Asthma Causation and the Gastrointestinal Microbiome and Metabolome – might there be a signal or is it just noise?

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The respiratory and gastrointestinal tracts (GIT) share common embryological origins and an intriguing, and commonly revisited, question is "do processes occurring somewhere along the length of the GIT cause asthma symptoms?" Gastroenterologists have been known to remind their pulmonology colleagues that the lungs are actually the larger of two appendices of the gastrointestinal tract. Might it be feasible that processes taking place in the GIT may cause respiratory symptoms?

There is no doubt that exposure of the upper GIT to peanuts in sensitised individuals can lead to asthma-like symptoms. Based on work done in animals, there is the plausible mechanism whereby acid in the oesophagus might cause cough via to gastro-respiratory neural connections but in humans, acid suppression does not improve asthma symptoms [1]. But what about asthma causation *de novo*? The only interventions to prevent the development of asthma included a multifaceted intervention which included prolonged breastfeeding, delayed weaning in infants and elimination of common food allergens until 12 months of age in those at increased risk for developing asthma [2,3]. So might GI exposures cause asthma? And if so by what mechanism?

The advent of techniques for describing whole communities of bacteria has led to considerable research activity which explores whether bacteria normally resident within humans (the microbiome) may be causally related to asthma. In the respiratory tract, asymptomatic nasopharyngeal carriage of *Streptococcus, Haemophilus* and *Moraxella* has been associated with increased risk for later asthma symptoms [4] and bronchial dysbiosis (i.e. an atypical bacterial community in the airways characterised by an increased burden of Proteobacteria) is associated with established asthma [5]. The GI microbiome may also be relevant to the development of asthma by acting as a source of lower airway bacterial colonisation and/or by influencing the development of atopy and/or by a "common mucosal response" (i.e. cross-talk between mucosal surfaces) [5]. The figure summarises these proposed mechanisms.

Proving whether the GI microbiome might cause asthma is a huge challenge for many reasons, including sampling at the correct time in the life course and analysing the complex GI microbiome. Additionally there are the usual epidemiological challenges of confounding (e.g. by smoking, poverty, diet), reverse causation, bias in recruitment, loss to follow-up and small sample size leading to false positive findings. In addition to these seemingly endless hurdles, there is no gold standard definition of asthma.

There are at least three ways to overcome some of these challenges and improve our understanding of asthma microbiome biology: (i) explore biological mechanisms to support direct causation/pathogenesis (ii) explore the disease longitudinally, preferably commencing pre-morbidly, to ensure signals are consistent throughout and (iii) analyse microbial signals with respect to objective disease markers longitudinally to demonstrate mathematical correlation as the disease flares or remits over time and with treatment. The landmark study of Arrieta and colleagues [6] is an excellent example of the first of these approaches in this field; here five GIT bacterial genera (*Veillonella*, *Lachnospira*, *Rothia*, *Faecalibacterium* and *Bifidobacterium*) were associated with a lower incidence of atopy and wheeze in infants, and so presumed to be associated with protection from asthma development. The offspring of mice colonised with these organisms were then shown to have ameliorated airway inflammation in an ovalbumin stimulation experiment. Whilst the mechanism of protection was not identified in this study, it neatly demonstrated a biological link between the organisms detected in a population study and protection against the clinical outcome associated in an animal model. The ongoing Genetics, Environment, Microbial Project in inflammatory bowel disease is a good example of the second approach [7]. The study by Quince et al [8] is an example of the third approach and links longitudinal changes to the microbiome in Crohn's disease before and after exclusive enteral nutrition and compares this change to an index of disease activity (calprotectin).

An article published in this edition of the *Journal of Allergy and Clinical Immunology* [9] has taken the first of the above approaches and explored the relationship between the GIT microbiome and faecal metabolome (i.e. small molecules encompassing metabolites produced by the host, the gut microbes or derived from diet) and asthma symptoms at age three years. In a cross-sectional analysis, using samples from 361 participants in a birth cohort initially numbering 806, the authors found associations between asthma symptoms and increased abundance in stool samples of the *Christensenellaceae* family, and also with reductions in 45 metabolomic signals (placed in 11 clusters) which were detected in the plasma. Following adjustment for multiple testing, associations between asthma and metabolites remained significant for only five molecules. The authors noted no associations between asthma and other bacterial populations nor between asthma and the 692 other molecules detected. There were associations between a diet rich in meat and some metabolites. In the longitudinal analysis breast feeding was associated with 11 plasma metabolites at three years associated, and some of these metabolites were also associated with current asthma symptoms; these metabolites explained approximately 20% of the apparently protective relationship between breastfeeding and asthma (odds ratio 0.36 in this cohort). Although the authors claim to have integrated omics datasets, their analyses relied on multiple correlations.

The paper by Lee-Sarwar [9] raises more questions than it answers, particularly in the context of metabolome. For example, it is plausible that blood metabolome in three year olds may be truly related to historical breast feeding since what you eat today will affect your blood metabolome within the next 30 to 5 hours only and your gut microbiome over the next two to four days? Did the plasma metabolites originate from gut microbes, the host or their diet? if so how many other metabolites were removed from the portal vein by the liver? the authors imply that several of their metabolites are microbially originating but that this is unlikely to be the case, particularly for PUFA which the gut microbiota are unlikely to produce. In other conditions, metabolites are usually raised in association with chronic inflammation so why were metabolites reduced in association with

asthma? An unexpected finding that *Christensenellaceae* is associated with increased symptoms since it is usually associated with reduced risk for other outcomes, particularly obesity[10].

As one of the first in its field, only time will tell whether this was a well-done proof-of-concept study which informed future research towards novel insights into the development (and prevention) of asthma. At this point in time, this remains a comprehensive assessment of the GI metabolome and GI bacterial community in the context of early asthma symptoms. As the authors point out, this analysis needs replication in other populations since many findings may be false positive results.

Finally, we should not forget our experience with *Helicobacter pylori* and peptic ulcer disease. The discovery of this one organism, colonising the previously unrecognised microbial niche of the human stomach, completely transformed the paradigm of a chronic inflammatory "non communicable" disease and often treated by major surgery such as partial gastrectomy or vagotomy. Peptic ulcers are now cured by acid suppression and a short course of antibiotics. One promise of microbiome research is the discovery of other such microbially-mediated diseases, with the prospect of similar paradigm shifts in prevention or therapy. Could asthma be a future candidate for such a change?

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FIGURE LEGEND

Schematic diagram summarising the possible mechanisms for the gastrointestinal bacterial community to cause asthma. 1. Common mucosal response, here shared properties of and cross-talk between the large intestinal and respiratory mucosa lead to similar bacterial communities at both sites. 2. The intestinal bacteria directly influence the bacterial community in the lower airways (likely by fecal-oral transmission). 3. The metabolome, determined in part by lower intestinal bacteria, predisposes to asthma. 4. Lower intestinal bacteria stimulate immune deviation to an atopic T_H2 phenotype during the neonatal and period which predisposes to asthma. Any one or combination of these mechanisms (or none) may be active.