



Rhinosinusitis and asthma-microbiome and new perspectives

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Purpose of review

Microbiome is one of the new perspectives in human health research, including airway diseases. There are several publications about the relationship of the microbiome and allergic diseases. Although pathogenesis of chronic rhinosinusitis (CRS) as well as its relationship with asthma has been widely investigated, the relationship of the microbiome and CRS is not yet well known.

Recent findings

The relationship between the hygiene hypothesis and microorganisms inside the human body and in the environment around it has been clearly shown. Furthermore, several researchers have reported that the microorganisms in the gut play a major role in regulating the immune cells that are of relevance to asthma and allergic diseases, such as Th1, Th2, Th17, Treg and dendritic cells as well as Toll-like receptors. Reduced contact of people with natural environmental features and biodiversity may adversely affect the human commensal microbiota and its immunomodulatory capacity. Some studies have shown a close relationship between CRS and *Staphylococcus aureus*, anaerobes and so on in the nasal cavity or paranasal sinuses, although the relationship between CRS and microorganisms in the gut has not been demonstrated.

Summary

In this review, we summarized about the microbiome, mainly in asthma and allergic diseases. The relationship between asthma and CRS has been clearly shown, and in particular, CRS with nasal polyps (CRSwNP) has been considered to be Th2-dominant. Studies examining environmental microbial exposure in populations at risk for CRS are necessary to improve our understanding of the role this factor plays in disease development.

Keywords

asthma, chronic rhinosinusitis, hygiene hypothesis, immune cells, microbiome

INTRODUCTION

Chronic rhinosinusitis (CRS) is an important public health problem and has a major impact on the quality of life [1–4]. The studies of CRS have been limited by access to tissue, the complexity of the sinonasal physiology, a lack of available biomarkers, the absence of useful animal models, a paucity of cohorts with biological samples for analysis, and limited well designed clinical trials or investigations of the immune function. Therefore, novel strategies for identifying biological mechanisms underlying this disease are much needed.

A number of studies have shown abnormalities in immune responses in CRS [5], and recent studies have highlighted the close relationship between the epithelium and immune system. Indeed, Th1, Th2, Th17, T regulatory (Treg) cells, neutrophils, and eosinophils as well as their associated cytokines and mediators have all been implicated in the

resultant cellular and molecular immunopathology [6–13]. Moreover, common to these studies is the conclusion that the dysregulation of immune responses contributes to the striking inflammation seen [7]. Factors that affect these responses may be involved in the persistent inflammation in CRS.

If we could understand what stimuli generate the hyperinflammatory responses in the sinonasal mucosa, we could better understand what factors

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KEY POINTS

- One of pathogenesises of CRS has been considered as an imbalance of Th1 and Th2 functions, and CRSwNP is said to be associated with asthma.
- Changes of human lifestyle lead to changes in bacterial composition in the gut, which results in inducing one's immune tolerance.
- Microorganisms in the gut, or peroral intake of microorganisms can promote proliferations of Th1, Th2, Treg, Th17, dendritic cell, and so on, which leads to correct Th1/Th2 balance.
- In CRS patients, *S. aureus* and anaerobes among others, which have been detected by DNA sequencing, may be considered to change immune function in sinus mucosa.

affect this process. The aforementioned work has been performed under the prevailing framework of CRS as an inflammatory disease. Early efforts toward identifying the genesis of this inflammatory response initially focused on bacteria using culture techniques to assess the presence of organisms. Although studies show that some patients yield positive cultures from sinus samples, it has been difficult to generate support for this idea because a high proportion of CRS cultures fail to grow any organisms or yield bacteria with no obvious relationship to disease. The variability in recovery may result from differences in the methodologies used for collection, processing, transportation and cultivation, patient heterogeneity, geographical characteristics, and prior therapy, but also reflects the fact that many organisms do not grow in culture.

One must consider that culture-based methods of assessing the presence of bacteria fail to recognize a large set of organisms that are present in the human body. For example, gut cultivation-based technologies limit analysis because more than 80% of the estimated species are not readily cultivated [14]. The cultures also fail to provide information on the communal composition and structure, phenotype, function, and gene expression of these organisms in their natural habitats. Therefore, we cannot discount the hypothesis that organisms from the sinuses that do not grow in culture influence the inflammation in CRS, either directly or indirectly, through effects on other organisms involved in disease. Similarly, environmental factors, such as smoking may affect the composition of these bacteria.

Studies on the total genomes of bacteria inhabiting the body, termed the microbiome, have recently been performed at National Institutes of Health. To elucidate the normal human microbiome,

samples were collected from 242 healthy Americans (129 men and 113 women) [15[¶]]. Tissue samples were collected from 15 and 18 sites of the body from men and women, respectively. A maximum of three samples were collected from the oral and nasal cavities, skin (posterior regions of the bilateral ears and medial sides of the bilateral elbows), lower intestine (feces), and female vagina. Total microbial DNA was purified from each sample and analyzed using a DNA base sequencer. To identify the bacteria-specific gene (variable ribosomal RNA gene termed 16S rRNA), 3.5 trillion base pairs of genomic sequence data were arranged. Only bacterial DNA base sequences could be analyzed without analyzing human genomic DNA sequences. In addition, metabolic activities encoded by these microbial genes could be analyzed by metagenomic sequencing (or determination of the total DNA base sequences of the microorganisms). This Human Microbiome Project (HMP) demonstrated the presence of more than 10000 microbial species in the human body, and it was estimated that 81–99% of the total microbial species inhabiting healthy individuals were identified.

Analysis of the microbiome in various diseases has also been progressing. For example, on analysis of the microbiome in the nasal cavity of children with fever of unknown origin, which frequently occurs in children, a maximum of five times larger amount of viral DNA was detected in nasal cavity samples from children with fever than in those without fever, and DNA of a broad range of viral species was detected [16[¶]]. Many studies have recently been performed on the function of the intestinal microbiome in Crohn's disease [17[¶]], ulcerative colitis [18[¶]], and esophageal cancer, function of the skin microbiome in psoriasis, atopic dermatitis, and immunodeficiency, function of the urogenital microbiome in pregnancy [19[¶]], sexual history, and surgery for phimosis, and function in many childhood diseases, such as pediatric abdominal pain and enteritis and the serious condition of premature babies with intestinal dysfunction [20[¶]]. There are still many unclear points with regard to the microbiome function in CRS, but allergic diseases and asthma are essential factors that need to be investigated regarding the development of CRS, and to understand the association between allergic diseases and asthma and the microbiome function, it is very important to elucidate the pathology of CRS.

HYGIENE HYPOTHESIS

The hygiene hypothesis is very important to consider the development of allergic diseases and asthma. In 1989, Strachan proposed that a lack of exposure to microbial environments in childhood

because of the 'clean environment' accompanying the advancement in hygiene leads to the poor development of immunity and increases the risk of allergic diseases [21]. This hygiene hypothesis is based on the observation that the risk of allergic diseases is low in children with many siblings and those who have grown up on farms with much contact with domestic animals. However, no direct relationship between infection and allergy was shown at that time, and this was a simplified observation. Later, a lower incidence of allergic and autoimmune diseases in individuals with numerous exposures to parasites was demonstrated by epidemiological and experimental studies [22]. In 1998, it was clarified that changes in the intestinal bacterial composition in childhood caused by changes in lifestyle influence the tolerability of mucosal immunity and induce 'distorted' immune reactions, connecting the hygiene hypothesis with the relationship with microorganisms [23]. Two cross-sectional surveys have recently been performed in a total of 16 000 children, and it was clarified that the development of asthma was inhibited in children with a more marked exposure to bacteria and fungi [24²²].

ALLERGIC DISEASES/ASTHMA AND INTESTINAL BACTERIA

Putting it simply, immunotolerance represents the biological capacity to identify antigens and exhibit defensive actions against these. Collapse of this tolerance is closely involved in the pathogenesis of various diseases. Acquired immunity plays an important role in the differentiation of self and nonself, but intestinal bacteria contain abundant nonself antigens including those derived from food regulating the body, and constantly present antigens to acquired immunity. Symbiotic microorganisms in the intestine develop a mechanism of inhibiting unnecessary inflammation by inducing immunotolerance through coordination with the natural immune system or regulation of the acquired immune system [25].

Allergic diseases and asthma are considered to be induced by excess reactions of Th2 cells. Th2 cells are characterized by IL-4, IL-5, IL-9, and IL-13 production, and these cytokines form and modulate the pathology of allergic inflammation. Not only Th2 cells but also Th1 cells are involved in the pathogenesis of asthma [26]. Studies have shown the increasing role of Th17 and Th9 cells in asthma [27–29]. Tregs are important for the regulation of immunotolerance and play an important role in the modulation of inflammatory reactions [30–32].

Toll-like receptors and nucleotide binding oligomerization domain-like receptors are expressed in the intestinal epithelium, and dendritic cells are activated through these and regulate immunotolerance. It has been shown that intestinal bacteria, such as *Lactobacillus* and *Bacteroides*, promote Treg expression in the body and enhance the secretion of IL-10 and TGF- β [33,34]. Furthermore, it has been clarified that the oral ingestion of bacteria corrects the Th1/Th2 balance [35] and promotes differentiation into Th17 cells, suggesting that Th17 cells are involved in defense against pathogens reaching the intestine [36]. The close involvement of these immunocytes in changes in intestinal bacteria has been shown, indicating that the intestinal bacterial balance is strongly associated with the development of allergic diseases and asthma.

EFFECTS OF MICROBIOME ON CHRONIC RHINOSINUSITIS

It has been clarified that various factors are involved in the pathogenesis of CRS [37²]. For example, the relationship between CRS with nasal polyps (CRSwNP) and IgE for *Staphylococcus aureus* super antigen, as well as Th1/Th2 imbalance due to a decrease in Tregs is widely known. In addition, CRSwNP is closely associated with the development of asthma, based on which it is easy to imagine that the intestinal bacterial balance is involved in the development of CRS. However, there has been no report on the association between CRS and the intestinal bacterial microbiome. The association between CRS and bacteria in the nasal cavity and paranasal sinuses has been investigated in many studies, but gene analysis of these bacteria, that is, the microbiome, has only been performed in only a few studies [38–40,41²²]. The first reported study was performed in 2003, in which a bacteria-specific gene, 16s rDNA, was amplified from the mucosa and maxillary sinus lavage of 11 patients with maxillary sinusitis. Bacterial genes were amplified in four patients and identified as *S. aureus*, gram-positives, gram-negatives, and anaerobes. However, no fungus was detected. Later, two articles were published in 2010. In one article, bacterial gene analysis was performed using the mucosal samples. Bacterial genes were amplified in all 18 patients, and *S. aureus* and a lot of Coagulase-negative staphylococci were detected in many samples, but more anaerobes were detected. The involvement of anaerobes in the development of CRS has been suggested, and this study demonstrated it at the gene level. In contrast, maxillary sinus lavage was analyzed, and a total of 142 bacterial genes were amplified

including many genes of indigenous bacteria in the oral cavity. In a recently reported study, cotton swabs of 15 CRS patients were analyzed, and more than 50 000 bacterial genes were detected in total. It was clarified that the incidence of asthma and the association with the diversity of the *S. aureus* gene increased as the diversity of bacterial genes in the samples increased. The relationship with *S. aureus* super antigen, which is considered to be the cause of CRS, and the development of asthma is also assumed on the basis of these viewpoints.

CONCLUSION

In the present review, we outlined existing information on CRS, allergic diseases, asthma, and the microbiome. There is still much to do in order to improve our understanding of the role this factor plays in disease development with regard to CRS. We need to understand the relationship of CRS not only with the intestinal bacterial microbiome but also with the microbiome in the nasal cavity and paranasal sinuses better, because of the difficulty in collecting samples from the paranasal sinuses without contamination, and inconsistency of the analytical methods due to the fact that no sampling methods have been established. As the recent progression in analytical methods has facilitated the investigation of microbiomes, further studies investigating the relationship between CRS and asthma in regards to the microbiome will be warranted.

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Conflicts of interest

There are no conflicts of interest.

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- of special interest
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