Determinants and duration of impact of early gut bacterial colonisation

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Abstract

Background: An increasing numbers of studies show low diversity of the gut microbiome in those with chronic diseases such as obesity, inflammatory bowel disease, and allergy. Manipulation of the microbiota may promote health. However, the adult microbiota is stable and may be difficult to change. Understanding the fixed and modifiable factors which determine colonisation in early life may provide strategies for acquisition of a health-promoting microbiome.

Summary: Not enough is known about the long-term effects of established determinants of gut colonisation, including delivery mode, perinatal antibiotics and infant diet. It has been suggested that weaning onto solid diet containing non-digestible carbohydrates and cessation of breastfeeding are key stages in the colonisation process. In addition, the microbiome of the placenta, amniotic fluid and breast milk, alongside vaginal and faecal bacteria, may aid the transfer of maternal bacteria to the infant. However, methodological issues such as contamination during collection and/or analysis should be considered.

Key messages: The factors determining early colonisation are becoming more evident. However, longitudinal studies of microbiome maturation into late childhood and adulthood are required. The nutrition and health status of the mother before, during and after birth may be major factors in the early colonisation of the infant.

Introduction

Knowledge of the bacteria in the human gut has increased substantially since the microbiome, the collective genome of the gut microbiota, has been elucidated and elaborated in different populations [1,2]. There have been several studies implicating the gut microbiota in inflammatory bowel disease [3], irritable bowel syndrome [4], obesity [5], allergy [6], auto immune disease and many other potential conditions including brain disorders [7]. The number and types of bacteria in the gut are important but their metabolic activity is also key to their impact in the body. Similar metabolic activities may be provided by different groups of bacteria and the exact species responsible for a particular function may not be as important as the metabolic capability of the consortium. The metabolic profile of the microbiota may be influenced by diet as well as other environmental and host factors. Understanding the fixed and modifiable factors which determine which bacteria are present and the resultant metabolic profile is key to determining the role of the bacteria in promoting health or causing disease and should indicate possible interventions and treatments for combatting a range of conditions.

The gut microbiota is thought to be quite stable in adulthood [8] although more studies are needed to really establish this. There are many barriers to new colonisation by bacteria in the adult human including gastric acid, bile acids, pancreatic enzymes and most importantly the colonisation resistance of the host microbiome. These factors make it very difficult for bacteria from the diet or environment to establish long term in the human intestine. This is well demonstrated by the transient residence of probiotic bacteria in the human gut despite ingestion in large numbers. These barriers to colonisation are reduced in the new-born when gut function including acid secretion, pancreatic function and the gut associated immune system are immature. They may be reduced in the adult by antibiotics, disease, and gut washout for example. Faecal transplantation is being increasingly explored to deal with dysbiosis in different conditions such as persistent Clostridia difficile infection [9]. However, it is more likely that early intervention in the first year of life will be successful in changing the gut microbiome than strategies employed in adulthood. The initial colonisation and establishment of bacteria in the gastrointestinal tract in early life is likely to be a major factor in determining the adult gut microbiome. Thus, early events in the first year of life may programme the microbiome and its activities into adulthood and if so have major influence on metabolism and disease risk.

Factors affecting early colonisation

There have been many studies which considered early colonisation of the infant gut [10-12]. As techniques have improved and gene sequencing has made more detailed analysis of the microbiota and microbiome possible, the factors which influence colonisation have been come more clearly established. The infant gut, mostly sterile at birth, is exposed to bacteria in the birth canal and from the mother's faeces during delivery. Infants born by caesarean section do not have this exposure and nearly all studies, regardless of technique used, report significant differences in the faecal bacteria of infants born vaginally or by caesarean section [10-13]. The nature and extent of these differences vary between studies and may be related to population variation and local obstetric practices. It is not clear how long these differences in the microbiota persist as not many longitudinal studies have been carried out. In Gronlund's study [13] it was suggested they lasted at least a month. The INFABIO study reported significant differences persisted 4 weeks after the start of weaning [10] but the impact of caesarean birth diminished over the first year and only few metabolic differences were observed at one year (Edwards et al., unpublished data). Another major factor determining early colonisation is the use of perinatal antibiotics. These may be given to the infant after birth but also to the mother around the time of birth and during lactation. There are initially major impacts of these antibiotics on the gut bacteria but the effects which were still seen at 6 weeks [10] were not maintained in the longer term. Country of birth was a major influence on the colonisation of the infant gut which persisted beyond other factors. In the INFABIO study of infants from five European countries (Sweden, UK, Germany, Italy and Spain) followed from birth to one year, there was a North-South gradient of the bacterial colonisation in the gut with more bifidobacteria dominated microbiota in the North and more bacteroides predominant in the South. These differences were still evident 4 weeks after the start of weaning [10] and may persist longer as geographical differences are reported in several studies [1,2]. Host genetics may be a key factor in early colonisation but while some studies have shown greater similarity in the microbiota of identical twins [14] than fraternal twins and siblings others have not [15].

Transfer from mother before and during birth,

During birth the infant should be exposed to the mother's vaginal bacteria and the microbiota from the faeces of the mother. This could provide the infant with bacteria the mother has acquired which have 'worked' in her environment and therefore may be of benefit to the infant. It has been long believed that the infant is born from a sterile environment in utero and the first bacteria are encountered in the birth canal. However, there is increasing evidence that bacteria can be present in placenta, cord blood and amniotic fluid [16,17]. The presence of bacteria in utero may also occur via vaginal transfer and contamination either during sample collection or during analysis must be ruled out [18]. If this placental transfer is established then its importance in overall gut bacterial colonisation and the factors controlling the process need to be explored.

Transfer from mother during breast feeding

It is well established that breastfed infants have more bifidobacteria in their microbiota than formula fed infants and this may be due to a variety of factors in human milk including oligosaccharides, lactoferrin, and low iron levels. However, a clear breast milk microbiome has been established in several studies [19]. Cabrera-Rubio et al measured the human milk microbiome at three different time points and related the composition to BMI, weight gain and mode of delivery. They found the milk of obese mothers had a less diverse microbiome than normal weight mothers and there was a major difference in the breastmilk bacteria of mothers with normal delivery and those undergoing elective caesarean but not emergency caesarean [19]. In a Chinese study of the microbiota in breastmilk of mothers measured from birth to 2 months of age, the milk was expressed with or without aseptic cleaning of the breast before collection. There was much greater diversity of the breastmilk microbiome of those who did not clean the breast suggesting a significant contribution from the skin of the breast and they found no effect of stage of lactation or delivery mode [20].

Another source of bacterial transfer may be from oral contact. One mode of transfer is parental behaviour around pacifier use. Some parents may suck the pacifier before giving to the child and pass more bacteria to the infant. Hesselmar et al [21] found a distinct pattern of colonisation in infants at 4 months of age between those whose mothers cleaned the pacifier by sucking and those that did not.

Impact of weaning diet.

There is considerable impact of the post weaning infant diet on the microbiota of the infant with diversity increasing as the child is weaned [10]. Development of fermentation capacity for a range on non-digestible carbohydrates increases during weaning with polysaccharides fermentation developing more slowly than oligosaccharides [22, 23]. Some bacterial enzymes and products such as β glucuronidase and butyrate do not increase substantially until later in the first year of life and cessation of breastfeeding, rather than introduction of solids, has been reported to be a major influence on development of the adult style microbiota [24]. Thus, diet during this period may influence maturation of the microbiota.

Inclusion of prebiotics and probiotics in the infant diet has been shown to increase the bifidobacterial and lactobacilli populations but it is not clear if this persists long after the prebiotics and probiotics have been discontinued. However, there have been longer term impacts reported on eczema after mothers and high risk infants were given probiotics [25].

There is a lack of human dietary and probiotic intervention studies over the longer term to determine if the differences seen in individuals with very different habitual diets can be related to early colonisation events, long-term modifiable dietary patterns or to a whole range of host factors.

How can we study the gut microbiome and its activities?

Although study of bacterial colonisation using microbiome analysis, including transcriptomics, can help unravel the factors influencing early colonisation and later health, it is very difficult to access events and microbiota populations in the proximal colon where most bacterial metabolism of dietary constituents, host secretions and cellular remnants occurs. Key differences in the production of bioactive molecules, such as short chain fatty acids, which modulate host metabolism and function may be missed. The bacteria can live in different niches and biofilms and may be associated with the mucosa, mucin layer or food remnants. The vast majority of studies have concentrated on faecal samples to analyse the gut microbiome and its metabolites, with a limited number of studies using gut mucosal samples and colonic contents. Studying the metabolism of the bacteria in the proximal colon in vivo is particularly difficult without invasive techniques which may disrupt normal metabolism, using intubation or capsules. Metabolomic studies may be useful but are still

difficult to interpret. Stable isotope studies have great potential to trace bacterial metabolism, but very few studies have been carried out so far.

Early microbiome and later disease

The role of early bacterial colonisation in the fine tuning of immune function has been explored since the initial proposal of the hygiene hypothesis which noted that allergy was higher in populations with smaller families, more urban environment and less rigorous use of vaccines and antibiotics. Altered immune function and development of allergy have been clearly linked to early colonisation [26, 27]. In addition, the association between the gut microbiome and obesity in adults has led to studies exploring the development of the microbiome and obesity in children. Despite evidence suggesting that a gut microbiome with lower numbers of bifidobacteria in infancy is related to later obesity [5, 28], children with hyperphagic disorders [29], including Prader Willi syndrome, who developed obesity had more similar gut bacterial activity to normal obese children than those with the same condition but who had not become obese indicating that the bacterial differences may have been caused by the obesity and not vice versa.

Conclusion

The gut microbiome has been related to a range of different acute and chronic diseases which stimulates consideration of strategies to modify/optimise the bacterial profile and activities. However, the adult microbiome is believed to be remarkably stable and has its own colonisation resistance which means encroachment by novel species including pathogens and probiotics remains transient. Changing initial colonisation during birth and infancy by controlling for factors such as perinatal antibiotics, mode of delivery and infant diet may be more successful in the long term but this still needs to be fully established in prospective long-term studies. The impact of maternal diet and health status as well as placental and breastmilk microbiomes also need to be considered.

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Conflicts of interest

Professor Edwards is chair of the working group on Early bacterial colonisation and potential implications later in life for ILSI Europe and has taken part in a workshop for Unilever.