

Additionally, patients with abundance expression of *Corynebacterium tuberculo* at the time of endoscopic surgery had an optimal outcome, while patients with high *S. aureus* had a suboptimal outcome (35). Coherently, an increase of nasal *S. aureus* carriage and the presence of *S. aureus* enterotoxin-specific IgE antibodies have been described in the mucosa of CRSwNP subjects and perennial allergic rhinitis patients (33, 37), and this is even higher in CRSwNP patients with asthma comorbidity and aspirin sensitivity. The further analysis on antibiotics resistance bacteria in allergic rhinitis highlighted that an increase of methicillin resistant staphylococcus aureus burden provides an insight on the difficulty of eradicating the bacterium (38).

Viruses and type 2 responses in allergic airway diseases

HRV, as one of the most common viruses in human airways, is associated with the development and exacerbation of allergic asthma. Epithelial cell derived cytokines such as IL-33, thymic stromal lymphopoietin (TSLP) and IL-25 are critical in propagating a type 2 immune response by affecting Th2 cells, innate lymphoid type 2 (ILC2) cells and dendritic cells (39). HRV infections induce mucosal IL-33 production in vivo, which can further initiate type 2 cytokine release from Th2 cells or ILC2 cells (40). HRV-induced IL-25 amplified type 2 responses via activation of IL-25 receptors on murine Th2 cell, ILC2s and basophils (41). Elevated levels of TSLP in allergic asthma subjects positively correlated with the severity of asthma (42), but so far there is no direct evidence showing the effect of HRV infection on TSLP production. In a recent study, CD11b⁺ exudative macrophages bearing M2 macrophage markers have been identified as a new source for IL-13 production in an OVA asthma mouse model after HRV infection (43).

Respiratory syncytial virus (RSV), a member of the paramyxoviridae family, is also associated with type 2 responses in allergic airway diseases. An increased level of IL-13 was observed in the lungs of BALB/c mice, mediated by IL-33 and TSLP production after intranasal RSV incubation (44, 45). Furthermore, the interactions between a glycosyltransferase lunatic fringe and Notch ligands 4- Notch were reportedly responsible for the augmented Th2 inflammation in mouse during RSV asthma exacerbations (46). Thus, IL-33, TSLP and Notch signaling might be the possible therapeutic target in RSV-induced Th2 responses. In addition to the classical Th2 cells and ST2L⁺ cells, a novel human subset of IL-6Rα^{high} effector memory

CD8⁺ T cells was identified to exclusively release IL-5 and IL-13 after HRSV infection (47). IL-6Rα^{high} effector memory CD8⁺ T cells were detected in high numbers in the peripheral blood of asthma subjects (47), suggesting a role of the cells in promoting Th2 responses in asthma (**Figure 1**).

Similarly, IFV may induce the release of IL-33 from Natural killer T cells and alveolar macrophages in mice, leading to a robust IL-5 release from ILC2 cells (48). Not only viruses but also viral nucleotide analogues can contribute to IL-13 expression by triggering mast cells in mice (49). Taken together, the effect of viruses including HRV, RSV and IFV on type 2 responses are well established in the lower airways, but their role in upper airways awaits further investigation.

Bacteria and type 2 responses in allergic airway diseases

Airway bacteria such as *Moraxella pneumoniae*, *Corynebacterium pneumoniae*, *Streptococcus pneumoniae* and *S. aureus* are associated with the development and exacerbations of asthma, but the underlying molecular mechanisms have not yet been unveiled. Endonasal instillation of ovalbumin (OVA) plus staphylococcal enterotoxin B (SEB) induced a murine pulmonary inflammation, which is characterized by an increase in the number of eosinophils and the production of Th2 cytokines (50). It has been reported that exposure of mouse splenocytes and peritoneal macrophages to CpG oligodeoxynucleotides from *Streptococcus thermophilus* enhanced IL-33 expression (51), which may further initiate Th2 responses. However, not all bacteria exhibit the ability to enhance Th2 response; *Haemophilus influenzae* infection synergized with OVA mouse model of asthma elevates Th17 responses, not Th2 responses (52).

In upper airways, our group has proved that SEB induces considerable release of Th2-associated cytokines including IL-4, IL-5 and IL-13 and also enhances eosinophilic inflammation in human nasal tissues (53-56) (**Figure2**). Without the effect of the enterotoxin, CRSwNP tissue exposure to *S. aureus* alone led to IL-5 expression via TLSP and IL-33 productions (data not published). Even though IL-6Rα^{high} effector memory CD8⁺T cells are involved in virus-induced Th2 response, they also trigger IL-5 and IL-13 expressions in response to the bacterial super-antigens SEB and TSST-1(47). In mouse, an adhesion molecule of *S. aureus* “iron-regulated surface determinant A” mediated IL-4 secretion from splenocytes

(57). In summary, the effect of bacteria on Th2 response especially in upper airways implicates the important role of bacteria in allergic airway diseases.

Type 2 cytokines upon viral and bacterial infection

In allergic airway diseases, IL-4 is critical for Th2 cell differentiation and causes a switch to IgE production by differentiating B cells (58). IL-5 is associated with eosinophil development and activation (59). IL-13 regulates IgE isotype switching in B cells, as well as activating mast cells, eosinophils, and neutrophils (60, 61). As mentioned above, microbial infection can induce type 2 cytokine releases. Simultaneously, type 2 cytokines also can exaggerate inflammation in response to respiratory infection. IL-4 and IL-13 pre-treated human airway epithelial cells promoted higher expression of pro-inflammatory chemokines such as IL-8, CXCL9, CXCL10 and CXCL11 in response to polyriboinosinic polyribocytidylic acid stimulation (62). IL-13 enhanced goblet cell differentiation(63), and the induction of mucous metaplasia inhibited ciliogenesis and ciliary beat frequency, thereby changing the apical membrane structure which increased susceptibility of human airway epithelium to HRV16 infection(63, 64). HRV16 infection in turn resulted in further dysfunction of ciliary clearance and hyper-secretion of mucus, aggravating the inflammation in airways (65). Additionally, IL-4 and IL-13 increased HRV16 replication by reducing the interferon producing capacity of human bronchial epithelial cell line (66). Thus, type 2 cytokines increase susceptibility to viral infection in airways via changing the epithelial structure and the production of interferons.

In the upper airway, a disruption of the tight junctions such as occludin and zona occludens-1, and an irregular expression pattern, were observed in the IL-4–stimulated primary nasal epithelial cells (67). Zona occludens-1 expression became discontinuous in human nasal epithelial cells exposed to the *S. aureus* conditioned media (68), however, there is no direct evidence showing a correlation between the reduced tight junctions and higher susceptibility to bacterial infection. Indeed, IL-13, not IL-4, markedly down-regulated antimicrobial protein psoriasin expressions in human nasal epithelial cells (69), which resulted in an increase in susceptibility to bacterial infection. In contrast, IL-4 and IL-13 exposure to

human bronchial epithelial cells during mucociliary differentiation contributed to the antimicrobial defense against *Pseudomonas aeruginosa* infection via induction of antimicrobial peptide human beta defensin-2(70). Therefore, the multifaceted role of Th2 cytokines has been shown in anti-microbial protein production.

Ways to block type 2 responses in allergic airway disease

As mentioned above, there is a link between infection and type 2 response. Type 2 cytokines and their binding receptors, mechanisms of inducing type 2 cytokines during infection and microorganisms become potential targets to block human type 2 responses allergic airway diseases (**Figure 3**). Intranasally pretreated anti-IL-4 and anti-RSV siRNAs reduced eosinophils in broncho-alveolar lavage fluid of a mouse model of asthma during asthma exacerbations (71). This model provides promising concepts on “silencing” approaches such as siRNA and DNazyme against viruses or bacteria to be used in humans to diminish type 2 allergic immune responses. Likewise, non-cytotoxic anthraquinone derivatives ameliorated IL-4 and IL-13 expressions through de-phosphorylation AKT in an OVA-asthmatic mouse model with RV exacerbations (72). Anti-IL-25 antibody had a similar inhibitory effect on Th2 inflammation induced by murine pneumonia viruses, but it also promoted a Th17 response (73). Based on the findings in a mouse model, monoclonal blocking antibodies against type 2 cytokines are used in asthmatic patients in clinic trails. Targeting IL-13 (lebrikizumab) or both IL-4 and IL-13 signaling have demonstrated therapeutic benefits for treating moderate-to-severe asthma patients with increased eosinophils or periostin (74, 75). Additionally, neutralization of IL-5 by mepolizumab is a promising intervention for patients with severe eosinophilic asthma (76, 77). Furthermore, blocking a subunit of IL-4 receptor by dupilumab can also efficiently inhibit both IL-4 and IL-13 signaling in patients with persistent, moderate-severe asthma (78). Omalizumab, a humanized monoclonal antibody to block the interaction between IgE and the high affinity IgE receptor (FcεRI), can significantly improve lung function and markedly advance asthma control in severe asthma patients (79). FcεRI activation is also associated with reciprocal down-regulation of type I interferon production during viral asthma exacerbations (80). Thus, omalizumab blocks type 2 responses in allergic asthma and secondarily downregulating FcεRI on dendritic cells may enhance type I interferon-dependent viral clearance. The expression and the production of Th2 cytokines is also controlled

by the transcription factor human GATA binding protein 3 (GATA3), which is necessary for the differentiation of Th2 lymphocytes. Applied in an asthma model in humans, GATA3 specific-DNAzyme, a DNA molecule that destroys GATA3 RNA, led to the reduction of Th2 inflammation (81). In the upper airways, the monoclonal antibodies mepolizumab, reslizumab, omalizumab and dupilumab have been subjected to proof-of-concept studies. Our group showed that patients with severe CRSwNP do experience a significant reduction of polyp score and an improvement in quality of life by the treatment with these monoclonal antibodies (82). MiR-143, with a direct inhibitory role on IL-13 receptor $\alpha 1$ chain expression, suppresses IL-13-induced inflammatory cytokine and mucus production in nasal epithelial cell from allergic rhinitis patients (83). Specific immunotherapy for 3 months also can inhibit Th2 response in allergic rhinitis patients by modulating T cell immunoglobulin mucin domain molecule 4 (TIM4)/TIM1 interaction on dendritic cells (84).

The interaction between virus and bacteria

Most of the investigations focused on the effect of virus alone or bacterium alone in allergic airway diseases, whereas little is known about viral and bacterial co-infection. In the clinic, virus and bacteria co-infections increased the readmission risk of asthmatic patients (85), and a high frequency of co-infections was observed in asthma exacerbation patients (14). In mouse model, preceding virus infection may enhance the bacterial adherence such as galectins for *pneumococcal* adhesion (86), and depress the recruitment of neutrophils and natural killer cells which play an important role in the bacterial clearance (87, 88). Conversely, exposure of *H. influenzae* to primary human epithelial cells significantly enhanced the binding of HRV16, probably through up-regulation of intercellular adhesion molecule and toll-like receptor -3 expression (89). Our recent study also showed that herpes simplex virus type 1 infection facilitates invasion of *S. aureus* into the nasal mucosa and CRSwNP tissue (90). In humans with acute respiratory illnesses, however, children with HRSV and *Mycoplasma pneumonia* co-infection had more severe airway inflammation than those with HRSV infection alone (91), bacteria (*influenzae*, *catarrhalis* and *pneumoniae*) are more likely to be detected among virus-negative specimens compared to virus-positive burden (92). Similarly, mice with pre-symptomatic influenza infection are less susceptible to secondary MRSA infection (93), due to the persistence of IL-13 signaling that was advantageous for

resolving MRSA infection. These observations suggest a profound deference of host defense upon different pathogen species infection. Further investigations addressing potential interactions between specific viruses and bacteria in allergic airway diseases are therefore warranted.

Perspectives

In this paper, we reviewed evidence whether viruses and bacteria initiate or maintain type 2 responses in the airways. Those infectious agents are commonly associated with Th1 and Th17 responses. We here stress that apart from allergens, specific organisms and viruses also may play a critical role in the induction of type 2 immune responses. We assume that one single event will not be enough to initiate and certainly not to maintain Th2 inflammation, but rather that multiple hits are necessary over time, involving microbes, allergens and environmental factors (**Figure 4**). The impact of such events probably depends on the quantity of viruses and/or bacteria and the time course, e.g. high numbers of microbes may hit the airway mucosa during an infection, and low numbers may persistently exert pressure as colonizers, biofilm formers or intra-mucosal intruders. Furthermore, the immune status of the airway mucosa will make a difference, with a Th1/Th17 biased mucosa being more resistant to Th2 pressure than an already Th2-biased inflammatory status. As we have discussed, these different Th milieus may co-exist, and thus the balance among Th1, Th2, and Th17 responses may determine the course and speed of disease development. Finally, a Th2 response may establish itself within the mucosa, which leads to further negative effects; a Th2 bias induces a deficit in defending the mucosa against viral and bacterial infections. For example, alternatively activated macrophages in a Th2 biased environment do not adequately phagocytose and kill *S. aureus* and other germs (50).

Some references indicated that there is no difference of the prevalence rate of viruses and bacteria between allergic airway diseases and controls. The following limitations have to be taken into account: When compiling the literature, the clinical phenotypes and mucosal endotypes of airway diseases were not always clarified; general conclusions might therefore be difficult to make. When taking CRS as an example, CRSwNP and CRSsNP exhibit totally different inflammation patterns, but are often not differentiated in the literature (15). The microbial identification in samples from CRS patients without further phenotype definition is of limited value. Although culture-independent techniques have the ability to detect more microbes than culture techniques, culture-dependent methods so far remain a better

approach to identify clinically relevant microbes. In this review, we were only able to find one paper on airway microbiome (6), which mentions additional culture dependent approaches to confirm findings. Furthermore, microbiological data is influenced by various parameters. Samples from different parts of the airways, under the influence of medication especially the use of antibiotics, an acute respiratory infection occurring in the intervening period between sampling, and sampling in different seasons may affect results. Therefore, standardized microbiome sampling and evaluation are necessary to obtain useful data. Future prospective studies using these criteria need to be performed to fully describe the role of viruses and bacteria in the initiation and persistence of Th2 diseases. In the absence of effective anti-viral therapies, targeting pathogenic bacteria for manipulating the microbiome by antibiotics may be an alternative approach. In contrast, several epidemiological studies reported that antibiotic consumption in the first year of life was associated with a risk of asthma in later life (6, 94). Due to the “common mucosal response” concept, which introduces the idea that alterations in immune function of the respiratory tract are linked to the immunomodulatory activity of the gut microbiome, oral administration of probiotics was used for treating allergic airway diseases by changing the gut microbiome. Some investigations have shown that oral administration of probiotics and prebiotics may benefit allergic rhinitis patients (95-97). Moreover, the local nasal administration of *Lactococcus lactis* NZ9000 can affect local and systemic immune responses against *Streptococcus pneumonia* (98). Probiotics seem a promising therapeutic approach for allergic airway diseases, but there are still many controversial issues in this field. The meta-analysis by Elazab *et al.* has demonstrated that early probiotic administration does decrease atopic sensitization, but it does not reduce the risk of developing asthma (99). The differences in study design and the usage of different probiotic strains or combinations can lead to completely different results. Adequate controlled experiments with standardized criteria using specific strains, dosages and timing are anticipated to provide further insights.

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Table 1. The prevalence of viruses in airways.

Characteristic of subjects	Sample source	Identification techniques	The common viruses	Ref.
Healthy infants (<12m) during acute respiratory infection	nasopharynx secretion	RT-PCR	HRV (40%); HRSV (11%);	6-7
Asthmatic children (3-16y) during asthma exacerbation	nasopharynx secretion	RT-PCR; VNT	HRV (30.3%); HRSV (40.9%); IFV (15.9%); HSV-1 (56.8%);	10-13
Asthmatic adults during asthma exacerbation	nasopharynx secretion	RT-PCR	HRV-A,B,C (10.4%) HRSV-A (10.4%); Metapneumovirus (6.3%);	14
Chronic rhinosinusitis patients	nasal tissue or epithelial cell scraping	RT-PCR	Picornavirus (29%); HRSV (15%); IFV (20%); Parainfluenza viruses-3(18 %);	15-16
Allergic rhinitis patients	nasal swab	RT-PCR	HRV (14.1%);	23

HRV: human rhinovirus; HRSV: human respiratory syncytial virus; IFV: influenza viruses; VNT: virus neutralization test; RT-PCR: reverse transcription polymerase chain reaction; 16S rRNA: 16S ribosomal RNA, m: month; y: year

Subjects	Sample	Identification technique	Bacterial species	Ref.
Healthy infants (2m) without acute respiratory infection	nasopharynx secretion	16S rRNA pyrosequencing; bacterial culture	<i>Staphylococcus</i> (41%); <i>Corynebacterium</i> (22%); (main bacteria)	6, 29
			<i>Alloiococcus</i> (14%); <i>Moraxella</i> (9%); <i>Haemophilus</i> (9%);	
Healthy infants (12m) without acute respiratory infection	nasopharynx secretion	16S rRNA pyrosequencing	<i>Moraxella</i> (41%); <i>Alloiococcus</i> (26%); (main bacteria)	6,
			<i>Staphylococcus</i> (11%); <i>Corynebacterium</i> (12%);	

Healthy children (24m)	nasopharynx secretion	bacterial culture	<i>Streptococcus pneumoniae</i> (72.8%); <i>Moraxella catarrhali</i> (78.6%); <i>Haemophilus influenzae</i> (57.3%); <i>Staphylococcus aureus</i> (3.9%);	25
Healthy adults (18-66y)	middle meatus swab	16S rRNA pyrosequencing; RT-PCR	<i>Staphylococcus epidermidis</i> (96.4%); <i>Staphylococcus aureus</i> (67.9%); <i>Propionibacterium acnes</i> (92.9%);	34
Asthmatic patients (>18y) during asthma exacerbation	nasopharynx swab	RT-PCR	<i>Streptococcus pneumoniae</i> (18.8%); <i>Haemophilus influenzae</i> (12.5%);	14
Chronic rhinosinusitis without asthma patients	sinus swab (ethmoid region)	RT-PCR	<i>Staphylococcus</i> (19.3%); <i>Fusobacterium</i> (9.0%);	35,36
Chronic rhinosinusitis with asthma patients	sinus swab (ethmoid region)	RT-PCR	<i>Staphylococcus</i> (28.4%); <i>Ralstonia</i> (8.2%); <i>Acinetobacter</i> (2.3%);	35,36

Table 2. The prevalence of bacteria in airways.

RT-PCR: Reverse transcription polymerase chain reaction; 16S rRNA: 16S ribosomal RNA, m: month; y: year.

Figure legends:

Figure 1 Viral-induced type 2 responses in the airways. Human airway epithelial cells produce IL-33, IL-25 in response to human rhinovirus (HRV) (41-42). IL-33 and IL-25 subsequently drive IL-13 (yellow balls) and IL-5 (green balls) productions via binding to their receptors ST2L and IL-25R respectively on Th2 cells, type 2 innate immune cells (ILC2s) and basophils (42). Human respiratory syncytial virus (HRSV) also can induce type-2 cytokine release in airways from IL-6R α^{high} effector memory CD8 $^{+}$ T cells (48).

Figure 2 Bacterial induced type 2 responses in the airways. After binding to toll-like receptor 2 (TLR2), *Staphylococcus aureus* (*S. aureus*) induces type 2 cytokines productions via thymic stromal lymphopoietin (TSLP) and IL-33 release from human airway epithelial cells (data not published). The staphylococcal enterotoxin B (SEB) induces type 2 cytokine releases by affecting the differentiation of T helper (Th) 2 cells and IL-6R α^{high} CD8 $^{+}$ cells (48).

Figure 3 Strategies to block type 2 immune responses in the airways with viral and bacterial impact. Microorganisms, epithelial cell derived cytokines such as TSLP and IL-33, type 2 cytokines such as IL-4,

IL-5, IL-13 and their receptors are targets to inhibit type 2 biased immune response in airways (79-82). TLR2: toll-like receptor, siRNA: small interfering RNA, TSLP: thymic stromal lymphopoietin, TSLPR: TSLP receptor, ST2L: IL-33 receptor, ILC2: type 2 innate immune cells, IgE: immunoglobulin E, GATA3 DNzyme: GATA Binding Protein 3 Deoxyribozymes.

Figure 4 Initiation and maintenance of Th2-biased immune responses require multiple hits. Low numbers of viruses and /or bacteria may persistently exert pressure as colonizers, biofilm formers or intra-mucosal intruders. And high number of microbes may hit the airway mucosa during an acute infection exacerbation and result in plenty of Th2 cytokine release. The balance among Th1, Th2, and Th17 responses, the status of airway mucosa and the some environment are also involved in Th2 biased immune response initiation or maintenance in airways. HRV: human rhinovirus, HRSV: human respiratory syncytial virus, *S. aureus*: *staphylococcus aureus*, Th: T helper.







