The small intestine microbiota, nutritional modulation and relevance for health
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The intestinal microbiota plays a profound role in human health and extensive research has been dedicated to identify microbiota aberrations that are associated with disease. Most of this work has been targeting the large intestine and fecal microbiota, while the small intestine microbiota may also have a profound impact on various aspects of the host’s physiology, including immune, metabolic and endocrine functions. This review highlights the recent advances made in the study of the human small intestine microbiota. In addition, it describes recent human and animal studies that underpin the importance of this part of the intestine for health of the host organism.

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Introduction
The different regions of the human intestine harbor distinct bacterial communities that vary in density and diversity [1]. The fecal microbiota composition has been associated with host health and disease, and its easy accessibility has stimulated its characterization in various human cohorts [2]. In contrast, limited information is available for the human small intestine (SI) microbiota characterized by its relatively low-density (10^2–10^7 cells/g), which is largely due to the rapid luminal flow and secretion of bactericidal substances (e.g. bile acids) in this part of the intestinal tract [3]. The SI is responsible for approximately 90% of the overall energy absorption from the diet [4] facilitated by the large surface area of its mucosa. Adequate absorptive capacity depends on maintenance of mucosal integrity that is constantly challenged by its exposure to the luminal milieu, including the microbiota. Mechanisms that contribute to this mucosal integrity, include the production of antimicrobial peptides (AMPs), a physical mucin barrier, and mucosal antibodies. Especially the terminal ileum is associated with the largest mass of lymphoid tissue in the human body, that is, the gut-associated lymphoid tissue (GALT) and Peyer’s patches [5], which are essential for maintenance of a microbiota-accommodating immune homeostasis via dedicated luminal antigen-sampling (see also Box 1).

As a consequence of the fundamental role of the SI in the host’s nutritional- (metabolic) and immune- status, it is highly plausible that this region of the intestine, and its endogenous microbiota, have profound influence on host physiology [6,7]. This review describes the state of our knowledge of the SI microbiota and the role of this region of the intestine in host health.

Sampling of the small intestine
The SI is poorly accessible in healthy individuals and collection of luminal samples during routine gastroendoscopy and colonoscopy practices is challenging [8,9,10**]. As an alternative, intraluminal naso-ileal catheters facilitate the collection of such samples [1,11**]. However, volunteers are fasted before placing such catheters and sampling may require luminal flushing, which may interfere with the accurate determination of the ‘normal’ SI microbiota in human individuals. Moreover, the invasiveness of the procedure requires gastroenterologist-supervision and is incompatible with repeated sample collection over time, limiting these measurements to single time-points, and hindering the study of microbiota dynamics and diet responses. Recent advances in the design of remote-control drug delivery capsules [12], may be adjusted to enable future sampling of the lumen from the human SI, using less invasive procedures. However, the high cost of these devices may hinder their application in larger scale dietary trials in human, while their small sample-size provides technological challenges for the analysis of the material obtained. Moreover, obtaining relevant, and unchanged
samples from the SI in these devices requires adequate quenching of reactions in the sample during the remaining transit time through the rest of the intestinal tract.

An alternative to obtain SI samples makes use of ileostomy subjects, which are individuals that underwent colectomy and have their terminal ileum connected to an abdominal stoma [1,13,14]. Non-invasive and repetitive sampling of the small intestinal lumen is possible in these subjects, without the use of catheters or other devices that may disturb the physicochemical and ecological characteristics of the samples obtained. Thereby, this human model enables the analysis of SI microbiota dynamics as a function of time and/or diet. Importantly, the microbial composition of ileostomy effluent was reported to resemble that of jejunal and proximal-ileum content in healthy individuals, indicating that the ileostomy effluent can be used as an adequate model for the proximal SI microbiota [1,11**].

The human small intestinal microbiota

Microbiota composition analysis of ileostomy effluent revealed that individual subjects harbor a distinct microbiota. Contrary to the more stable fecal microbiota [15,16], the SI microbiota displays pronounced compositional fluctuations during the course of several days and even within a day, which most likely reflect the subject’s dietary variation [13]. The microbiota can encompass subject-specific genera like Clostridium, Escherichia, and Turicibacter in variable amounts, but Streptococcus and Veillonella species are consistently encountered in this ecosystem, albeit in variable relative abundance per individual. The Streptococcus and Veillonella genera were also among the most active members of the ileostoma effluent microbiota, indicating that they are canonical and active SI commensals (B. van den Bogert, PhD thesis, Wageningen University, 2013). Cultured isolates of the SI streptococci belonged to the S. mitis, S. bovis, and S. salicarius species groups, and exhibited substantial divergence in their carbohydrate import and metabolism [14,17]. Such characteristics reflect their SI adaptation, in which a variety of diet-derived carbohydrates is encountered [17*]. Veillonella species are renowned utilizers of lactic acid as a carbon and energy source and are predicted to ferment the lactic acid produced by the carbohydrate-fermenting streptococci (Figure 1; [18]).

Metatranscriptome analysis of ileostomy effluent underpinned the metabolic focus of this microbial ecosystem on transport and metabolism of ‘simple’ carbohydrate substrates. These functions were predominantly assigned to Streptococcus, although also other genera (e.g. Escherichia) expressed these activities, especially in samples with relatively low streptococcal abundance. These findings suggest that the SI microbiota is dedicated to the utilization of (diet-derived) simple carbohydrates and that this task is not restricted to, but predominated by only few phylogenetic groups [11**] (unpublished data).

Microbiota-driven SI immune development in health and disease

Throughout the host lifespan, complex-signaling and dynamic-signaling interactions shape the interplay between host and microbiota [19], including spatial and temporal variations within the different regions of the intestine [20,21]. Exposure of the newborn to the maternal microbiota initiates the intestinal colonization by the microbiota of which the composition varies dramatically during the first year of life, after which it stabilizes to establish a complex and host-specific microbial community [22]. The microbiota drives rapid maturation of the neonatal immune system, initially by activating innate immune functions followed by development of the adaptive immune system [23]. Eventually, in healthy hosts, these processes lead to a state of mucosal homeostasis that accommodates the intestinal microbiota. Germfree (GF), gnotobiotic, and genetically modified (GM) animals have been instrumental in our understanding of the mechanisms that underlie the intimate coexistence of the microbiota with its mammalian host [24]. In particular, GF and GM mice have underpinned the role of specific host-genes in the interplay between (specific) microbial groups and the mouse physiology. These
models have been used to study the influence of microbiota on different domains of host physiology, including immune and metabolic homeostasis and more recently mood and behavior, in healthy-animals [25**] as well as diseased-animals, including models for inflammatory bowel disease, obesity, diabetes and autism [26,27**]. Gnotobiology combined with genomics-based approaches has accelerated our understanding of the strongly intertwined microbiota-host communication networks that are launched at birth, and have profound effects in development with potential long-term consequences for health and disease susceptibility of the host organism [28**,29].

Recent studies indicate that the naive mucosa is primed to launch transient inflammatory cascades upon colonization to promote early immune responses, followed by desensitization to suppress excessive responses [30,31,32]. Transient priming of the naïve intestinal epithelial cells (IECs) may involve close contact with specific microbiota members, as demonstrated for segmented filamentous bacteria (SFB) [33*]. In response to colonization, IECs enhance mucin and AMP secretion to reduce microbial contact, whereas secretion of pro-inflammatory cytokines by IECs induces the development of effector T cells repertoires (Figure 2). In parallel, IECs simultaneously stimulate anti-inflammatory responses [34], that drive multiple tolerance mechanisms, which are crucial for immune balance by suppressing excessive effector T-cell responses. Moreover, the development of immunoglobulin (Ig)A+ producing plasma cells is induced to ensure an abundant supply of secretory (sIgA) that further limits microbial contact with the epithelia [35]. The tightly regulated immune development process in response to microbial colonization is essential to establish homeostatic coexistence and may be most amenable
Multifactorial cross-talk between the microbiota and local and systemic host processes. Colonization of the gut microbiota launches complex communication networks with the host immune, metabolic and neuroendocrine systems, which ultimately impacts the overall wellbeing of the host organism.
to diet or microbial modulation during a limited postnatal time-window [36,37]. Disruption of intestinal host-microbe homeostasis can drive pro-inflammatory cascades, including the induction of IL-17 and IL-22 production, neutrophil recruitment and the expansion of effector T cell repertoires (T_{eff} and T_{h17}) [38]. These responses [39] can lead to intestinal conditions that skew the microbiota composition toward increased abundance of pathobionts [40] and reduced abundance of more tolerogenic microbial groups [41], which can enhance inflammatory responses and/or promote IEC hyperproliferation, increasing the risk for the development of inflammatory diseases and cancer [42,43].

The molecular mechanisms by which microbes can positively or negatively affect inflammation-disease risk remains limited due to our incomplete understanding of the molecular triggers and corresponding cascades involved in the establishment of homeostasis versus disease-development. Further insight in the molecular interactions between the microbiota and the (SI) mucosal immune system can enable the development of more effective prophylactic and therapeutic strategies in the context of inflammatory diseases [44].

**Microbiota impacts on SI metabolism and gut-brain signaling**

The gut microbiota affects host metabolism through various physiological processes, including the production of short-chain fatty acids (SCFAs). Emerging evidence shows that SCFAs produced by the microbiota interact with host enteroeococrine cells (e.g. L cells) by modulating G protein coupled receptor (GPR41, GPR43) signaling [45], impacting on the production of glucose homeostasis modulators like peptide YY (PYY) and glucagon-like peptide (GLP)-1 [46]. SCFAs may further control intestinal gluconeogenesis (IGN) via different mechanisms, in which butyrate can activate IGN gene expression via a cAMP-dependent mechanism and promote activates IGN gene expression via a gut-brain neural circuit involving Gpr41 [47**]. Moreover, specific probiotic interventions (L. acidophilus) were shown to modulate transcriptional patterns in the duodenal mucosa associated with enteroendocrine function via an unidentified mechanism [48,49].

Various studies have focussed on the influence of the intestinal microbiota on metabolic control in the host [50]. A recent study revealed that a metabolic shift from oxidative toward glycolytic energy supply and anaerobic metabolism in the jejunal mucosa were strongly intertwined with immune-system related responses within the same tissue. These metabolic responses appeared to be coordinated through transcription regulation signatures that were significantly associated with glucose and energy homeostasis and type 2 diabetes (T2D) [28**][Figure 2]. The possible controlling role of the proximal SI and its microbiota is in agreement with the observation that bariatric surgery, performed in obesity and metabolic syndrome patients, induces changes in the gut microbiota [51,52]. These patients commonly suffer from low-grade inflammation, coinciding with increased intestinal permeability, endotoxemia, and insulin resistance [53]. The bariatric surgery not only leads to weight loss in these patients, but also drastically improves their insulin sensitivity [54], possibly via FXR-signaling and the subsequent changes in circulating bile acids [55**]. Importantly, a recent fecal transplantation study concluded that fecal transfer of intestinal microbiota from lean donors increased insulin sensitivity in individuals suffering from metabolic syndrome. These health improvements were associated with increased abundance of butyrate-producing bacterial groups in both fecal and SI samples obtained from these subjects, implying a casual role for the microbiota in the observed effects [10**].

Overall, the microbiota has a profound impact on the metabolic state of the host, and the SI and its endogenous microbiota is likely to play a pivotal role in metabolic regulation. Dietary or microbial interventions that aim to change the interplay of the microbiota with the proximal SI mucosa appears a plausible approach to modulate diseases like T2D and/or other disorders associated with metabolic syndrome.

**Targeting the SI ecosystem to improve consumer health**

Diet is a major driver of population dynamics of the SI microbiota, which may strongly influence the interactions between the microbiota and the mucosa in the SI, which can impact on the host health [50,57]. This notion is supported by recent *in vitro* studies that highlighted the considerable variation in immunomodulatory capacities of streptococcal isolates from the SI (unpublished data) and the proposed role of SI butyrate-producing microbes in T2D control [10**]. These diet-induced effects in the small intestine may be anticipated to be much more pronounced as compared to the large intestine, since this intestinal region is the site where the diet first encounters the microbiota. Further insight in the *in vivo* interaction mechanisms between the SI microbiota and mucosa, can guide the rational design of diets aiming to modulate mucosal and systemic physiology via modulation of the endogenous microbiota. Alternatively, interventions with probiotics can transiently impact the SI microbiota by strongly overcrowding the endogenous microbial populations (Figure 1), and were shown to significantly affect transcriptional patterns within mucosal tissues that can be correlated to predicted, and in several cases experimentally verified, physiological impacts on both metabolic and immune functions [48,49]. Again, overcrowding the microbiota in the small intestine is very feasible, while
achieving similar microbe domination in the densely colonized large intestine seems barely feasible.

Taken together, dietary modulations of the SI microbiota are highly feasible and can affect both local and systemic processes in the host. An important future challenge lies in the elucidation of mechanisms underlying these effects, with the aim to harness their potential for the rational design of food compositions that contribute to the maintenance or improvement of human health.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

● of special interest
** of outstanding interest


5. Emund A, Schute A, Johanson ME, Gustafsson JK, Hansson GC: Studies of mucus in mouse stomach, small intestine, and colon. I. Gastrointestinal mucus layers have different properties depending on location as well as over the villus's parietes. Am J Physiol Gastrointest Liver Physiol 2013, 305,G331-G337.


This study underpins the causal role that the microbiota can play in metabolic syndrome associated diseases, by improving insulin sensitivity through fecal transplantations.


This study describes the small intestine microbiota in humans, including the microbiota of healthy individuals and long-term ileostoma subjects, and employs metagenomic approaches to illustrate the main evolutionary driving forces that shape this microbial community.


This study compares the genomes of human small intestine streptococcal lineages, and highlights their functional and genetic diversity in relation to the small intestine habitat.


This study demonstrates that the host-microbiota specificity during developmental processes in early life, highlighting that host organ specific intestinal microbes, including the segmented filamentous bacteria, are required to drive full immune maturation.


Elegant study that illustrates the role of the microbiota in the modulation of behavior, using a mouse model that is now to display features of autism.
spectrum disorder. In addition, the study identifies metabolite profiles and microbial species that can play a role in the animal’s behavioral abnormalities.


Mouse conventionalization study that highlights the importance of the proximal regions of the small intestine in the metabolic response to colonization of the intestine, illustrating the metabolic reorientation of local mucosa upon conventionalization as well as core regulatory signatures that drive these changes and relate strongly to glucose homeostasis and insulin control.


An elegant study that describes the molecular signatures and mechanisms that drive the mucosal responses of the intestine to the colonization by a specific microbial group; the segmented filamentous bacteria.


A study that strongly supports the prolonged effects of early life immune development on immune status during later stages of life.


This study illustrates the importance of differential regulation of intestinal gluconeogenesis (IGN) by the microbiota fermentation products propionate and butyrate, which impact on processes involved in glucose homeostasis and body weight control via distinct mechanisms, both involving IGN.


Study that investigates the mechanism underlying the body weight reduction and improvement of insulin sensitivity associated with vertical sleeve gastrectomy, demonstrating that alterations in circulating bile acids and FXR signaling play an important role.
