



The Intestinal Microbiome: Exploring a New World

The whole of the bacteria hosted in our intestine, or the microbiome, forms a vital critical mass that interacts with our body – “for better or worse” – starting from birth. As recent studies have shown, the intestinal microbiome appears to have significant connections to gluten-related disorders.

This issue of the DSI Forum explores the different perspectives on the relationships between the microbial flora populating our intestine – known as the intestinal microbiome – and gluten-related disorders. The first article discusses celiac disease and the microbiome as two apparently distant “worlds” that are actually surprisingly linked.

A glance at PubMed, the most complete medical-scientific database worldwide, shows that there has been an exponential growth in the number of publications on the intestinal microbiome since the early 2000s, jumping from 35 articles in 2004 to 1,656 in 2014! In 2014 alone, no fewer than 21 articles dealt with the relationships between the “bacterial world” of the intestine and celiac disease. Such an increase is primarily due to the development of new technologies in molecular genetics that

enable us to analyze the intestinal bacterial flora quickly, easily and accurately. One of the most relevant topics today in the field of celiac disease is the impressive increase in the frequency of this illness over recent decades. This is a phenomenon that cannot be ascribed to changes in genes, which require much more time to take place, but only to environmental changes. These environmental changes include changes in diet, lifestyle, the spread of infections, and the bacterial population that has settled in our intestine.

As a paediatrician, I am particularly interested in the potential relationship between certain factors in early life, such as type of birth (natural or Caesarean), infant feeding, intestinal infections and the use of antibiotics, the intestinal microbiome and the risk of developing celiac disease. The analysis of these aspects

could help us to better understand the “recipe” for the genetic and environmental cocktail that leads to the development of gluten-dependent disorders – not just celiac disease, but also non-celiac gluten sensitivity. In terms of treatment, an understanding of persistent changes in the microbiome, known as dysbiosis, could foster the implementation of new treatments aimed at improving quality of life in people suffering from gluten-related disorders.



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Gut Microbiota in Health and Disease

Ms Wilson's article reviews the influence of the environment on the gut microbiome and discusses potential dietary avenues to improve it.



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Gut microbiota functions are varied and still being explored.

As new evidence and better analytical techniques emerge, more information is becoming available about our gut bacteria. It is becoming clear that the type and relative amount of bacteria present in our gut plays an important role in both health and disease. Biotech companies are investing more in technologies to target this 'microbiome' as a potential moderator of our gut health and our innate immune system. The increase in incidence of immune mediated diseases and neurological disorders cannot be explained by shifts in human genetics.¹ Dysbiosis and loss of diversity is something that is now being commonly traced to these diseases as we look to our 'other genome' for clues. What has become abundantly clear from the work thus far conducted in metagenomics is that greater bacterial diversity or 'gene richness' is strongly associated with better health.

INFO

Schlüsseldefinitionen

Microbiome: the collective name for the gut microorganisms.

Dysbiosis: one or more potentially harmful microbial organisms predominating the gut microbial population.³

Metagenomics: the field of study which compares entire genomes.

Phyla: a taxonomic term for dividing organisms into groups with others of similar properties.

Enterotype: a term to divide humans into groups based on the gut bacteria present.

Transcriptome: the genes which have been expressed as proteins i.e the active part of the genome.

Typical bacterial species

Mouth
(approx. 10^{11} bacteria)

Streptococci
Veillonella
Actinomyces
Porphyromonas
Fusobacteria
Eubacterium
Prevotella

Stomach and Duodenum
(10^1 – 10^3 bacteria)

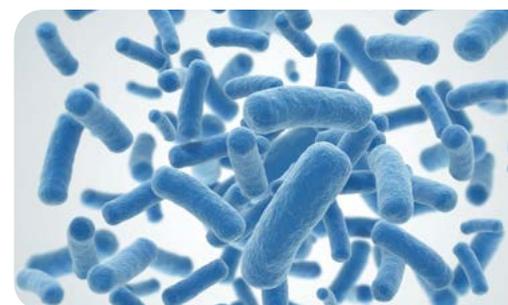
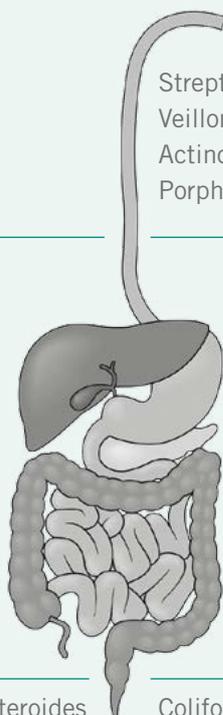
Lactobacilli
Streptococci
Candida
Helicobacter pylori

Small intestine
(10^3 – 10^4 bacteria)

Lactobacilli
Coliforms
Streptococci
Bacteroides
Bifidobacteria
Fusobacteria

Colon
(10^{10} – 10^{12} bacteria)

Bacteroides	Coliforms	Staphylococcus
Bifidobacteria	Clostridia	Peptococci
Streptococci	Veillonella	Peptostreptococci
Eubacterium	Lactobacilli	Ruminococci
Fusobacteria	Enterococci	



What species makes up the Microbiome?

Although great inter-individual variation exists at species level – most people have around 160 species from a possible 1000 – the phyla represented in the microbiome are quite narrow. Qin et al (2010) have identified a core set of bacteria present within all people. Three main enterotypes have been configured for the human microbiome.² The marker genera for which microbiome a subject belongs to are the Bacteroides, Prevotella and Ruminococcus (the latter group is further associated with the presence of the Methanobrevibacter).¹

Function

The functions of the gut microbiota are still being fully elucidated but some key aspects are: immune signaling and modulation; production of nervous system messengers; production of essential vitamins; regulation of fat metabolism; production of short chain fatty acids (SCFA) specifically butyrate, and branched chain fatty acids. Depending on the substrate fermented, other products of the microbiome include hydrogen, carbon-dioxide and methane gasses, ammonia, amines and phenolic compounds.³



The symbiosis between humans and the gut microbiota is becoming ever more apparent. The term 'superorganism' has been coined as the human body starts to be considered more a conglomerate organism of our own transcriptome and far greater transcriptome of the gut microbiota. The genes encoded by our gut bacteria outstrip our own by greater than 100-fold.⁴ It is therefore not surprising that much focus of disease cause, prevention and cure is now being placed on this 'other genome'.

The enteric nervous system (ENS) is sometimes referred to in literature as the 'second brain'. This is due to it consisting of over 200 million neurons.⁵ The ENS sends signals from the gut to the brain via endocrine, neuronal

Both nature and nutrition can affect our microbiome.

and immune afferent signaling.⁵ In addition, the gut associated lymphoid tissue (GALT), which regularly samples and responds to signals from within the intestinal lumen, is considered the body's major defensive organ against infection.⁵

The combination of interactions between the ENS, the microbiome and the GALT has great potential to effect changes to physical, immunological and emotional wellbeing.

What shapes the Microbiome?

The microbiome develops from birth. Route of delivery and early feeding affects the initial development of the microbiome. Weaning and childhood environment (rural or urban) likely has an impact on the development of the mature microbiome. Studies of isolated population groups in Africa show divergent and unique bacterial colonization from that of a Western cohort. These findings indicate that

Environment plays a role in microbiome colonization.

the environment is a strong driving force for colonization.⁶ Twin studies have also revealed that at least with some taxa there is a definite genetic influence on species abundance.⁷ The spouses of identical twins also showed positive correlations which adds to the concept that both nature and nurture can affect gene richness of the gut microbiota.⁸

In the elderly the microbiome changes again, although it is not clear why this occurs. A reduction in butyrate producing bacteria is seen within the elderly population and a reduction in gene richness of the microbiome. Elderly living within the community maintain greater gene richness, thought to be an artefact of a more varied diet, than elderly in long term care.⁹

How do changes affect us?

Dysbiosis is associated with several disease states.³ A recent review was published on diseases and the associated alterations in bacterial populations.⁴ New technologies have allowed a type of bacterial fingerprint to be developed for certain diseases which may provide both a powerful and non-invasive diagnostic tool and a potential target for therapy in treating these conditions.

In obesity studies, subjects with reduced gene richness showed greater overall adiposity, insulin resistance and dyslipidaemia and a more pronounced inflammatory phenotype.¹⁰ Those with lower gene richness also gained more weight over time. Goodrich et al (2014) provide insight into the heritability of an obesogenic microbiome and the potential influence of methanogens and *Christensenella* spp. on metabolic disorders.

Studies in infants with genetic disposition to celiac disease (CD) show a reduction in Actinobacteria (which includes Bifidobacteria) and an increase in Firmicutes and Proteobacteria spp.¹¹ This said, a definitive link between microbial alterations and development of CD disease has not been shown.¹²

Dysbiosis has been identified in microbiome sequencing in irritable bowel syndrome (IBS).¹³ Further studies have identified distinct differences in gut bacteria in different IBS subtypes in both luminal and mucosal populations.¹⁴⁻¹⁶ A recent pilot study in pediatrics has identified that the microbiome may be indicative of the likelihood of the low FODMAP diet being effective in symptom alleviation.¹⁷

Qin et al (2012) identified antagonistic behavior between beneficial and harmful bacteria in type 2 diabetes. A decline in butyrate producing bacteria may be an indicator of increased risk of developing obesity related co-morbidities.¹⁸

How can we improve the Microbiome?

Dietary studies have shown the power of dietary manipulation in altering the microbiome¹⁹ and this is one area with great potential. Feeding the host and feeding the microbiota is the obvious way in which manipulation of the microbiota may come about. A high fat-high protein diet has been linked to the *Bacteroides* enterotype, and a carbohydrate rich diet corresponds to the *Prevotella* enterotype.²⁰ Short term dietary changes (~10 days) were shown to change the composition of the microbiome but not significantly affect the identity of the enterotype. *Faecalibacterium prausnitzii*, *Bifidobacterium* and *Clostridium* cluster XIVa have all been shown to be elevated by high fiber dietary supplements and these are three groups which are generally associated with better health.^{1,21}

Other studies have definitively shown that prebiotics and probiotics to varying degrees are useful tools in promoting beneficial bifidobacteria and lactobacilli. Several studies have shown mechanisms by which different species of Lactobacilli and Bifidobacteria not only confer beneficial effects on the host but also inhibit attachment and activity of invading enteropathogens (Reviewed in);⁴ It may be that in the future more strains of bacteria (e.g. *Akkermansia muciniphila* and *Christensenella minuta*) will be targeted by both prebiotic and probiotic supplementation.⁴

Fecal microbiota transplantation is another technique for rapid correction of a disordered microbiome. Trials for intractable *C.diff* infected patients have shown promising results thus far. Lean donor fecal microbiota transplantation showed an improvement in insulin resistance in patients with metabolic syndrome²²





– lending support to the growing concept that dysbiosis plays an important role in the development of obesity related disorders. Antimicrobial strategies for modulation of the microbiome may have therapeutic potential in the future however current knowledge of the efficacy in this area is based on mice models and therefore not presently a recommended strategy.⁴

A varied diet including all food groups should be utilized to provide alternating substrate and reduce the likelihood of unfavorable species becoming dominant within the gut. For

perturbations in the microbiome brought on by antibiotics or a bout of gastroenteritis the use of prebiotics and probiotics are likely to be useful and are considered to be safe for general use. Stool diagnostics capable of detecting a decrease in diversity may be a useful tool for early disease prediction and preventative measures.

REFERENCES

- Blottiere, H.M., et al., Human intestinal metagenomics: state of the art and future. *Curr Opin Microbiol*, 2013. 16(3): p. 232-9.
- Qin, J., et al., A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*, 2010. 464(7285): p. 59-65.
- Roberfroid, M., et al., Prebiotic effects: metabolic and health benefits. *British Journal of Nutrition*, 2010. 104(S2): p. S1-S63.
- Walsh, C.J., et al., Beneficial modulation of the gut microbiota. *FEBS letters*, 2014. 588(22): p. 4120-4130.
- Al Omran, Y. and Q. Aziz, The brain-gut axis in health and disease, in *Microbial Endocrinology: The Microbiota-Gut-Brain Axis in Health and Disease*. 2014, Springer. p. 135-153.
- De Filippo, C., et al., Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences*, 2010. 107(33): p. 14691-14696.
- Goodrich, J.K., et al., Human genetics shape the gut microbiome. *Cell*, 2014. 159(4): p. 789-799.
- Nelson, K.E., et al., *Metagenomics of the human body*. 2011: Springer.
- Claesson, M.J., et al., Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proceedings of the National Academy of Sciences*, 2011. 108(Supplement 1): p. 4586-4591.
- Le Chatelier, E., et al., Richness of human gut microbiome correlates with metabolic markers. *Nature*, 2013. 500(7464): p. 541-546.
- Olivares, M., et al., The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease. *Gut*, 2014: p. gutjnl-2014-306931.
- McLean, M.H., et al., Does the microbiota play a role in the pathogenesis of autoimmune diseases? *Gut*, 2014: p. gutjnl-2014-308514.
- Rajilic-Stojanovic, M., et al., Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology*, 2011. 141(5): p. 1792-801.
- Saulnier, D.M., et al., The intestinal microbiome, probiotics and prebiotics in neurogastroenterology. *Gut Microbes*, 2013. 4(1): p. 17-27.
- Parkes, G.C., et al., Distinct microbial populations exist in the mucosa-associated microbiota of subgroups of irritable bowel syndrome. *Neurogastroenterology & Motility*, 2012. 24(1): p. 31-39.
- Sundin, J., et al., Altered faecal and mucosal microbial composition in post-infectious irritable bowel syndrome patients correlates with mucosal lymphocyte phenotypes and psychological distress. *Aliment Pharmacol Ther*, 2015. 41(4): p. 342-51.
- Chumpitazi, B.P., et al., Gut microbiota influences low fermentable substrate diet efficacy in children with irritable bowel syndrome. *Gut microbes*, 2014. 5(2): p. 165-175.
- Qin, J., et al., A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*, 2012. 490(7418): p. 55-60.
- Cotillard, A., et al., Dietary intervention impact on gut microbial gene richness. *Nature*, 2013. 500(7464): p. 585-8.
- Wu, G.D., et al., Linking long-term dietary patterns with gut microbial enterotypes. *Science*, 2011. 334(6052): p. 105-108.
- Shen, Q., L. Zhao, and K.M. Tuohy, High-level dietary fibre up-regulates colonic fermentation and relative abundance of saccharolytic bacteria within the human faecal microbiota in vitro. *European journal of nutrition*, 2012. 51(6): p. 693-705.
- Vrieze, A., et al., Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology*, 2012. 143(4): p. 913-6.e7.

The influence of the microbiome on gluten-related diseases

The increasing prevalence of food intolerances, especially in relation to certain carbohydrates, represents a global health problem.¹ Moreover, intolerance of gluten and gluten-bound substances, e.g. amylase-trypsin inhibitor (ATI) is blamed² for intestinal (e.g. meteorism, pain, constipation, diarrhea) and extra intestinal symptoms (e.g. fatigue, headaches, joint pain, skin irritation) in affected patients.³ The pathogenesis of food intolerances is blamed on factors such as changes in the composition of the intestinal flora and its influence on mucous membrane immune tolerance.⁴

The intestinal barrier is important to maintain homeostasis in the intestine. If intestinal imbalance occurs, the intestinal barrier can be attacked and become permeable as part of leaky gut syndrome. It has been proven that there is an association between leaky gut syndrome and the development of gastrointestinal disease and possibly food intolerances.⁵

The microbiome of the human digestive tract therefore also appears to play an important role in influencing wheat/gluten-associated disease.⁴

It is common knowledge that the intestinal microflora is dependent on many factors. The microbial composition in the small intestine is primarily determined by the competition between micro-organisms and the host to ensure rapid absorption and utilization of carbohydrates. The micro-organisms in the colon, on the other hand, are affected by both the complex utilization of carbohydrates and competition among themselves.⁶

Nutrition plays an important role in this case. Mouse studies have already demonstrated that diet can rapidly alter the intestinal microbial composition.⁷

Numerous publications demonstrate the presence of streptococcus sp., E.coli, clostridium sp., GC-rich organisms, bacteroides uniformis, blautia glucerasea and bifidobacteria in the small and large intestine, which prefer different substrates.⁸ It is interesting in this context that B. uniformis primarily utilises inulin, whereas other species primarily metabolise fructo-oligosaccharides or monosaccharides.⁹

An indication of the importance of microbial composition in celiac disease is provided by the fact that there is a different bacterial population in these patients compared to non celiac individuals. A significantly higher proportion of bifidobacterium bifidum and increased numbers of lactobacillus sp. were found in



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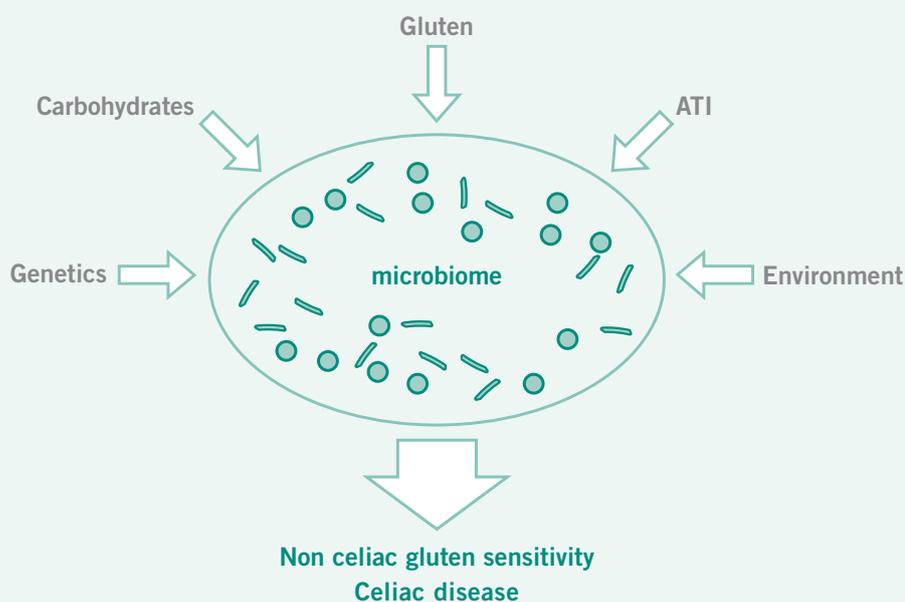
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Celiac disease patients
have a different bacterial
population when compared to
healthy individuals.



Influences on the microbiome:



There is an important link between celiac disease and the microbiome.

celiac patients, but their diversity decreased significantly after maintaining a gluten-free diet.¹⁰ Furthermore, it was demonstrated in-vitro that certain bifidobacterium strains reduced the inflammatory immune response triggered by gliadin peptides, thus exerting a protective effect.^{11,12}

In 2013, Wacklin et al. reported a possible link between the manifestation of celiac disease in the form of gastrointestinal or extraintestinal symptoms and the microbiome.¹³

In another study, the duodenal microbiome of celiac disease patients with persistent symptoms despite long-term GFD and normalized small-intestinal mucosa was studied in comparison with celiac disease patients without symptoms. It was determined that there

were differences in bacterial colonization of the small intestine between patients without symptoms and patients with persistent symptoms. They had a significantly increased amount of proteobacteria, whereas the number of bacteroidetes and firmicutes was reduced. Overall, the celiac disease patients with persistent symptoms exhibited reduced microbial diversity. In some subgroups of celiac disease there is thus evidence of a dysbiosis as a possible cause of recurrent symptoms, in which case new treatment approaches, e.g. in the form of pro- or prebiotics, would be possible.¹⁴

In addition, Smecuol et al. have already investigated the effect of the probiotic bifidobacterium infantis Natren Life Start Strain Super Strain on the clinical progress of untreated celiac patients. Of the 22 patients, 12 were given 2 B. infantis capsules and 10 were given 2 placebo capsules with meals. While taking the probiotic had no effect on intestinal permeability, symptoms of dyspepsia, constipation and gastro-esophageal reflux improved significantly in the B. infantis group. There was also a significant increase in MIP-1 β (macrophage inflammatory protein-1 β) in the probiotics group. Although this study indicates a possible mitigating effect of probiotics on some celiac disease symptoms, it requires confirmation by further studies.¹⁵

A recent study by Olivares et al. in patients with a high genetic risk of celiac disease revealed an altered microbial composition even in infancy and early childhood, which is an indication that the change in the microbiome might take place at a very early stage. Compared to infants without increased celiac risk, those individuals who were positive HLA-DQ2 carriers had a significantly higher number of firmicutes and proteobacteria, and





a smaller number of actinobacteria. The number of bifidobacteria species was also reduced. A genetic predisposition in the form of HLA-DQ2 thus appears to have an impact on the microbiome and could as such also contribute to the pathogenesis. This finding could be useful in determining the risk of celiac disease.¹⁶

Generally, this data indicates that a link exists between the pathogenesis and the symptoms of gluten-related diseases and the human microbiome. However, the extent to which certain bacterial species are involved in the pathogenesis of celiac disease or NCGS, and the extent to which damaged mucosa provide preferential living conditions for these bacterial species, is still unclear and requires further investigation. The initial data from Biesiekierski et al. (2013) allows us to speculate that fermentable carbohydrates are also the cause or at least an influencing factor in patients with NCGS. We therefore consider determination of the microbial colonisation of very interesting for the detection of any differences between these patients and healthy controls.

In the context of a controlled prospective study, we are therefore examining changes in intestinal microflora in patients with documented NCGS with a mixed diet, gluten-free diet and low-FODMAP diet to determine the influence of carbohydrate chains on bacterial growth and differentiation thereof. The comparison with a healthy control group and a control population with proven celiac disease is used for better differentiation between the bacterial strains that are responsible for the pathogenesis of NCGS.

The detection of a specific flora composition in patients with NCGS could represent an innovative approach for targeted probiotic treatment with few side effects.

REFERENCES

- Zopf Y. et al., The differential diagnosis of food intolerance. *Dtsch Arztebl Int.* 2009 May;106(21):359-69;
- Junker Y. et al., Wheat amylase trypsin inhibitors drive intestinal inflammation via activation of toll-like receptor 4. *J Exp Med.* 2012 Dec 17;209(13):2395-408.
- Volta U. et al., Non-celiac gluten sensitivity: questions still to be answered despite increasing awareness. *Cell Mol Immunol.* 2013 Sep;10(5):383-92.
- Galipeau HJ, Verdu EF. Gut microbes and adverse food reactions: Focus on gluten related disorders. *Gut Microbes.* 2014;5(5):594-605.
- Barbara G. et al., Mucosal permeability and immune activation as potential therapeutic targets of probiotics in irritable bowel syndrome. *J Clin Gastroenterol.* 2012 Oct;46 Suppl:S52-5.
- Zoetendal, E.G. and W.M. de Vos, Effect of diet on the intestinal microbiota and its activity. *Curr Opin Gastroenterol.* 2014.
- Ooi, J.H., et al., Dominant effects of the diet on the microbiome and the local and systemic immune response in mice. *PLoS One.* 2014. 9(1): p. e86366.
- Zoetendal, E.G., et al., The human small intestinal microbiota is driven by rapid uptake and conversion of simple carbohydrates. *ISME J.* 2012. 6(7): p. 1415-26.
- Tannock, G.W., et al., RNA-stable isotope probing (RNA-SIP) shows carbon utilization from inulin by specific bacterial populations in the large bowel of rats. *Appl Environ Microbiol.* 2014.
- Nistal, E., et al., Differences in faecal bacteria populations and faecal bacteria metabolism in healthy adults and celiac disease patients. *Biochimie.* 2012. 94(8): p. 1724-9.
- Medina, M., et al., Bifidobacterium strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients. *J Inflamm (Lond)*, 2008. 5: p. 19.
- Laparra, J.M. and Y. Sanz, Bifidobacteria inhibit the inflammatory response induced by gliadins in intestinal epithelial cells via modifications of toxic peptide generation during digestion. *J Cell Biochem.* 2010. 109(4): p. 801-7.
- Wacklin, P., et al., The duodenal microbiota composition of adult celiac disease patients is associated with the clinical manifestation of the disease. *Inflamm Bowel Dis.* 2013. 19(5): p. 934-41.
- Wacklin, P., et al., Altered duodenal microbiota composition in celiac disease patients suffering from persistent symptoms on a long-term gluten-free diet. *Am J Gastroenterol.* 2014. 109(12): p. 1933-41.
- Smecuol E, H.H., Sugai E, Corso L, Cherňavsky AC, Bellavite FP, González A, Vodánovich F, Moreno ML, Vázquez H, Lozano G, Niveloni S, Mazure R, Meddings J, Mauriño E, Bai JC., Exploratory, randomized, double-blind, placebo-controlled study on the effects of Bifidobacterium infantis naten life start strain super strain in active celiac disease. *J Clin Gastroenterol.* 2013. 47: p. 139-147.
- Olivares, M., et al., The HLA-DQ2 genotype selects for early intestinal microbiota composition in infants at high risk of developing coeliac disease. *Gut.* 2015. 64(3): p. 406-17.

The importance of the microbiota in the pathogenesis and treatment of celiac disease

The composition of the gut microbiome is influenced by many factors. The microbiota may also be a key influencer in overall health and disease outcomes.



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Man is not a single living being; he lives in a community with trillions of bacteria and other micro-organisms. The gastrointestinal tract is most densely populated: it has an estimated 100 trillion (10^{14}) micro-organisms, which are referred to in their entirety as the intestinal microbiota (formerly intestinal flora). The number of microbial cells in the intestine is ten times greater than the number of human somatic cells. Because they have about 150 times more genes than the human body, they have an enormous metabolic activity. Their metabolic products and neurotransmitters have a close interdependency with the somatic cells both inside and outside the gastrointestinal tract. They support the digestive functions, help ward off pathogenic micro-organisms and contribute to the development and maintenance of the immune system and the intestinal barrier. The intestinal barrier is a complex system that separates the intestinal lumen from the inside of the body and is composed of the following elements:

Mechanical: Epithelial cells with tight junctions, mucus

Humoral: Defensins, immunoglobulins, cytokines

Immune cells: Specific and non-specific immune cells

Muscle cells

Nerve cells

The intestinal microbiota is involved in metabolic processes and can modulate the barrier function. In addition to a balanced microbiota, another important protection mechanism for a functioning intestinal barrier is the regulation of paracellular passage through tight junctions.

In recent years, scientists have achieved great progress in the study of microbiota thanks to the application of methods of analysis from molecular biology. The 1000 types of bacteria that occur in the intestine can be divided into a total of six different subgroups. Up to 90% of the intestinal bacteria belong to the groups of firmicutes and bacteroidetes, followed by proteobacteria, actinobacteria, verrucomicrobia and fusobacteria. The majority of micro-organisms are found in every human intestine. These are referred to as the core microbiome. Every person also has a variable part. This makes up the individual microbiota. The composition and activity of the microbiota are influenced by several factors, including type of birth (vaginal or C-section), genes,





age and lifestyle. Medicines (e.g. antibiotics) and diet play an important role: factors such as quantity and type of fiber and fermented foods consumed are important. According to recent studies, the composition of the microbiota plays an important role in the maintenance of health. The different species of bacteria can have both protective and harmful effects. Certain pathogenic bacteria can, for example, cause local inflammation, weaken the intestinal barrier and increase permeation of substances including gluten.

The composition and activity of the microbiota are influenced by several factors, including type of birth, genes, age and lifestyle, medicines (antibiotics) and diet.

Celiac disease and microbiota

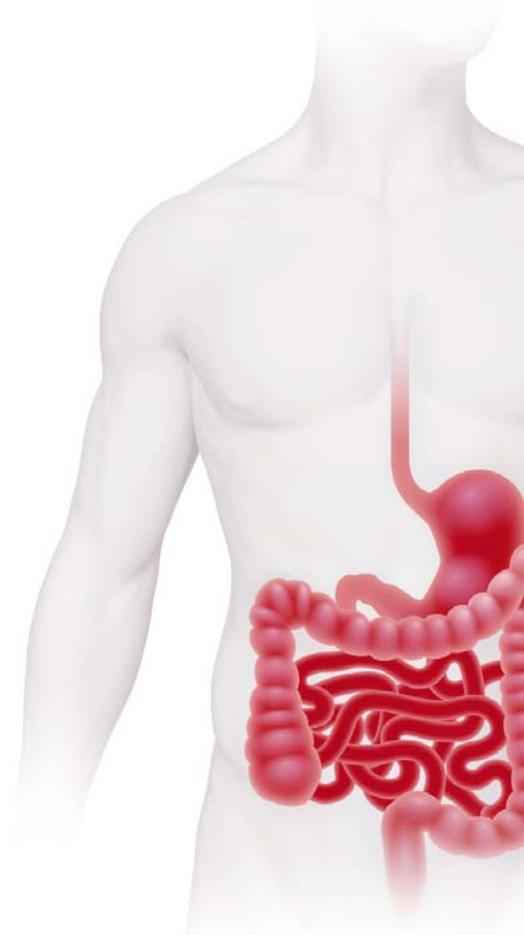
It is known that missing peptidases in the human intestine mean that gluten is incompletely digested and that gluten peptides are absorbed via Tsmall intestine mucosa. Furthermore, there is growing evidence that a change in **intestinal permeability** due to increased permeability of tight junctions (TJ) is a major factor in the pathogenesis of celiac disease. This makes it simpler for the remaining oligopeptides to be absorbed into the lamina propria and trigger the inflammation typical of celiac disease. It is still unclear whether disorders of the intestinal barrier are the primary cause or the consequence of celiac disease. However, it has been possible

to demonstrate that in the case of persons suffering from celiac disease, gliadin is a strong stimulus for the release of zonulin. This protein increases intestinal permeability by facilitating the absorption of macromolecules via TJs.

On the other hand, there is evidence that **changes in the intestinal microbiota** can lead to increased intestinal permeability and therefore may be involved in the pathogenesis of celiac and allergic diseases. There are, however, only a few studies on the role of the microbiota in the pathophysiology of celiac disease. It is believed that in the case of genetically susceptible patients, gram-negative bacteria are involved in the loss of gluten tolerance. Comparative studies between children with celiac disease and healthy control groups detected a lower number of lactobacilli and bifidobacteria in the children with celiac disease. However, it is unclear whether a change in the microbiota is the primary cause or the

Comparative studies between children with celiac disease and healthy control groups detected a lower number of lactobacilli and bifidobacteria in the former.

consequence of celiac disease. In biopsy specimens of the duodenum of untreated children with celiac disease, more gram-negative bacterial strains were detected compared to treated children and healthy control groups, which suggests a change in the microbiota as a result of this disease.





Celiac disease and probiotics

Currently, the only therapy there is for celiac disease patients is a strict lifelong gluten-free diet (GFD), which includes avoiding traces of gluten. In the long term, this is difficult for many of those affected, especially without concomitant dietary advice. Despite complaints and the risk of complications and long-term

Composition and activity of microbiota are influenced by: genetics, type of birth, age, lifestyle and intake.

consequences (e.g. malignancies, refractory celiac disease) 30% to 50% of patients do not maintain a strict gluten-free diet. Given the knowledge of changes in the intestinal microbiota in persons with celiac disease, the following studies have demonstrated that the **use of probiotics** may constitute a promising approach to concurrent therapy of celiac disease:

In 2006, De Angelis et al. examined **combination preparation** VSL#3, which contains 8 different probiotic strains (e.g. bifidobacteria and lactobacilli). They demonstrated that compared with isolated strains and other commercially available products that were tested, the combination of these probiotic strains can split gliadin peptides more effectively, i.e. gliadin peptides are easier to digest with the aid of this probiotic combination preparation.

In the case of PBMCs*, De Palma's research group (2010) was able to reduce the secretion of interleukin-12 and IFN-gamma (pro-inflammatory cytokines) under the influence of gluten in-vitro, using specific **bifidobacteria**. This observation suggests an anti-inflammatory effect of the investigated bifidobacteria.

Lindfors et al. (2008) demonstrated that the **bacterial strain b. lactis** can prevent the toxic effect of wheat gliadin on epithelial cell cultures at doses of 106 and 107 CFU** per ml, but not at 105 CFU per ml.

In a mouse model by D'Arienzo et al. (2011), a milk product with the **L. casei ATCC 9595** strain (Actimel) enhanced the intestinal barrier function and prevented the intake of gliadin in the lamina propria.

* PBMC: peripheral blood mononuclear cell
** CFU: colony-forming units





NCGS and probiotics

As yet there is no practical study on the influence of the microbiota on the pathogenesis of this new disease in the specific case of gluten/wheat sensitivity. Unlike celiac disease, an innate immune response is suspected in the case of NCGS. This is activated by gluten or wheat, but does not alter the intestinal mucosa or its permeability. However, there are indications of increased intestinal permeability in patients with neurological symptoms such as schizophrenia or autism and suspected NCGS.

INFO

Non-celiac gluten sensitivity (NCGS)

is a reaction to gluten or wheat in the absence of celiac disease or a wheat allergy. [Felber et al. 2014 (S2k-Leitlinie Zöliakie), Sapone et al. 2012, Catassi et al. 2013]

The aforementioned studies demonstrate that certain strains of bacteria aid the digestion of gliadin peptides. This may mean that patients with NCGS can as in the case of celiac disease benefit from a concomitant course of probiotics. Further studies are required for specific recommendations.

In the case of some diseases (e.g., antibiotic-associated diarrhoea, irritable bowel syndrome, ulcerative colitis, pouchitis) there are studies that demonstrate clinically relevant efficacy of probiotics. However, the underlying mechanisms are still unclear. There are currently few studies on the relationship between celiac disease and microbiota and the use of probiotics in the treatment of celiac disease. Since many effects of probiotic micro-organisms are strain-specific, evidence that has been obtained with a specific bacterial strain or preparation/product for celiac disease cannot necessarily be transferred to other strains of probiotics. Further investigation of the underlying mechanisms of action is necessary. Due to the many positive experiences and lack of side effects, the trial use of probiotic foods is recommended.

INFO

Probiotics

are live micro-organisms that offer the host a health benefit if they are taken in sufficient quantities (FAO/WHO 2002). These are special types of non-pathogenic bacteria (in particular lactobacilli and bifidobacteria) that are especially resistant to acids and therefore to a large extent survive the passage through the stomach and small intestine. Probiotics are available in the form of medicines, food and food supplements. Commercially available foodstuffs with live cultures include yogurt, drinking yogurt and mixed milk products. There are freeze-dried forms of probiotic bacteria found in products including cereals and baby food. Although the Health Claims Regulation (Regulation EU No. 432/2012 of the Commission of 16 May 2012) states that these foods may not use the term "probiotic" or claim a health effect, this does not rule out efficacy.

Probiotic bacteria temporarily settle in the intestine and produce organic acids (e.g. butyrate). This reduces the pH, which repels pathogenic bacteria. Some probiotic bacteria strengthen the intestinal barrier, e.g. by inducing the formation of a defense from mucosal cells. An intact intestinal barrier ensures that nutrients can cross the intestinal wall, but that pathogenic bacteria and toxins are repelled.



INFO

Useful websites

Research on microbiota and health:
www.gutmicrobiotaforhealth.com

Human microbiome project:
www.hmpdacc.org

German Society for Mucosal Immunology
 and Microbiome (DGMIM):
www.dgmim.de

Advice for the selection and the use of probiotics:

Choosing the right probiotic !

Effects are strain-specific

Sufficiently high bacterial count of 10^8
 and 10^9 CFU per day

Recommend products that also contain
 bifidobacteria

At the beginning of therapy: Take with
 meals and refrain from products with pre-
 biotics such as inulin and oligofructose;
 to decrease the potential of simultane-
 ous malassimilation of carbohydrate. Re-
 frain from those with lactose or fructose

Slowly increase dosage (start with $\frac{1}{2}$ the
 amount)

Duration of use:

Continuously for at least 4 to 8 weeks

Change the preparation or product if
 there is no significant effect after 8
 weeks



REFERENCES

Binns N: Probiotics, prebiotics and the gut microbiota. ILSI Europe Concise Monograph Series 2013.

Bischoff, SC (Hrsg.): Probiotika, Präbiotika und Synbiotika. Georg Thieme Verlag, Stuttgart, 2009.

Bischoff SC, Köchling C: Pro- und Präbiotika. Akt Ernährungsmed 37, 287-306, 2012.

Bischoff SC, Barbara G et al.: Intestinal permeability – a new target for disease prevention and therapy. BMC Gastroenterology 14: 189, 2014. doi: 10.1186/s12876-014-0189-7.

Blaut M: Interaktion von Ballaststoffen und Mikrobiota. Aktual Ernährungsmed 40, 43-49, 2015.

Catassi C, Bai JC, Bonaz B et al.: Non-celiac gluten sensitivity: The new frontier of gluten related disorders. Nutrients. 2013, 5, 3839-3853; doi:10.3390/nu5103839.

Catassi C, Elli L : How the diagnosis of non celiac gluten sensitivity In Vorbereitung 2015.

Charisius H, Friebe R: Bund fürs Leben. Warum Bakterien unsere Freunde sind. Carl Hanser Verlag München, 2014.

De Angelis, M, Rizzello C. G., Fassano A et al: VSL#3 probiotic preparation has the capacity to hydrolyze gliadin polypeptides responsible for celiac sprue. Biochim. Biophys. Acta. 2006; 1762: 80-93.

Drago S, El Asmar R, Di Pierro M et al.: Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. Scan J Gastroenterol. 2006; 41: 408-419.

Fasano A: Physiological, pathological, and therapeutic implications of zonulin-mediated intestinal barrier modulation: living life on the edge of the wall. Am J Pathol. 2008; 137: 1243-1252.

FAO/WHO: Probiotics in food. Health and nutritional properties and guidelines for evaluation. FAO Food and nutrition paper 85, 2002.

Felber J, Aust D, Baas S et al.: S2k-Leitlinie Zöliakie. 2014. URL: www.awmf.org/leitlinien.

Körner U, Schareina A: Nahrungsmittelallergien und -unverträglichkeiten in Diagnostik, Therapie und Beratung. 2. überarb. u. erw. Auflage. 2015 (in Vorbereitung).

Meddings J: The significance of the gut barrier disease. Gut. 2008; 57: 438-440.

Moraes L. F. de Sousa, Grzeskowiak L.M. et al: Intestinal Microbiota and Probiotics in Celiac Disease. Clinical Microbiology Reviews 2014; 27: 482-489.

Sapone A, Lammers KM, Casolaro V et al.: Divergence of gut permeability and mucosal immune gene expression in two gluten-associated conditions: celiac disease and gluten sensitivity. BMC Medicine. 2011, 9:23 (9 March 2011).

Sapone A, Bai J, Ciacci C: Spectrum of gluten-related disorders: consensus on new nomenclature and classification. BMC Medicine. 2012, 10:13 (7 February 2012).

Schuppan D, Zimmer KP: Diagnostik und Therapie der Zöliakie. Deutsches Ärzteblatt 2013; 110: 835-846.

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News



Book, enjoy, relax.

Schär Glutenfree Holidays is the new online travel portal, which allows your celiac patients to book their next trip in moments, most importantly guaranteeing safe gluten-free meals for the duration of their trip. The travel portal currently offers an attractive selection of destinations in Italy, from which you can choose your favorite trip. We are constantly adding more and more fantastic holiday locations to

suit you. Experts in gluten-free disorders and several years' experience in travel planning: Schär works with a reliable tour operator and offers beautiful accommodations. Whether you're looking for a beach holiday, a ski trip or a city break: on Schär Glutenfree Holidays you have a whole host of options to choose from. We hope you have a fantastic trip! More information: www.holidays.schaer.com

May was a busy Celiac Awareness Month with many activities for both consumers and Health Care professionals.

Dr Schar sponsored "The Supermarket Dietitians Symposium" in St Petersburg Florida in March of this year. The supermarket dietitian has a key role in the health and wellness of the American consumer. Indeed, the retail dietitian has the opportunity to highlight appropriate food choices at a crucial decision making point for most consumers. In recognition of this important role we developed several ways to partner with the retail

dietitian at several chains: Shop Rite, Hy-Vee, Weiss Markets, and Giant Eagle, just to name a few, to provide educational materials and support especially during Celiac Awareness month. In addition to provided educational materials highlight health gluten free choices we provided our Gluten Free starter kit. The kit includes samples of our bread, cookies and pasta, coupons, and a DVD of our YouTube series Better Without.



One of our events included a free one-hour continuing education webinar with Barbara Ruhs, MS, RD, LDN on "Supermarket Nutrition: Promoting Health Through Shopper Engagement". A recorder version of the webinar

is available through the Dr Schar Institute. Check out our DSI website for more details on this and other upcoming events. You will find the webinar here: www.bit.ly/1LbX2Sr

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