Microbiota of the alimentary tract of children

-implications for allergy and inflammatory bowel disease

Akademisk avhandling

Som för avläggande av doktorsexamen vid Sahlgrenska akademin, Göteborgs Universitet, kommer att offentligen försvaras i föreläsningssalen, våning 3, Guldhedsgatan 10A, Göteborg

Torsdagen den 2 oktober, kl. 9:00

av

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Avhandlingen baseras på följande arbeten:

I. Hesselmar B., Sjöberg F., Saalman R., Åberg N., Adlerberth I., Wold AE. **Pacifier Cleaning Practices and Risk of Allergy Development.** Pediatrics 2013; 131: 1-9

II. Sjöberg F., Nowrouzian F., Rangel I., Hannoun C., Moore E., Adlerberth I., Wold AE. **Comparison between terminal-restriction fragment length polymorphism (T-RFLP) and quantitative culture for analysis of infants' gut microbiota.** J Microbiol Methods 2013; 94: 37-46

III. Sjöberg F., Barkman C., Östman S., Nookaew I., Adlerberth I., Saalman R., Wold AE. **Altered composition of duodenal microbiota of children with newly diagnosed inflammatory bowel disease**. In manuscript

IV. Sjöberg F., Nookaew I., Adlerberth I., Wold AE. **Comparative analysis of infants' gut microbiota by next generation sequencing and quantitative culture.** In manuscript

UNIVERSITY OF GOTHENBURG
Göteborg 2014

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Abstract

Allergy, which is the most common chronic disease in Swedish children and adolescents, is associated with a high standard of living and Western lifestyle. According to the hygiene hypothesis, allergy is due to inadequate stimulation of the immune system by microbes during early childhood, leading to failed maturation of the immune system. The incidences of inflammatory bowel diseases, i.e., ulcerative colitis and Crohn's disease, have also increased dramatically in Western countries over the last few decades, and currently, these diseases are often diagnosed already in childhood. Epidemiological evidence suggests links between alterations to the intestinal microbiota and these diseases. Thus, studies of the composition of the bacterial microbiota in infants and young children are of relevance for the pathogenesis of both allergic diseases and inflammatory bowel diseases.

In this thesis, quantitative culture and DNA-based methods are compared for their abilities to characterize the gut microbiota in infants. Terminal-Restriction Fragment Length Polymorphism (T-RFLP) is based on differences in the 16S rRNA gene sequences between bacteria, as revealed by differences in fragment sizes after restriction enzyme digestion. A database was constructed to identify bacteria based on their fragment sizes. Multi-parallel sequencing of the 16S rRNA gene by pyrosequencing and T-RFLP were compared for sensitivity with quantitative culture of infant fecal samples. Bacterial genera that were present at $>10^6$ colony forming units/g feces, as determined by culture, were generally readily detected by DNA-based methods, with the exception of bifidobacteria, which generated only one sequence read per 10^8 viable bacteria. Clinically and immunologically relevant facultative bacteria, e.g., staphylococci, were often missed by the DNA-based methods due to having low counts in the fecal samples. The studies presented in this thesis indicate that cultivation and molecular-based assays are complementary in generating an overall picture of the complex gut microbiota.

In the ALLERGYFLORA cohort study, T-RFLP was used to analyze the salivary microbiota in 4-month-old infants whose parents had the habit of "cleaning" their pacifier by sucking on it, and of control children whose parents did not have this habit. Sharing of the pacifier between parent and infant was associated with reduced risk of the child developing an allergy and altered salivary microbiota in the child. We hypothesize that the oral bacteria transmitted from the parents stimulate the child's immune system in such a way that allergy development is avoided. Samples of the duodenal fluids of children with newly diagnosed and untreated inflammatory bowel diseases (ulcerative colitis and Crohn's disease) and controls (having functional bowel disorders without signs of intestinal inflammation) were analyzed by culture and pyrosequencing. The microbiota of children with ulcerative colitis displayed lower bacterial diversity than that of the control children, and certain bacterial groups were less abundant in the former group. Taken together, the studies presented in this thesis suggest that the compositions of the commensal microbiota in the oral cavity and small intestine affect the risk of developing immunoregulatory diseases, such as allergies and inflammatory bowel diseases.

Keywords: Infant, gut flora, oral flora, allergy, duodenum, inflammatory bowel disease, culture, T-RFLP, pyrosequencing.
ISBN: 978-91-628-9090-2 (Tryckt) **ISBN:** 978-91-628-9147-3 (e-pub)