Introduction

Over time, humans have evolved a relationship with microorganisms in the environment whereby interactions with these bacteria, viruses and fungi are necessary to normal human immunity. A microbiome in a given organ or system consists of the entire microbial constituents (commensal flora) and their genomes, the surrounding environmental conditions and their interactions with the host.

There is a microbiome everywhere in the body. The gastrointestinal (GI) microbiome is the easiest to study and largely influences development of early immunity. Microbiomes are also present in the respiratory tract (including the lower respiratory tract), skin, genitourinary tract and even the blood stream. Much of our immunity from chronic inflammatory disorders hinges upon having a healthy microbiome.

Professor Robin Green believes that knowledge and understanding of the microbiomes of the body is “still in its early days”. He shared his experience of what is known about the microbiome and respiratory inflammation at the recent Cipla Respiratory Symposium (17 March 2018).

KEY MESSAGES

- There is a microbiome everywhere in the body
- Much of human immunity from chronic inflammatory disorders hinges upon having a healthy microbiome
- Interactions between the host immune system and intestinal bacteria shape immune system development
- Many factors of modern living contribute to dysbiosis, creating an inflammatory milieu associated with chronic inflammatory conditions
- Of the four main drivers of asthma development, three are microbiome interdependent
- The upper airway microbiome differs between chronic rhinosinusitis ( CRS) and allergy
- The microbiome in CRS is influenced by the presence and type of asthma
- More research is required for the use of probiotics in airway disease prevention.
The GI microbiome is inherited orally from the mother during vaginal delivery, to start colonising the infant gut. The largest microbiome in the body, there are more than 100 trillion microbes from over 1000 species in a functional gut microbiome, with influence on health and disease much less dependent on a single species than the entire range of species acting together. It is plastic, adapting to changes in the environment (e.g. diet); with different microbes facilitating particular functions, such as the fermentation of dietary fibre by *Bifidobacterium* and *Faecalibacterium prausnitzii* to make short chain fatty acids (SCFAs) that serve as energy sources and as anti-inflammatory agents.

Host-commensal interactions extend beyond the local intestinal environment and shape the repertoires of immune cells at extra-intestinal sites such as the lungs. An increase in gut immune T-Reg cell numbers is seen with increasing microbial diversity. When the gut microbiome goes wrong, many chronic inflammatory conditions such as asthma, allergy, metabolic syndrome, obesity, hypertension and diabetes are associated.

### Respiratory microbiome

By the time humans are a few months old there should be a rich and healthy microbiome in the respiratory tract, allowing for robust and rapid immune responses. The gut microbiome reaches the airway through translocation and micro-aspiration (inhalation/exhalation) and there is also evidence that it is transported through the blood stream to the lung, with continuous recolonising of the lower respiratory tract from the surrounding sites in a dynamic and transient manner.

The respiratory microbiome has recently been characterised using culture-independent techniques (16S rRNA sequencing) and is dominated by the same major phyla as the GI microbiome (Firmicutes, Bacteriodetes and Proteobacteria), but with different relative abundances and reduced total bacterial burden.

Exposure to commensals in the developmental window of the newborn period directs lung-selective trafficking of group 3 innate lymphoid cells (ILC), a group of sentinel cells that maintain mucosal homeostasis. This is mediated by intestinal dendritic cells, which induce expression of the lung-homing signal CCR4 on ILC3. Lung-selective trafficking of ILC3 promoted the resistance of newborn mice to pneumonia. This may explain the association between widespread use of antibiotics and an increased risk of pneumonia in newborn infants.

### Gut-airway axis

The gut-airway axis refers to the cross-talk between the gut and lung microbiota. The gut microbiota can influence the lung microbiota by modulating lung immunity through production of bacterial ligands, bacterial metabolites and immune cells that circulate through the blood to reach the lungs. These directly influence the lung immune response and composition of the lung microbiome, protecting against chronic inflammatory disorders such as allergy, asthma and infectious diseases.

Microbial communities in the lower airways and lungs are shaped by microbes present in the oral cavity and upper airways, where microbes arrive through inhalation of the surrounding environment or microaspiration of both the upper gut and upper airway microflora. The lung microbiome is likely to be important in shaping the innate and acquired immune responses in the lungs through their interactions with airway epithelium and immune cells. There is also the possibility that innate and acquired immunity could in turn regulate the lung microbiome (Figure 1).
The microbiome and respiratory inflammation

Dysbiosis

In a healthy microbiome, normal flora attach to specific pattern recognition receptors, inducing changes in the epithelium and submucosa to produce anti-inflammatory mechanisms (immune cells and cytokines) that protect us from chronic immune disease (Figure 2). In a disturbed microbiome (dysbiosis), the number or make-up of organisms has been destroyed. Pathogens bind to different epithelial receptors and start to interact with cells and cytokines that create an inflammatory milieu (Figure 3). The bidirectional relationship between the GI and respiratory microbiomes via the gut-airway axis sees many chronic GI diseases having a respiratory component and vice versa.

Figure 1. Gut-airway axis

Figure 2. Healthy airway microbiome
The microbiome and respiratory inflammation

Causes of dysbiosis

Ethnogeography, the interaction between genetics and the environment, gives rise to different microbiomes in different populations and between individuals. Many factors are implicated in dysbiosis. Early life circumstances contributing to dysbiosis are birth by elective Caesarean section (Box 1), birth in hospital (exposure to resistant microbes), unnecessary use of antibiotics in paediatrics and artificial feeds (replacement of breastmilk with formula). Other influencing factors are urban living, age, diet, nutritional status, cigarette smoking and pollution. Urbanisation has had the consequence, for example, of decreased exposure to healthy microbes from farm animals. Allergy is much less common in those born and having lived in a farming community.

Dysbiosis in asthma

Epidemiological studies demonstrate a steady increase in incidence of allergic disease, including asthma, in developed countries. The four main drivers of the development of asthma are genetic predisposition and early life allergy, recurrent viral infections of the lower respiratory tract and presence of bacteria in the lower respiratory tract. The early life factors are microbiome interdependent variables, implying that the microbiome is critically important to the development of asthma. Recent experimental data that suggest a lack of proper exposure to varied, mostly gut-associated, microbes in early life may be a contributing factor to dysbiosis and the development of asthma.

Factors favouring microaspiration of GI and upper airway secretions into the lungs (ciliary damage, reduced cough reflex and gastroesophageal reflux) could, in combination with environmental factors, contribute to lung dysbiosis in asthma. This is characterised by an increase in bacterial communities such as Proteobacteria, Firmicutes and Actinobacteria.
In the lower respiratory tract, a proposed cycle of dysbiosis leads to increased lung inflammation and immune dysfunction, which contributes to the initiation of allergic asthma and the various traits of severe asthma. Allergic asthma could be initiated through activation of the innate and acquired immune system by components of the bacterial wall or bacterial products within the airways. Induction of a chronic inflammatory process might form the basis for worsening of established asthma with exacerbations. This inflammatory process can encourage specific bacterial communities that in turn contribute to further microbial dysbiosis (Figure 4). The presence of *Haemophilus parainfluenzae* has the capacity to directly induce corticosteroid resistance in macrophages, contributing to steroid insensitivity in patients with severe asthma.

**Asthma and allergic rhinosinusitis**

Asthma and allergic rhinitis frequently coexist. Up to 80% of patients with asthma have allergic rhinitis, 20% of patients with allergic rhinitis have concurrent asthma. Epidemiologic studies support the suggestion that allergic rhinitis should be suspected as a comorbid condition in most patients with asthma.

Due to multiple environmental influences, it is difficult to identify and define a ‘normal’ upper airway microbiome. The microbiome in the nose and sinuses in CRS is different to the microbiome in allergy. CRS specimen culture finds the usually expected bacteria that are treated for with antibiotics (*Staphylococcus*, *Streptococcus*, *Haemophilus* and the anaerobes *Prevotella* and *Peptostreptococcus*), although there is a large variability between studies. Viral 16S rRNA gene sequencing shows different results to culture. The anaerobic bacteria *Diaphorobacter* (78%) and *Peptoniphilus* (72%) have been identified.
in CRS, but not in control cases, whereas S aureus was found in half of CRS cases, but detected in all controls. Viral sequencing also revealed the presence of Pseudomonas, Citrobacter, Haemophilus, Propionibacterium, Staphylococcus and Streptococcus in the microbiome of CRS.

The microbiome in CRS also depends on presence and type of asthma. The sinus microbiome phylum levels in asthmatic CRS cases was more like controls than non-asthmatic CRS. A pattern was observed for Prevotella and Staphylococcus, which had a trend towards a lower abundance in asthmatic CRS compared to controls, but a trend towards higher abundance in the non-asthmatic CRS group.

Fungal species associated with CRS vary in different studies and are often found in normal controls. Common are Candida, Aspergillus and Mucor species. Using multiplex PCR for respiratory viruses, viral nucleic acid sequences were found in 64% of sinus scrapings and 50% of nasal lavage samples in the CRS group, significantly higher than healthy controls (30% and 14% in scraping and lavage respectively). Rhinovirus was most frequently detected.

### Using probiotics for airway disease prevention

The use of probiotics conveys advantage in other medical conditions but use in allergy prevention is still uncertain as we do not yet have the right dose, timing or combination of microbes for the intervention. Overall literature has shown an improvement in asthma and allergic rhinitis with probiotics and prebiotics. These findings are not consistent and considered preliminary. Administration of probiotics is beneficial for through the upper airways to specifically target the lung microbiome might be effective.

### Sepsis

Children with sepsis and severe acute respiratory distress syndrome (ARDS) have a pathological lung microbiome resembling that of the gut due to antibiotic use. Gut-associated Bacteroides were common, possibly due to antibiotic use. A pathological lung microbiome resembles that of the gut due to antibiotic use. A pathological lung microbiome resembles that of the gut due to antibiotic use.

### References

CpD QUeSTIoNnAIRe
Earn FrEE CPD Points

The microbiome and respiraTory inflammaTion
Earn FrEE CPD Points

Complete and email to admin@denovomedica.com, info@denovomedica.com or fax to 0866103395. Alternatively, you can complete this questionnaire online at www.denovomedica.com. Fill in your details using clear block letters and mark the answers with a tick (✓).

I agree that my CPD-accredited certificate will be forwarded to my e-mail address. ____________________________________________________________

(Signature of healthcare professional)

<table>
<thead>
<tr>
<th>First Name</th>
<th>Surname</th>
<th>Profession</th>
<th>Telephone</th>
<th>City</th>
<th>Sales Representative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Much of human immunity from chronic inflammatory disorders hinges upon having a healthy microbiome.
   ☐ A True ☐ B False

2. Which of the following statements is false?
   ☐ A The GI microbiome is the largest in the body
   ☐ B There are more than 100 trillion microbes from over 1000 species in a functional gut microbiome
   ☐ C The effect of microbiome on health and disease is more dependent on the dominant species than the entire range acting together
   ☐ D All of the above
   ☐ E None of the above

3. Which of the following statements is false?
   ☐ A Host-microbiome interactions extend beyond the local GI environment and shape the immune response at extra-intestinal sites such as the lungs
   ☐ B Increased gut T-Reg cell numbers are associated with greater microbial diversity
   ☐ C Dysbiosis of the gut microbiome is associated with chronic inflammatory conditions
   ☐ D All of the above
   ☐ E None of the above

4. Culture-independent techniques such as 16S rRNA sequencing have indicated that the respiratory microbiome is dominated by the same major phyla as the GI microbiome.
   ☐ A True ☐ B False

5. The gut microbiome reaches the airway via:
   ☐ A Translocation and micro-aspiration
   ☐ B Transport through the blood stream
   ☐ C All of the above

6. Which of the following statements is false?
   ☐ A The respiratory microbiome is dominated by the same major phyla as the GI microbiome
   ☐ B In newborns, lung-selective trafficking of ILC3 is mediated by intestinal dendritic cells
   ☐ C The lower respiratory tract is sterile

7. The bidirectional relationship between the GI and respiratory microbiomes via the gut-airway axis sees many chronic GI diseases having a respiratory component and vice versa.
   ☐ A True ☐ B False

8. Factors implicated in dysbiosis include:
   ☐ A Mode and place of birth, early life nutrition
   ☐ B Use of antibiotics
   ☐ C Urbanisation and pollutants
   ☐ D All of the above

9. Which factor driving the development of asthma is not microbiome interdependent?
   ☐ A Genetics
   ☐ B Early life recurrent viral infection of lower respiratory tract
   ☐ C Early life presence of bacteria in lower respiratory tract
   ☐ B Early life allergy
   ☐ D Early life allergy

10. Which of the following statements is true?
    ☐ A Factors favouring microaspiration of GI and upper airway secretions into the lungs could contribute to lung dysbiosis in asthma
    ☐ B Dysbiosis in asthma is characterised by increased communities of Proteobacteria, Firmicutes and Actinobacteria
    ☐ C A cycle of dysbiosis leads to increased lung inflammation and immune dysfunction that may worsen established asthma
    ☐ D All of the above

11. Steroid insensitivity in patients with asthma may be linked to the presence of Rhinovirus.
    ☐ A True ☐ B False

12. What percentage of patients with asthma have co-morbid allergic rhinitis?
    ☐ A 20% ☐ B 40% ☐ C 80%

13. Which bacteria have been identified in CRS but not in healthy control cases?
    ☐ A Diaphorobacter ☐ B Peptoniphilus ☐ C Staphylococcus ☐ D A and B ☐ E B and C

14. In what manner does the presence and type of asthma affect the microbiome phylum levels in CRS?
    ☐ A Asthmatic CRS cases resemble healthy controls more than non-asthmatic CRS
    ☐ B Non-asthmatic CRS cases resemble healthy controls more than asthmatic CRS cases

15. The common fungal species Candida, Aspergillus and Mucur are associated with the microbiome in:
    ☐ A CRS ☐ B Healthy controls ☐ C Both of the above ☐ D Neither of the above
Treating the full spectrum of organisms