Interaction between Nutrition, Intestinal Flora and the Gastrointestinal Immune System

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Introduction

The intestinal mucosa is the biggest surface of the body, which is constantly in close contact with a high number of different bacteria and food antigens. Furthermore it has to absorb nutrients and in this process to differentiate between those molecules which have to pass the mucosal barrier and be taken up as nutrition and those molecules and organisms which have to be kept out to maintain the sterile condition in the organism. This is a complex function which is regulated by different layers of the intestinal barrier as well as specific transport systems.

Structure of the Intestinal Barrier

The intestinal barrier consists of the mucous layer, the epithelial cells and the intestinal immune system. The mucous layer represents a physical barrier against bacteria as well as an immunological barrier due to its high content of secretory IgA. Specific mucins secreted into the mucous layer inhibit the adherence of bacteria to the epithelial cells [1]. The mucosal epithelium is also a physical barrier against invasion of bacteria. The epithelial cells are connected by specific structures the tight junctions regulating the intercellular influx of molecules. Located between the epithelial cells are the so-called M cells, specialized cells sampling luminal antigens like intestinal bacteria and presenting them to the intestinal macrophages in the submucosal department. Furthermore, dendritic cells enter in the tight junctions between epithelial cells, also sampling antigens and bacteria from the lumen. In rodents
translocation of bacteria through the epithelial cells of the intestine has frequently been described, however apart from intestinal infections this appears to be a rare phenomenon in man.

The intestinal immune system is the third part of the barrier, which can react to invasion of bacteria with inflammation or tolerance. In the healthy organism the intestinal immune system is tolerant against commensal luminal bacteria and food antigens. Furthermore the barrier function of the mucous and epithelial cells is sufficient, so that the intestinal immune system is not activated, as has been shown by measurement of cytokine production by mucosa-associated immune cells [2].

However there are different conditions in which the barrier function is disturbed, and a temporary or chronic activation of the intestinal immune system is observed [3]. During gastrointestinal infections, in malnutrition, critical illness, and in inflammatory bowel disease, the intestinal barrier appears to be leaky allowing bacteria to adhere to the mucosa or even invade the mucosa. Consequently the intestinal immune system reacts with inflammation. In self-limiting infections this inflammation is quickly downregulated by the release of anti-inflammatory cytokines [4]. However, in inflammatory bowel disease the downregulation of the inflammation is inadequate and a continuous inflammatory activation of the immune system results. This could be due to a genetically mediated disturbance of the intestinal barrier function allowing adherence or even translocation of bacteria without infection with pathogenetic strains [5]. A similar situation might also develop during critical illness and even during malnutrition-associated damages to the intestinal barrier. In vitro studies demonstrated different effects of different bacterial strains.

The functional assessment of the intestinal barrier is difficult due to its complexity. However so-called permeability tests using different sugars or polyethylene glycol (PEG) have been developed, which reflect the integrity of barrier function [6]. These tests seem to reflect clinically relevant changes in barrier function, however whether they allow conclusions on bacterial translocation is still an open question.

**Mucosal Flora in Health and Disease**

Although the concentration of bacteria in the human colon is about 10^{12}/ml, it appears that the colonic mucosa in healthy persons is sterile due to the excellent barrier function of the mucous layer. In endoscopic biopsies from the human colon of control persons none or only very few bacteria have been found [5]. In contrast in gastrointestinal infections and in inflammatory bowel disease, a high number of bacteria is adherent to the mucosa. Thereby inflammatory bowel disease might be a model for a disturbance of the intestinal barrier and might offer the opportunity to study immune reactions to...
chronic barrier leakiness. It appears, however, that the concentration and the composition of the mucosal bacteria in inflammatory bowel disease is different than in all other conditions [5].

Since the early studies on bacterial translocation and the development of the gut sepsis hypothesis, the question was raised whether malnutrition might lead to similar defects in the intestinal barrier and thereby also allow bacteria to adhere or invade the mucosa and lead to an inflammatory reaction or to an infection. Although bacterial translocation appears to be an infrequent event in man, it has been shown by permeability tests that intestinal permeability increases with the degree of malnutrition [7]. This increased permeability is also accompanied by HLA-DR expression, indicating that immune activation is taking place [7]. It is noteworthy that such findings have only been reported in patients and not in healthy malnourished people. In contrast, several articles have shown a reduction in intestinal permeability in underweight healthy persons such as anorectic women [8]. It might be concluded that malnutrition and a second insult such as disease stress is necessary to disturb the intestinal barrier and allow bacteria to adhere. This hypothesis is supported by data from animal experiments showing a deterioration in the mucous layer, followed by adhesion of bacteria to the intestinal epithelium of rats exposed to psychic stress [9].

Effect of Substrates and Bacteria on Barrier Function and Intestinal Flora

These changes seem to be influenced independently by the nutritional situation and the stress factor. Alscher et al. [10] demonstrated that injection of endotoxin was followed by an increase in intestinal permeability, compared to sham injection, in fed animals. In fasted animals, however, intestinal permeability was also increased after sham injection. By the combination of both endotoxin injection and fasting, the permeability increased significantly more than in fed endotoxin-injected animals. This experiment showed that both starvation and stress (endotoxin injection) independently disturb the intestinal barrier.

The mechanism of this barrier disruption was studied by Spitz et al. [11]. They demonstrated a significant reduction in the secretory IgA concentration in the mucous layer by injection of dexamethasone. This effect was further increased when animals were starved. They also showed that bacterial adherence correlated very well with the IgA concentration in the mucous layer.

It appears therefore that both malnutrition and disease stress impair the intestinal barrier; however, stress and malnutrition have a potentiating effect.

From these data it was concluded that nutrition in severely ill, and also in other malnourished patients, is essential to maintain the intestinal barrier and avoid gut-derived sepsis or complications. This hypothesis is supported
by a number of studies showing an improved barrier function and reduction of complications in patients with enteral nutrition as compared to starved patients. However, the question has to be raised, which route and composition of nutrition might be optimal to maintain the intestinal barrier and at the same time induce a positive reaction of the gastrointestinal immune system rather than continuous inflammation.

Luminal nutrition clearly appears to be better in maintaining the integrity of the intestinal mucosa than parenteral nutrition. Luminal nutrition leads to proliferation of the intestinal epithelium as has been shown in a number of studies. However, it is not only the mucosal integrity which is influenced by nutrition but also the composition of luminal flora. Schneider et al. [12] demonstrated in healthy controls and patients that both total enteral nutrition and total parenteral nutrition were followed by changes in the composition of the fecal flora. While the concentration of anaerobes was reduced by both total enteral nutrition and total parenteral nutrition, aerobes were only reduced by parenteral nutrition [12]. Such changes in the barrier and the composition of the intestinal flora appear also to be followed by immune reactions. Ikeda et al. [13] showed that the expression of MADCAM-1, an adhesion molecule in Peyer's patches, was significantly reduced by parenteral nutrition as well as by elemental diets and intragastric application of parenteral nutrition.

In addition to the substrate effects which were considered to be mediated by gut hormones, probiotics seem to have a profound effect on the intestinal barrier as well as the intestinal immune system. In the coculture model in which immune cells are cultured under a layer of epithelial cells, it has been shown that the addition of bacteria to the epithelial cell compartment does induce secretion of different cytokines by the immune cells, which do not have direct contact with the bacteria [14]. This raised the question whether changes in the luminal flora of the intestine might also have effects on the intestinal immune system. Actually Roller et al. [15] demonstrated that IgA secretion into the mucous layer as well as systemic IL10 secretion were increased by feeding pro- and prebiotics as well as symbiotics to animals. In contrast, the oxidative burst in intestinal immune cells was reduced. Some recent studies demonstrated that several probiotics have profound effects on the intestinal immune system when fed to IL-10 knockout animals [16]. These animals have an increased tumor necrosis factor (TNFα) secretion in basal conditions as well as after lipopolysaccharide (LPS) stimulation. By prefeeding them with probiotics, the TNFα production was reduced in both the basal state as well as after LPS stimulation. This effect apparently cannot only be induced by whole bacteria but also by bacterial DNA. Rachmilewitz et al. [17] showed that feeding of a 22-basepair-long bacterial DNA to animals with experimental colitis could significantly reduce disease activity, weight loss and inflammatory changes. Similar effects have been demonstrated by Jijon et al. [18] in HT29 cell cultures. They were able to demonstrate that
bacterial DNA inhibited proteasome activity, interferon-γ secretion and IL-8 secretion in those cells after infection with *Salmonella typhimurium* DNA [18].

**Clinical Consequences**

Of course, the question has to be raised as to whether the above-mentioned data are relevant in clinical situations for patients. There are few data on the effect of enteral nutrition on the intestinal flora or bacterial translocation in man. However Sedman et al. [19] showed no difference in the frequency of bacterial translocation in patients whether they were fed enterally or not. The clinical effects of enteral nutrition on outcome are limited and furthermore cannot clearly be attributed to an effect on the intestinal barrier or flora.

A number of publications, however, have recently shown that addition of bacteria or bacterial DNA to nutrition has a profound impact on the clinical course, infections and diarrhea in patients. Treatment with *Escherichia coli* Nissle maintains remission in ulcerative colitis as well as 5ASA medication [20]. The addition of *Saccharomyces boulardii* to enteral nutrition of critically ill patients significantly reduced the incidence of diarrhea [21]. Feeding of lactobacillus reduced the number of infections after liver transplantation as compared to treatment with antibiotics as well as enteral nutrition without probiotics [22, 23]. This appears to be an especially interesting study since it randomly compared patients who were fed standard enteral nutrition with patients who received the lactobacillus, indicating that the effect of the probiotics outweighs the effect of substrates. Also in acute pancreatitis the addition of lactobacillus to feeding significantly reduced the numbers of septic complications and positive aspiration cultures [24].

Therefore, the question that must be raised is whether enteral nutrition should be supplemented by probiotics and/or bacterial DNA in patients on long-term nutrition who run a higher risk for infection. However, although the positive effect of some probiotics has been demonstrated in several disease states, it is not clear which probiotic or which bacterial DNA would be most suitable as a general supplement to enteral nutrition. In vitro experiments as well as clinical studies have shown that not all probiotics act similarly in all situations. Care has therefore to be taken not to consider all probiotics equally. While positive effects have been demonstrated in different conditions with *E. coli* Nissle and VSL3 and some lactobacilli in some situations as described above, one study showed a negative effect with *Lactobacillus* GG in postoperative Crohn’s disease. The patients receiving *Lactobacillus* GG had more relapses than the control patients [25]. Before recommending probiotics, careful studies should therefore be performed.

However, due to the impressive effects in some studies, it appears to be quite important to further investigate the interaction between bacteria, the
intestinal barrier and intestinal immune system to improve the composition of enteral nutrition.

In summary a strong effect of both substrates as well as bacteria and bacterial DNA on the intestinal barrier and immune system has been demonstrated, and this appears to be clinically relevant. Although we do not yet know all the conditions in which such an effect might be crucial for the outcome of the patient, studies are essential to investigate which composition of enteral nutrition is advisable in which situation.

References


Discussion

Dr. Cynober: I was interested by the provocative data you showed us about the decrease in the lactulose mannitol test in patients suffering from anorexia nervosa, and a lot of data you show are based on this test. This test is based on the variations in lactulose and mannitol, which lead to variations in the ratio. I would like to know what your feeling is about this test and the interpretation and the degree of confidence we can achieve with this test?

Dr. Lochs: The major questions are, if permeability tests are reproducible and if they reflect clinically relevant changes of the intestinal barrier. The first question has been investigated and showed that the lactulose mannitol test is nicely reproducible in the same person. Regarding the second question, some studies e.g. the study by Welch et al. [1] showed a good correlation of intestinal immune reactions to the permeability test. Welch showed that HLA-DR expression and other inflammatory signs correlated with increased lactulose permeability index. Similar correlations have been shown with other permeability tests like the polyethylene glycol test. It has not been demonstrated that permeability tests correlate with bacterial translocation in humans, however this seems to be a rare event. Furthermore it is not clear if the translocation of whole bacteria is necessary to induce the complications attributed to increased intestinal permeability or if adherence of bacteria to the intestinal mucosa with initiation of a proinflammatory immune reaction and translocation of bacterial toxins like lipopolysaccharide are enough to induce infectious complications. In summary right now the permeability tests are the best parameter we have to investigate disturbances of the intestinal mucosa.

Dr. Schiffrin: I would like to ask you whether you see some differences in the studies that you have shown and those of Jijon et al. [2] with bacterial DNA from VSL3 on anti-inflammatory activities, and the study that has been reported by Rachmilewitz et al. [3] involving CpG motifs-Toll-like receptor-9 reactions and also anti-inflammation? Do you need to be very specific in the bacterial DNA that is interacting with the pattern recognition receptors to start this anti-inflammatory activity?

Dr. Lochs: I did not want to go into that because I thought, you would deal with it. The studies up to now used very different bacterial DNA or even parts of it like the study of Rachmilewitz et al. [3] who used a 22 base-pair CpG. There is evidence that certain motifs in the bacterial DNA seem to be responsible for the reaction with the Toll-like receptor-9. These motifs seem to be present in the DNA of different bacteria. This probably also explains the data on the reduction of atopia in children: if you need the mothers during pregnancy with probiotics their children get these motifs as well,
and this creates a different immune reaction. However, there might be much more DNA motifs which induce different immune reactions we have not yet investigated.

**Dr. Van Gossum:** You have shown that malnutrition may alter intestinal permeability, but is it really due to malnutrition itself or to the fact of not being correctly fed? That is the first question. The second question is about the role of intestinal content because there are many reports showing that, for example in Crohn's disease, with a stoma. If one takes biopsies from a segment distal from the stoma there is a decreased immune reaction. So what is the role of intestinal fluid, and not only the feeding itself? The third question, you didn't talk about any specific nutrients, such as the role of glutamine, for improving the intestinal permeability. Are there some data about that, could you speculate on that?

**Dr. Lochs:** I absolutely agree with Dr. Labadarios that malnutrition by itself is not enough to damage the intestinal barrier; there needs to be something else and apparently this is some inflammatory stimulus, and bacteria are essential for this inflammatory stimulus. This has been shown in patients, as you mentioned, if a stoma is created then these inflammatory changes are not found distally from the stoma. In animal experiments, if the animals are bred sterile they do not develop inflammation, but as soon as some bacteria are added to the environment then inflammation is created. Of course there are a number of substrates influencing the intestinal barriers but this will be covered in the next session. There are experiments on glutamine, on glycine, on other substrates influencing the barrier, and I think this is one of the major things we can do with nutrition, but I do not want to take this into my talk because I am sure that this will be coming.

**Dr. Labadarios:** I am interested in the study on the stressed rats you referred to and the apparent adhesion of microorganisms on the mucosa. You know there are stressed humans as well. The recent literature [4], actually indicates that stress is a significant component of the irritable bowel syndrome (IBS). Do you have any thoughts on this?

**Dr. Lochs:** It was in exactly this context that Soderholm et al. [5] conducted their study. They were looking for a model of IBS and found that these rats, if some stress was produced, developed neurological changes in the intestine similar to IBS, and then they found that bacteria adhered. Their hypothesis is that this happens also in men. Now we looked at the mucosa of IBS patients, and in fact a good number of IBS patients have bacteria on the mucosa similar to inflammatory bowel disease patients, so obviously in a good number of patients there is a breakdown of this barrier. If you look at other parameters what do you find histologically? Most of these patients have microscopic inflammation, so this fits very well. Hospitalization is usually a stress to the patient, and this might be some reason why the patients, even without malnutrition, do develop problems with their intestinal barrier when they get into hospital.

**Dr. Thomas:** Can you comment on the current thinking about the intestinal barrier and increased infection complications even in acutely ill patients? That seems to have gone back and forth in the literature over the last several years about the concept of early feeding, even hypocaloric feeding, decreasing septic complications.

**Dr. Lochs:** I think it is well established that feeding is really decreasing. The question which is raised now is how does the food have to be composed and do we need to add either probiotic bacteria or at least CpGs of probiotic bacteria? There are very nice studies comparing standard enteral nutrition versus probiotics on infections after liver transplantation, and during acute pancreatitis the probiotics do better than standard enteral feeding. So it seems that it is not just the food in the lumen, it has to be composed in a certain way to create certain immune responses.

**Dr. Elia:** Do you have any information about the effect of starvation and nutrition on the barrier function of other epithelial surfaces such as the respiratory epithelium or genitourinary epithelium? Are there common overacting concepts?
**Dr. Lochs:** I don’t know but that sounds like a very interesting question, if that is regulated in a similar way, I have no information about that.

**Dr. Lesourd:** Regarding the data you presented about the numbers of bacteria that link to the mucosa with a different regimen, it seems logical to me that when you re-feed just with food you probably have less adherence of the bacteria. But why is there so much difference between the two tube feedings, with percutaneous endoscopic gastrostomy and nasal tube feeding?

**Dr. Lochs:** This study was not an intestinal bacterial adherence. It was just in the oropharynx that pathogenic bacteria were found, not commensal but pathogenic bacteria. Apparently if the patient eats you find much less pathogenic bacteria in the oropharynx as compared to tube feeding. The interesting thing to me was that the tube which goes through the naso-oropharynx into the stomach causes the highest number of pathogenic bacteria being there, not adhering. The surface was not looked at, a swab was taken to look at the composition.

**Dr. Endres:** I enjoyed your talk very much; especially when you spoke about whether probiotic bacteria have to be alive. We know that from strain to strain it is different. It has been demonstrated with various bacteria that they can have some effects upon the immune system even after heat inactivation, whatever the effective agent may have been, e.g. cell membranes, DNA, etc. Thus not to find living probiotic bacteria in the feces of patients having received probiotics does not necessarily mean that there has not been a functional effect. What counts is the clinical effect which has to be proven. Would you agree that probiotic bacteria having to be alive is questionable nowadays?

**Dr. Lochs:** I agree, I think it is questionable. However, there seem to be different effects that can be created with live bacteria and with DNA. For example live bacteria change the composition of the mucins in the mucin layer. If some lactobacilli are added, for example mucin-5, which has the ability to avoid adherence is increased in comparison to other mucins. So it seems that the DNA or the CpGs are important for some immune reactions but live bacteria seem to interact with the mucosa and create other effects. We know very little about that but we do know that there are some effects dependent on live bacteria.

**Dr. Arnaud-Battandier:** I would like to know about food antigen when a patient in the intensive care unit or the ward has a leaky gut, what about gluten? Do you think gluten can provoke an immune reaction and then have a definitive consequence?

**Dr. Lochs:** There are two nice reviews about this question. The problem with gluten is that HLA DR2 is needed because gliadin has a form that fits in the HLA DR2 to be ideally presented. Therefore people who do not have HLA DR2 do not respond to gluten as HLA DR2-positive people do. But if you reduce the question and ask how the development of celiac disease is explained, then the hypothesis is that by some infection you reduce the barrier at some stage and then gluten enters and if the person is HLA DR2 positive the immune reaction starts. Does that answer your question?

**Dr. Arnaud-Battandier:** Yes partly. So this doesn’t happen in the majority of the patients, only in the DR2-positive patients?

**Dr. Lochs:** Only in the genetically predisposed patients, that is what we know right now.

**Dr. Van Gossum:** You mentioned malnutrition combined with any kind of stress for alteration of the intestinal barrier, but what about the role of drugs such as NSAIDs that elderly patients are taking a lot? We know that they could also alter the intestinal barrier.

**Dr. Lochs:** You are absolutely right. Some drugs constitute a classical stress to the intestinal barrier. This has been shown for NSAIDs but also for other medications. There are very nice experiments showing that, for example, antibiotics damage the barrier really profoundly. So there is a double effect with antibiotics: on the one hand you damage the barrier and on the other hand you keep the bacteria out.
Dr. Bowling: The gut is full of millions and millions of bacteria, most of which are commensal. How can you tell when you just do sampling if you are looking at commensal or pathogenic bacteria? We all have *Escherichia coli* in our gut, but what makes *E. coli* become pathogenic in healthy individuals, and is that relevant here?

Dr. Lochs: For research you can look at it using different probes. We use about 80 different probes for different species. The interesting thing is how the immune system knows this and it seems to be very nicely regulated. We are all tolerant to our own flora, and interestingly if the flora from a healthy person is brought into contact with the immune cells of another healthy person, the immune cells will be activated by the flora of the other healthy person but not their own flora. So there is a learning of tolerance in early childhood and whenever a foreign antigen arrives, it then activates the immune system. Of course there are bacteria which invade the cells and are immediately recognized as pathogenic. If bacteria are presented via the M cells they usually create tolerance, but if bacteria invade via the epithelial cells or via the tight junctions they create an immune response with secretion of IL1 or IL6.

Dr. Morley: One of the things I have always struggled with is the intestinal barrier. Is there another group of people besides the malnourished who have a very leaky intestinal barrier e.g. diabetics who are usually very obese and often over eat? I am wondering how you put the two very different groups or disparate groups together when you come up with theories of how this works?

Dr. Lochs: You are right, this is an interesting question, but diabetics are prone to infections and we have never really known why. Perhaps this disturbed intestinal barrier is contributing.

Dr. Armstrong: My question probably reflects ignorance. I have always had difficulty understanding the concept of a leaky gut as a cause of problems. It is actually a pathogenic mechanism or an indicator of a problem going on. The reason is that it strikes me that the gut always has bacteria as we heard; it is always repairing itself, and it is always potentially leaky. There are numerous things that can alter gut permeability: drugs, viral infections, celiac disease, a whole lot of things that can alter permeability which do not necessarily seem to lead inexorably to the sort of manifestations and diseases that we have heard of, and it may therefore be changes in gut permeability or other processes. We may be barking at the wrong tree by trying to improve or reduce permeability in some of the conditions that we heard about. I would be interested in your comments.

Dr. Lochs: I agree there is not enough evidence to show a causal relationship. However, it goes very nicely in parallel; so the leakiness of the gut goes in parallel with the increased complications and the tightening of the gut goes in parallel with a reduction in complications and infections. So it is the best parameter we have right now to look at, but I absolutely agree, it is not clear if that is the first step or if it is only one step in a series.

References