

Mucosal Flora in Crohn's diseases and ulcerative colitis

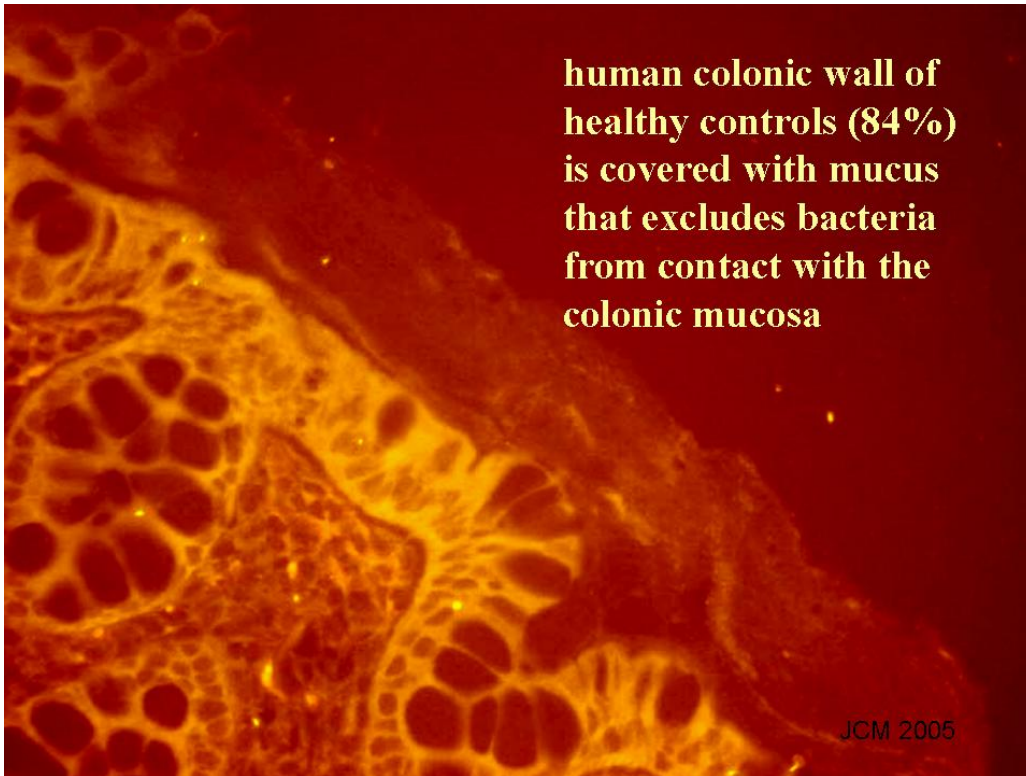
Alexander Swidsinski and Vera Loening-Baucke

Until the late 19th century, microbes were the major cause of death in humans. Ironically, the infectious nature of most diseases was not recognized. There was little treatment available and was mostly focused on strengthening the general immunity. This changed with the identification of pathogens by Pasteur and Koch. The knowledge about microbial infections quickly expanded and reduced dramatically the impact of pathogens on humanity. However these advances referred exclusively to mono-infections. The situation in medicine stayed unchanged for polymicrobial diseases. A polymicrobial involvement is suspected in caries, pharyngo-tonsillitis, vaginosis, inflammatory bowel disease (IBD) and colon cancer; Research data on coronary heart disease, stroke and autoimmune diseases suggest that pathogens trigger the illness, however positive proof and understanding of causality are lacking. The current medical strategies for handling of these diseases are therefore directed toward managing symptoms, conditioning immunity, and the search for the genetic background.

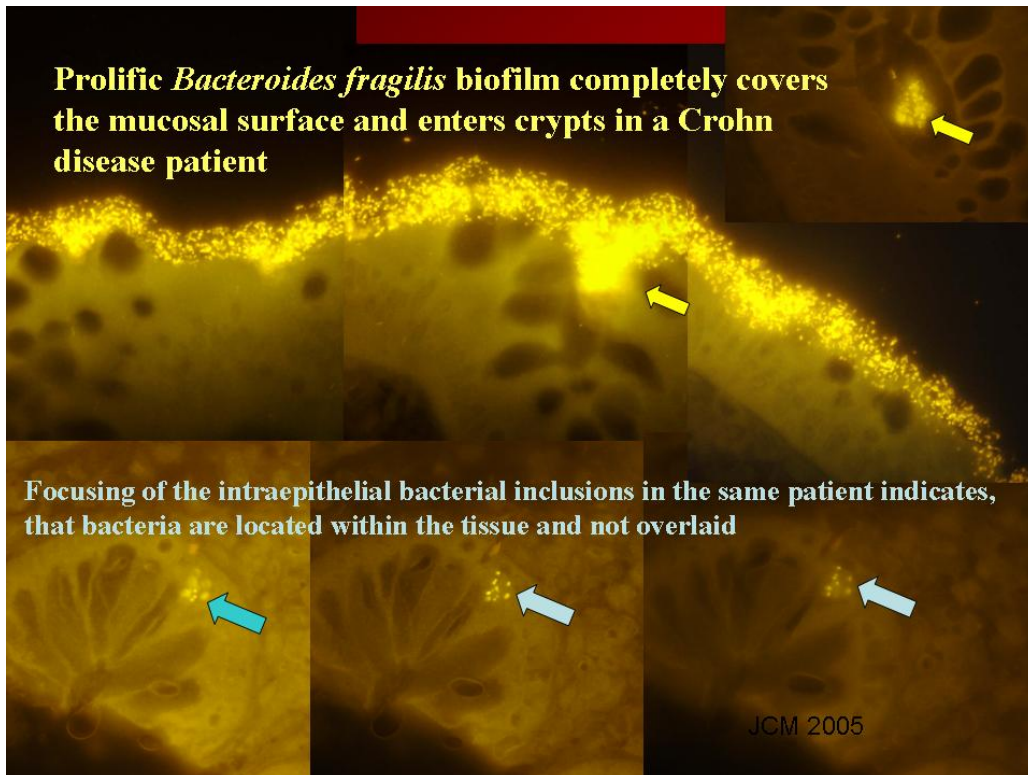
Most of the polymicrobial infections are probably not recognized. The reason for this unawareness is a lack of appropriate instruments. Since Robert Koch and Li Pasteur, we define a pathogen as a microorganism, which is isolated from a diseased person, absent in a healthy person and causes a disease upon transfection to a healthy person. The value of Koch's principles is however limited in case of polymicrobials. The polymicrobial community can not be grown elsewhere by transfection of single strains and the investigation of isolated microorganisms does not explain how the polymicrobial community functions or why it can grow under conditions, which are deadly for each of the constituents. We have to monitor their composite structure in relation to propagation, growth and response to environmental challenges in order to understand polymicrobial infections. Unfortunately we can not currently propagate most of the polymicrobial communities in culture. The environmental microbiologists, however, developed different tools to analyze microbiota in situ.

One of such methods is the ribosomal RNA fluorescence in-situ hybridization (FISH). Depending on metabolic activity, each bacterial cell contains 10^4 - 10^8 ribosomes. Each ribosome includes a characteristic RNA molecule. Some areas of the ribosomal RNA are strain-specific, other are more universal. Based on sequences of the ribosomal RNA, probes can be developed and synthesized to bind specifically to organisms of interest. Using probes labelled with different fluorescent dyes, we can simultaneously visualize different types of microbes within complex communities. Over 100 FISH probes are currently available and allow explicit analysis of intestinal bacteria. It is not necessary that the bacteria are alive at the time of the investigation. The FISH investigations can be carried out any time and repeated, if the material is properly fixated.

We have investigated biopsies from more than 10.000 patients and controls using FISH in order to search for microbial roots of inflammatory bowel disease. The most striking finding in these studies was a lack of contact between intestinal bacteria and the mucosa in normal subjects. In most healthy controls (84%), the intestinal wall throughout the ileum and colon was covered with mucus, which hindered bacteria from contacting the mucosal surface.

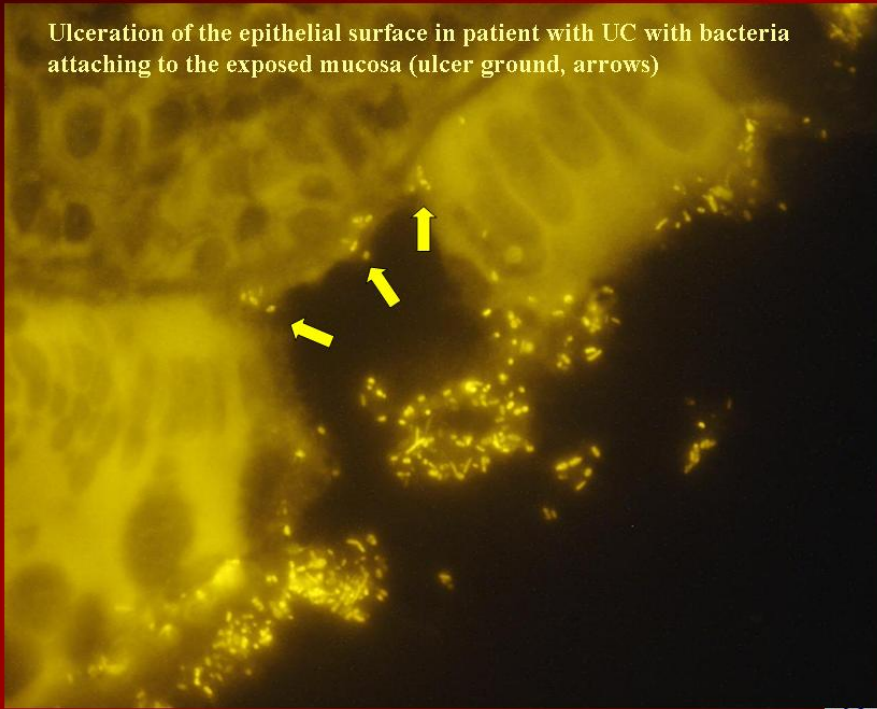


In contrast in nearly all patients with IBD we found a dense coating of bacteria on the intestinal surface. Bacteria adhered to epithelial cells, entered crypts and were sporadically found within cells (blue arrows). The intracellular bacteria were located mainly at the bottom of the crypts, which were in most cases empty of bacteria, but not in the columnar epithelium, which directly contacted the dense masses of bacteria.



Although adherent bacteria were present in nearly all (94%) IBD patients who had not been treated with antibiotics, the highest concentrations of mucosal bacteria were found, not in the inflamed regions of the intestine, but in less or macroscopically non-inflamed regions.

Ulceration of the epithelial surface in patient with UC with bacteria attaching to the exposed mucosa (ulcer ground, arrows)



Replace “UC” bei “in a patient with ulcerative colitis. Bacteria attach to the exposed....”

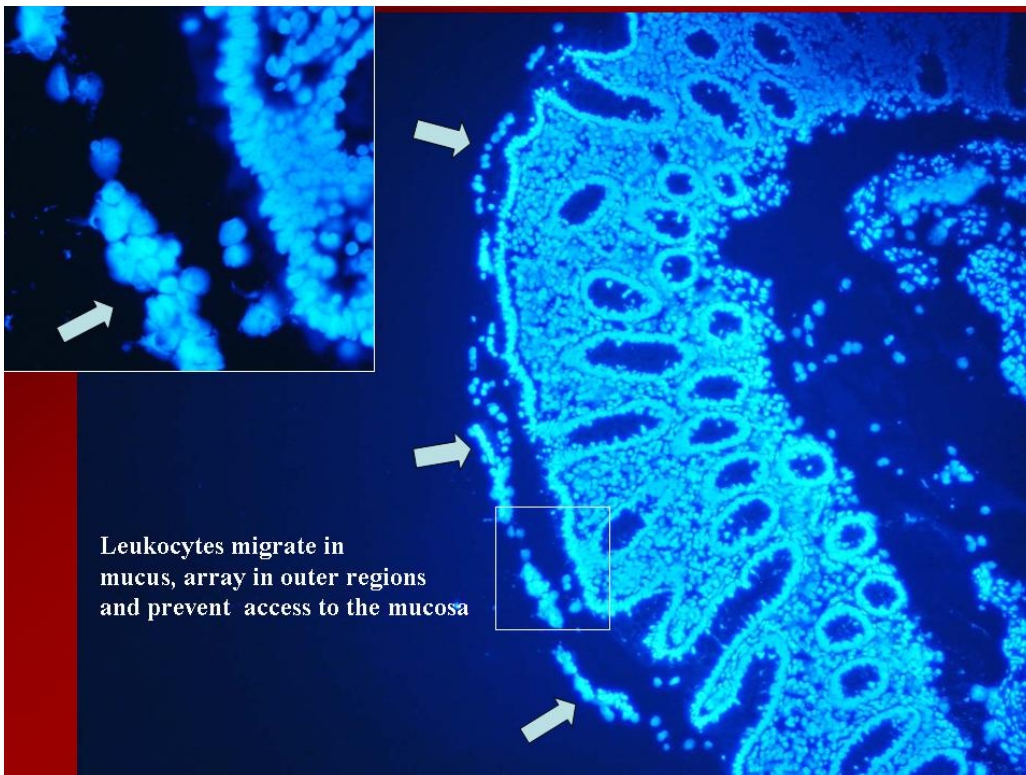
In inflamed regions, the bacterial concentrations were reduced due to leukocytes that migrated in the outer regions of the mucus, either preventing access to the mucus layer or exerting antimicrobial effects.

Gelöscht:

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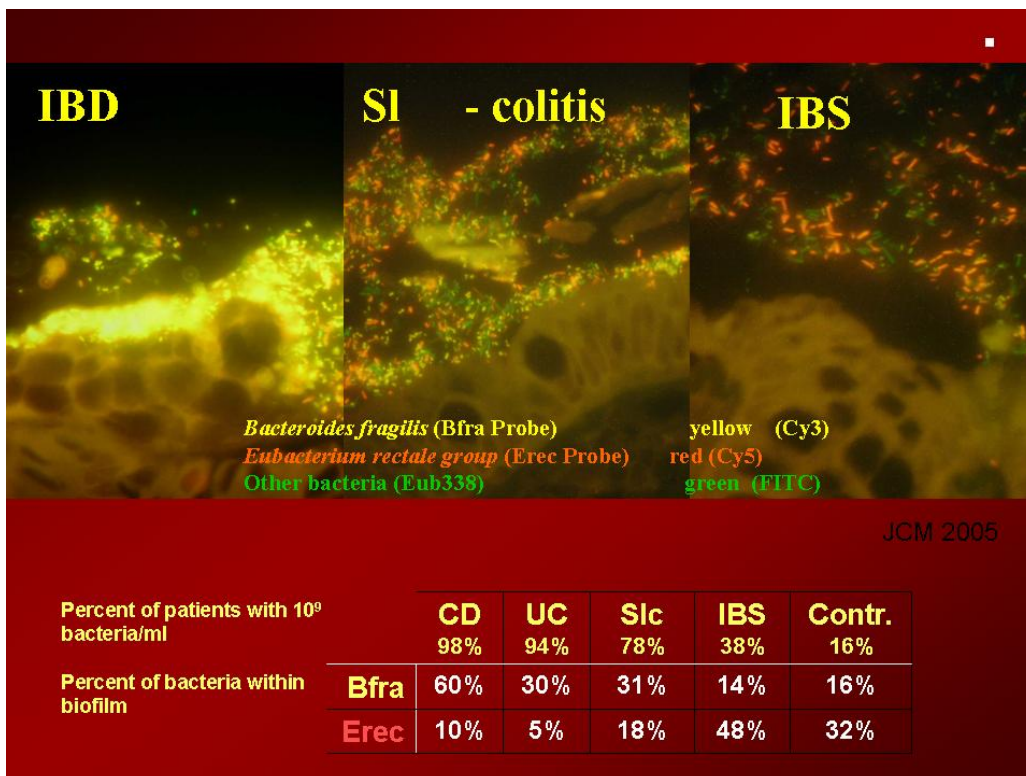
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Despite high concentrations of leukocytes and reduced numbers of bacteria in the mucus of inflamed gut segments in IBD patients, some of these bacteria reached the intestinal wall leading to development of ulcers, fissures, abscesses and deep tissue infiltrates.

The bacterial adherence to the mucosa was not IBD specific. Bacterial concentrations of 10^9 cells/ml or higher were found in nearly all patients with IBD but also in patients with self-limiting colitis (SI-colitis), celiac disease, HIV enteropathy, 62% of patients with acute diarrhea, 52% of patients with diverticulosis, 45% of patients with carcinoma or polyps, and in 38% of patients with irritable bowel disease (IBS). However, the mean density of mucosal bacteria was significantly lower in groups without intestinal inflammation and the composition of the biofilm was different.



Bacteria of *Bacteroides fragilis* group and *Enterobacteriaceae* were responsible for >60% of the biofilm mass in IBD, but only for 30% in self-limiting colitis. In contrast, bacteria that positively hybridized with the Erec (*Eubacterium rectale*) and Fprau (*Fecalibacterium prausnitzii*) probes accounted for >50% of the biofilm in IBS patients, but only for <30% of the biofilm in IBD. Bacteria other than *Bacteroides*, *Enterobacteriaceae*, *Fecalibacterium prausnitzii* or *Eubacterium rectale* were predominant in self-limiting colitis.

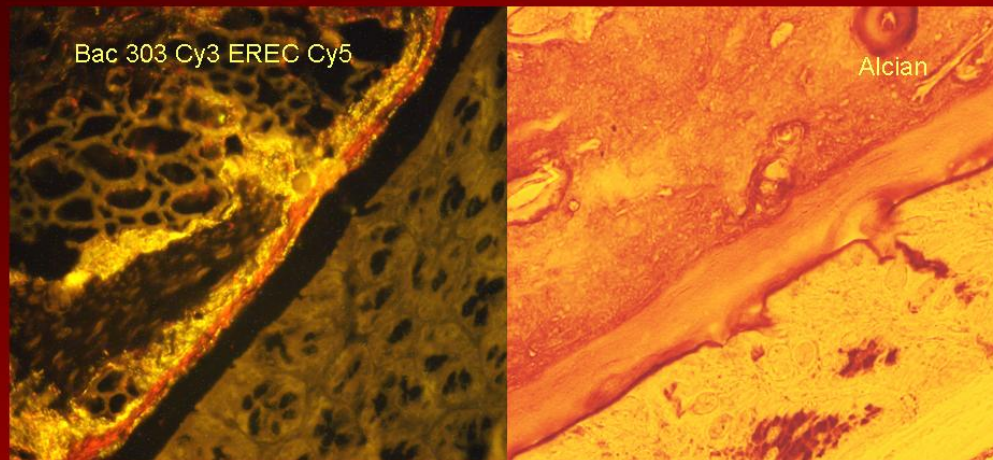
Human bowel is cleaned before the colonoscopy. To investigate the composition of the mucosa adjacent bacteria throughout the intestine without cleaning, we studied sections of whole mice intestine. For better understanding and comparison of the findings, only microphotographs hybridized with the same set of probes are shown throughout the presentation. Thus, in the following figures *Bacteroides* is Cy3-stained and appears yellow, *Eubacterium rectale* - *Clostridium coccooides* group (EREC probe) is Cy5-stained and has red fluorescence, and all other groups are FITC-stained and appear green. The colours are shown as they appear through the microscope or camera. Micrographs are not manipulated.

Small intestine of a healthy wild type mouse contains no bacteria which can be definitively detected by FISH, corresponding to a bacterial concentration of less than 10^6 bacteria/ml. The few microorganisms found were heterogeneously composed, random, and without signs of adhesion or contact with the intestinal wall. All of them were separated from the colonic wall by a mucus layer.



The large intestine of the healthy wild type mouse contains a highly concentrated mass of bacteria. In the distal colon a distinct mucus gap devoid of bacteria completely separates the colonic wall from the highly concentrated faecal biomass. The width of the mucus layer increases progressively from the middle to the distal colon. No bacteria contact colonic wall. The same segment stained with alcian blue demonstrates that the gap is indeed filled with mucus.

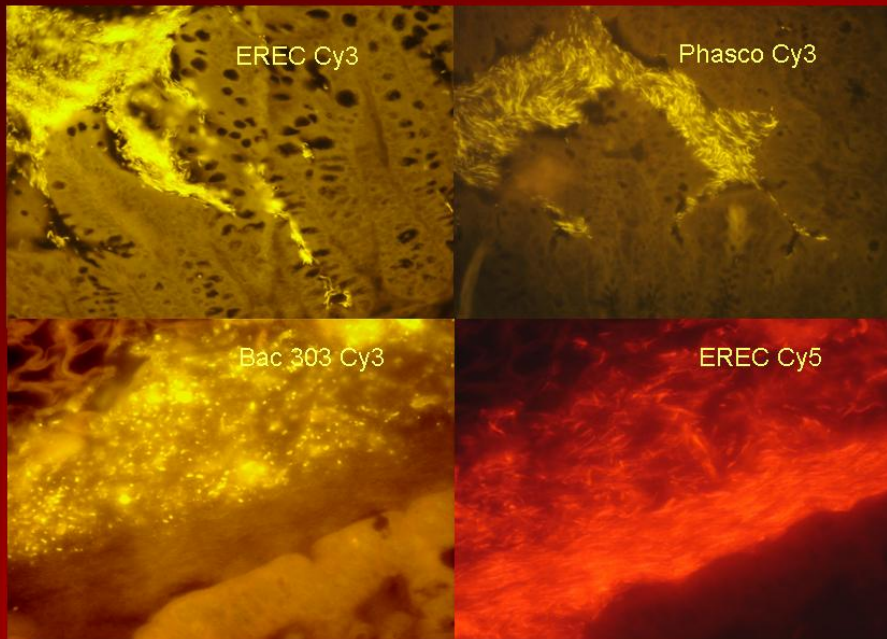
The situation in the distal colon of the mouse is obviously identical to the situation in human.



The mucus completely separates mucosa from feces in distal colon of mice similar to the situation in man

...from faeces in the distal colon...

In the proximal colon of the healthy mouse the situation was completely different to that observed in healthy human. Luminal bacteria directly contact the colonic wall in the healthy mouse. However, this contact is selective, while *Eubacterium rectale* contacts mucosa and enters crypts in large numbers, *Bacteroides* is separated from the colonic wall. The differences in arrangement of bacterial groups are especially obvious in multi colour FISH visualizing different species in different colours within the same microscopic field. *Eubacterium rectale* are condensed in extremely dense mats adjacent to the mucosa, which are clearly demarcated from the rest of the faeces and *Bacteroides*.



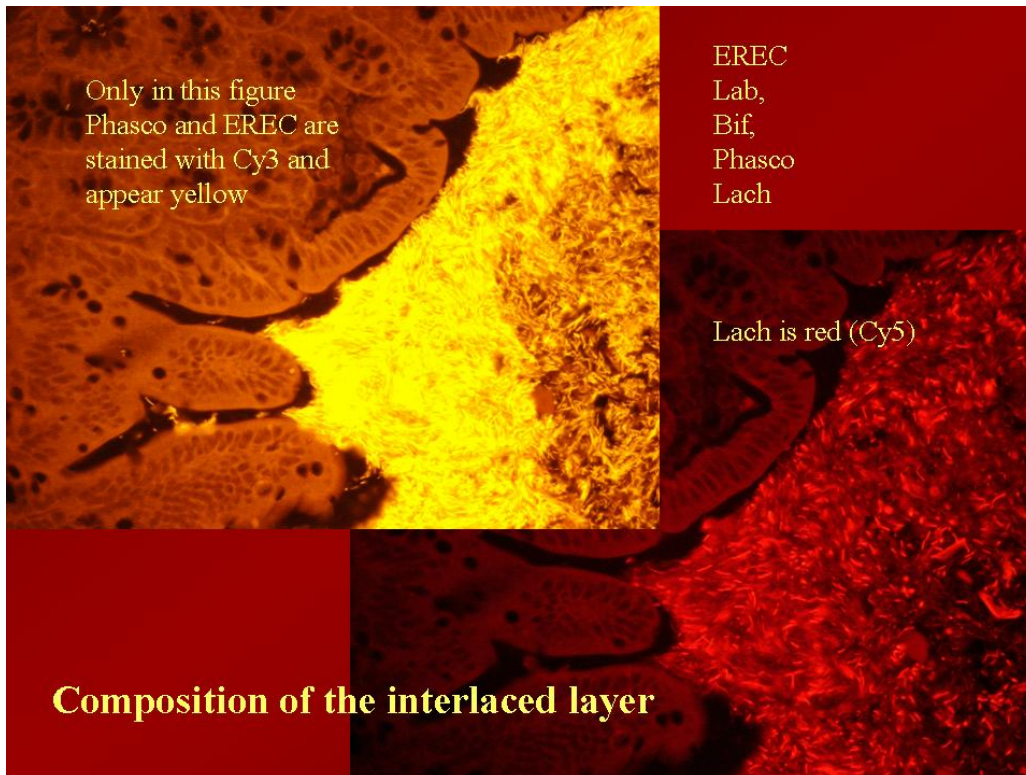
The separation of bacteria in the proximal colon of mice is selective, EREC enters crypts, *Bacteroides* has no contact with colonic wall

The first impression is that *Eubacterium rectale* hinders *Bacteroides* from contact with the mucosa. This impression is wrong.

Bacteria which were separated from the colonic wall were represented by *Bacteroides*, *Enterobacteriaceae*, *Clostridium difficile*, *Veillonella* and other groups. Typical for these groups was not the biochemistry or phylogenetic relationship, but the bacterial cell morphology of short coccoid rods.



Bacteria contacting the proximal colonic wall in mice were also represented by different groups belonging to *Eubacterium rectale* (EREC), *Bifidobacteriaceae* (Bif probe) *Lactobacillus* and other groups. Common for these bacteria was their shape of long often curly rods.

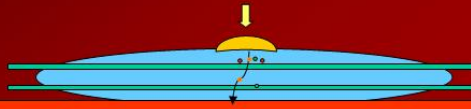


The bacterial shape is important for bacterial movements. Short rods are equipped with multiple pili. Pili enable movements in a watery environment but not in slime. Short rods have additionally flagella, which like propeller allow them to move through slim. Long curly rods use complex body movements to screw through gels of high viscosity, but are of no use in water. We testet the mobility of intestinal bacteria in vitro with a viscous gel layer containing different additives enclosed between two cellulose membranes which were placed on blood agar to attract bacteria as shown in the figure below. The viscosity of the gel was adjusted by varying the concentration of agarose from 0.5% to 2%. Mixtures of enteric bacteria were overlaid onto the simulated mucus. After 28 hours of anaerobic growth, membranes were fixed, sectioned, than examined by FISH using rRNA-directed oligonucleotide probes that identify specific groups of bacteria. Movement of bacteria through the simulated mucus were quantified.

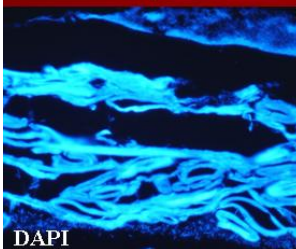
Mucus simulation in vitro

Native mix of fecal bacteria

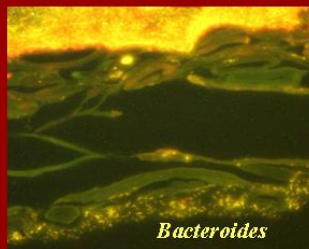
Two layers of cellulose covered with a LB-agarose gel of variable viscosity



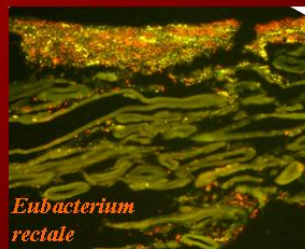
Blood agar plate



DAPI



Bacteroides



Eubacterium rectale

Examples of mobility

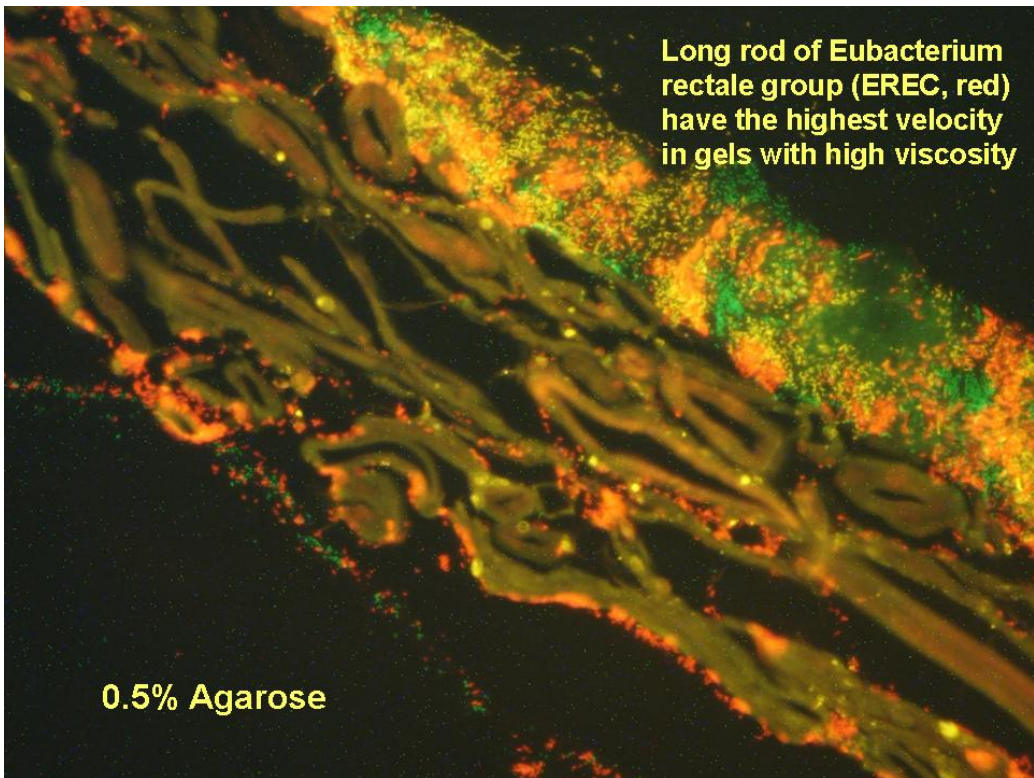
At agarose concentrations of 0.2% only small coccoid rods of the *Bacteroides* group moved. The long rods of *Eubacterium rectale* group were immobilized.

Bacterial velocity through gels of different viscosity is species specific. Small coccoid rods of the *Bacteroides* group have the highest velocity in gels with low viscosity

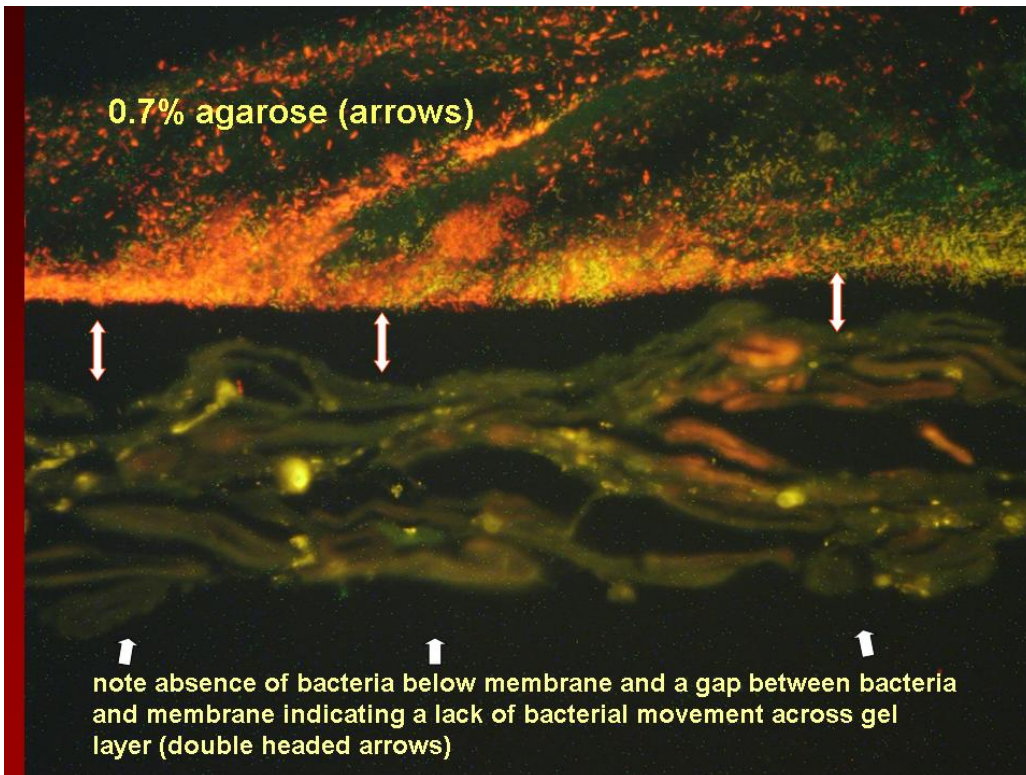
0.2% Agarose

Bacteroides was immobilized and only long rods of *Ebacterium rectale* moved at agarose concentration of 0.5%.

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The movement of all bacterial groups were inhibited at agarose concentrations of 0.7%.



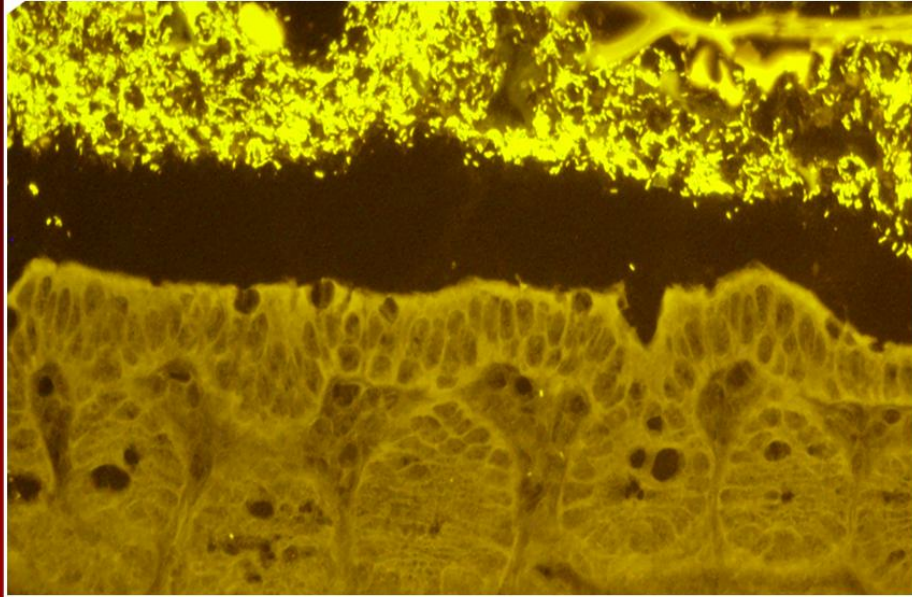
“Note the absence of bacteria below the membrane and a gap between the bacteria and the membrane which indicates a lack of bacterial movement across the gel layer”
 oder lass “note” aus

The segregation of bacteria in the proximal colon in mice in those bacteria contacting the mucosa and separated from the mucosa is therefore not a result of adherence of “probiotic” bacteria but is due to moderate viscosity of the mucus layer in this region, which permits bacteria with a long curly rod shape to move and contact mucosa but immobilizes the coccoid or short rod shaped bacteria.

The presence of the mucus barrier in the proximal colon of mice can be clearly demonstrated in germ-free mice mono-associated with *Enterobacter cloacae* – a bacterium with a short coccoid form.

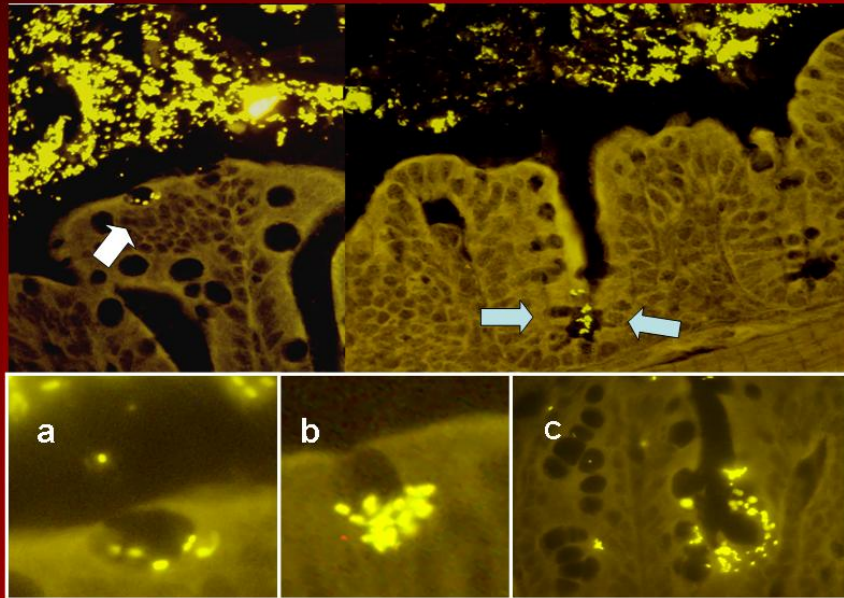
The distinct mucus layer and separation of bacteria from the colonic wall can be observed in both distal and proximal colon.

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- Distal colon of mice mono-associated with *Enterobacter cloacae*

However, while bacteria are perfectly separated in the distal colon, in the proximal colon some metastases of bacteria can be found inside of isolated vacuoles of the goblet cells, especially at the bottom of crypts.



Proximal colon of mice mono-associated with *Enterobacter cloacae*

The undifferentiated epithelial cells at the base of crypts are primarily mucus-secreting cells, whereas differentiated cells of the columnar epithelium are mainly absorptive cells, removing water and electrolytes from the mucus. The epithelial stem cells at the crypt base proliferate and replace surface cells within 4–8 days. The dissemination of *E. cloacae* in crypt bases and goblet cells outline zones of lower viscosity and confirms independently that during the journey from the crypt base toward the surface epithelium crypt cells become increasingly differentiated and absorptive. The absorptive cells of the crypt necks and of the epithelial cells of the columnar epithelium dehydrate the mucus layer. Dehydration makes the mucus layer solid and impenetrable for bacteria and protects sites of mucus production and the mucosa from encounters with potential pathogens. The lower viscosity of the mucus at the crypt base promotes emptying of crypts and prevents obstruction, but as a drawback it may make these types of cells more vulnerable to invasion by potential pathogens. Indeed, invasion of epithelial cells by *E. cloacae* was observed exclusively at the crypt bottom, whereas no *E. cloacae*-containing cells were observed within the cytoplasm of the columnar epithelial cells in mono-associated mice. Interestingly, crypt abscesses, which are typical histomorphologic findings in human self-limiting colitis and IBD, are also more abundant toward crypt bases.

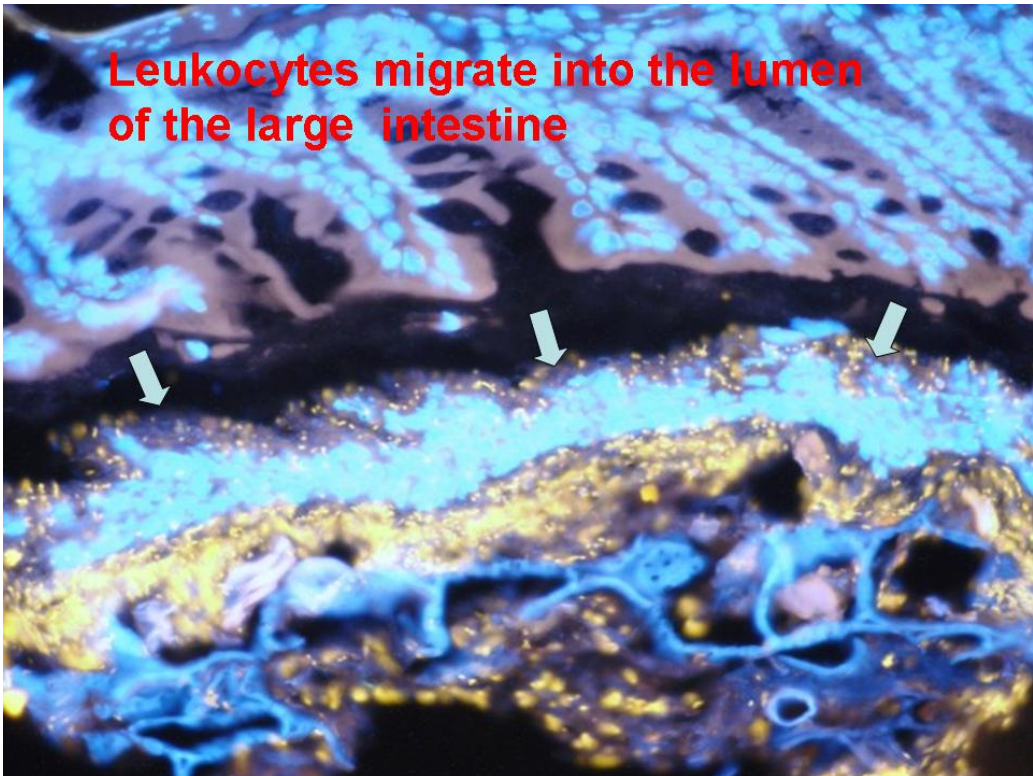
What happens if the viscosity of the mucus layer is reduced for example by addition of the detergents?

In in-vitro experiments the addition of dextrane sodium sulphate (DSS) makes the gels penetrable for bacterial movements at viscosity levels, which normally completely immobilise bacteria.

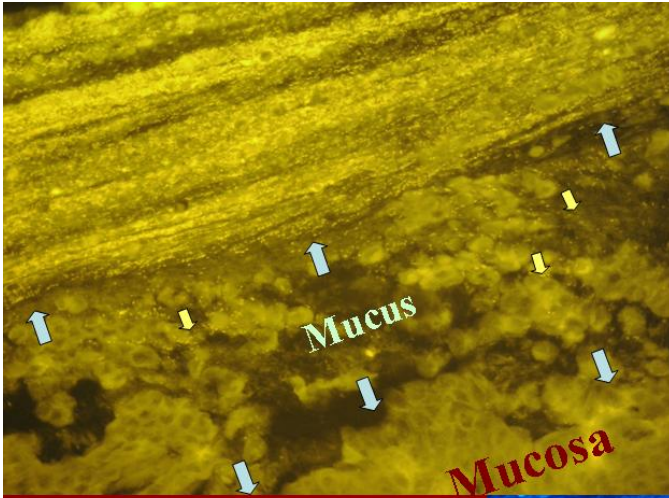


Replace “fecal” by “faecal”

In mouse, the addition of DSS to food leads to a colitis. In DSS colitis, leukocytes migrate into the colonic lumen and line up at the border between mucus and faeces.

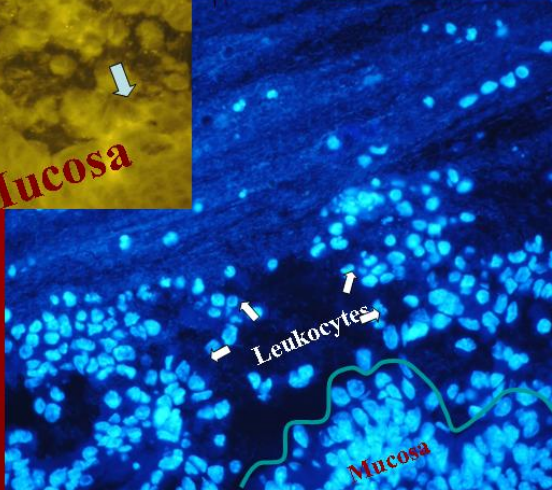


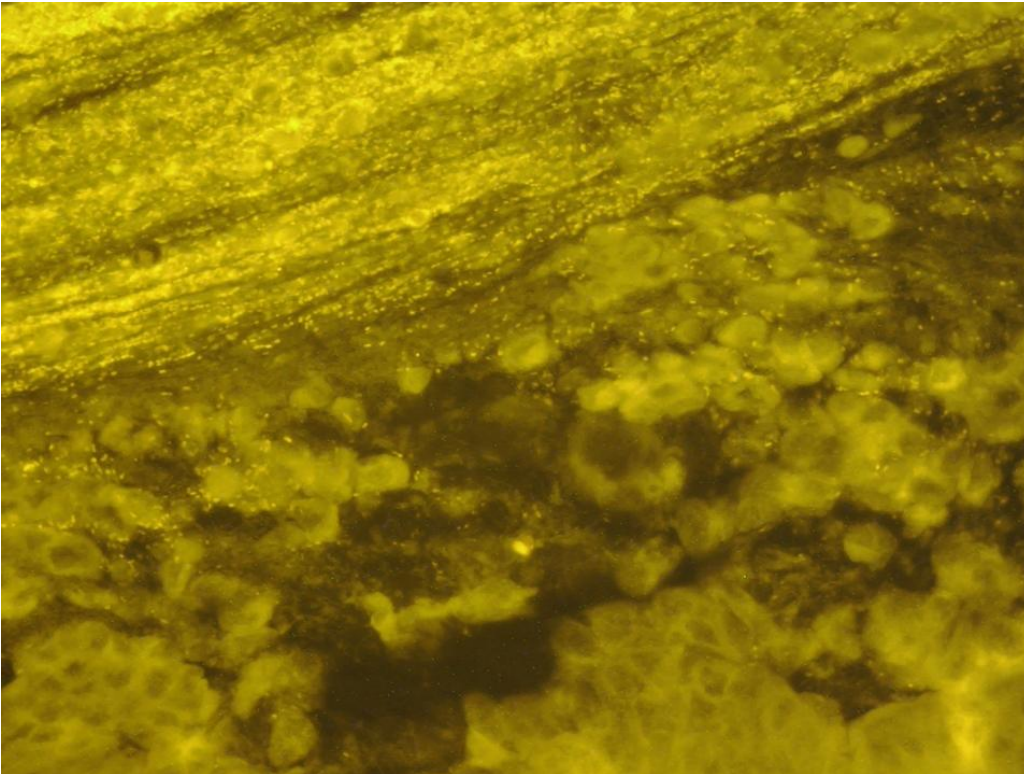
However even this leukocyte response can not stop the migration of bacteria toward the mucosa. One can clearly see at a larger magnification that even in the distal colon *Bacteroides* circumvents the leukocytes, passes through mucus, adheres to the mucosa, and causes deep tissue infiltration.



Bacteroides
crosses
mucus

The same microscopic field in DAPI shows leukocytes (large blue nuclei) migrating in mucus and hindering *Bacteroides* movement towards mucosa, normally only single leukocytes are present in mucus





The inflammation in the DSS animal model is restricted to the large bowel, although the substance is provided in the drinking water and should have theoretically the same effect throughout the intestine. However bacterial concentrations in the small intestine of mice are extremely low compared to bacterial concentrations in colon. Mucus barrier failure has therefore fewer consequences in a small intestine than in large intestine.

It has been previously assumed that the enormous masses of bacteria present in the intestine directly contact the intestinal wall. The non-pathogenic bacteria are tolerated, while the pathogenic bacteria are responded to. Dysfunction of the immunologic balance would lead to overreaction to normal non-pathogenic fecal components, thus initiating and sustaining chronic inflammation. Unfortunately, the residents of the large bowel can not be thus clearly divided into good and evil. However, many of indigeneous bacteria are pathogenic.

| *Escherichia coli* causes sepsis, *Bacteroides* causes abscesses, *Enterococci* cause endocarditis, *Clostridium histolyticum* causes gas gangrene. We call these bacterial groups normal inhabitants of the human colon since they can be found in every healthy person. Apathogenic are these bacteria in no way. Exactly seen most of the indigenous bacteria of the large intestine are just waiting of the opportunity to harm us. Let us assume that the host can recognize within the faecal mass more or less pathogenic bacteria and specifically hinder them on contact. This response has to eliminate single bacterial groups from the

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polymicrobial mixture without affecting all other bacteria – an implication which is difficult to believe.

The FISH analysis of the mucosal flora clearly indicates that the host does not tolerate the indigenous flora or its parts, it ignores it in whole. The bacterial concentrations within the large intestine can reach extremely high concentrations of 10^{11} bacteria/ml, but the mucus barrier efficiently separates colonic bacteria from the colonic wall making any response unnecessary.

Viscous mucus covers the intestinal wall, disables bacterial movements, and protects epithelial cells from contact with bacteria. Leukocytes migrate into and patrol within the mucus layer executing surveillance functions without any collateral damage. The sticky outer mucus surface offers the opportunity for probiotic strains to grow and build protective interlaced layers, making it even more difficult for pathogenic strains to reach the mucosa.

The inflammation takes place only after the mucus barrier is broken and the defence is overwhelmed.

Since the beginning of the 20th century, there has been a steady increase in reported cases of both Crohn's disease and ulcerative colitis and the peak has obviously not been reached. This increase in IBD is mainly affecting the developed world, especially populations with high living standard and urban areas.



Statistically the frequency of the disease correlates with introduction of tap water, soap and improvement of the living conditions. The hygiene hypothesis argues therefore, that improved hygiene and a lack of exposure to microorganisms of various types have sensitized our immune systems, leading to inadequate reaction to harmless bacteria in our environment. Out of this speculations have come recommendations to allow young children a reasonable amount of contact with dirt, pets, and other potential sources of infection as well as helminth therapy for IBD.

The statement that exposure to microbes in the city is lower than in the country population is basically wrong. The vegetables and fruits on our table are coming not from the beds and trees behind the cottage, but are imported from Greece, Portugal, New Zealand, South Africa, and Australia. They import a vast variety of microorganisms, that were previously unknown in the village. The mobility of the modern society has led to a profound and rapid exchange of bacteria worldwide which was never encountered in the suburban world.

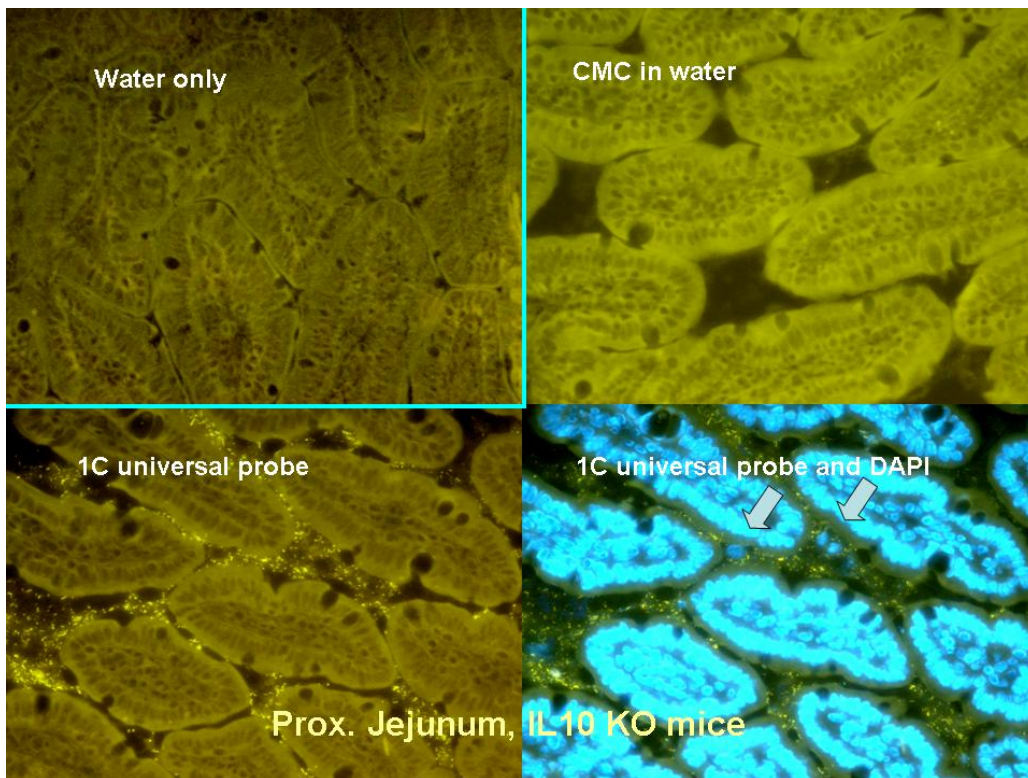
The in-vivo effects of the DSS detergent in mouse and in the mucus simulation model however reveal other possible potential side effects of cleanliness and urbanisation. Traces of the detergents that make our dishes shine are ingested with our food. The “cleaning” effects of

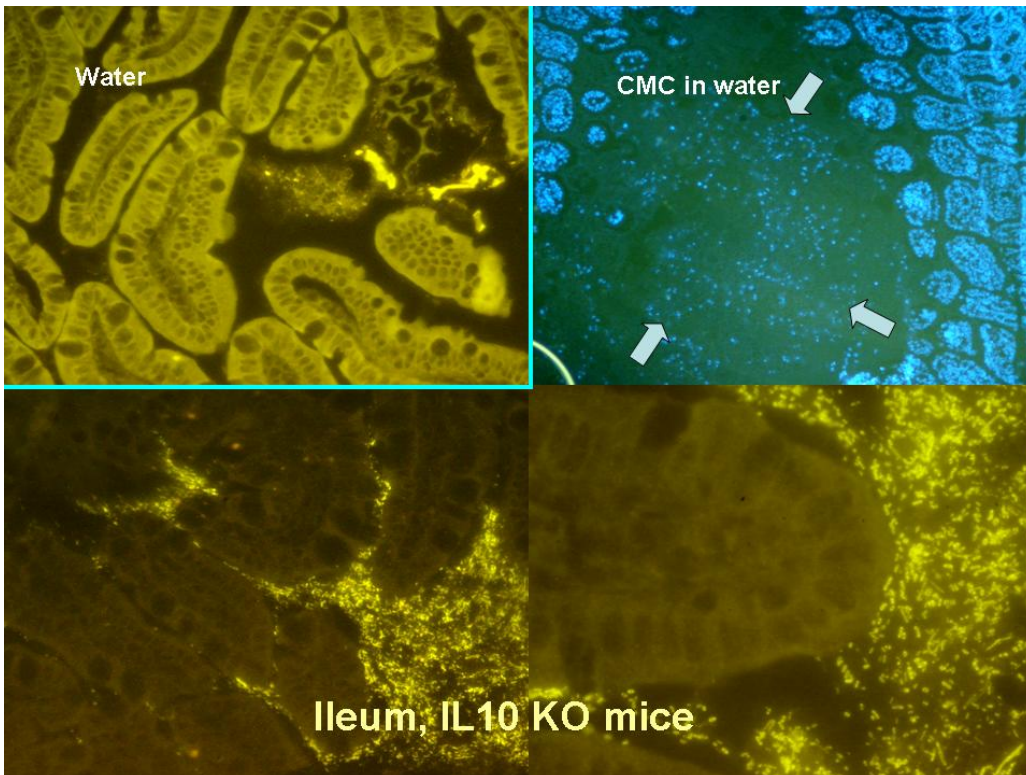
ingested home cleaning products on colonic mucus have been never investigated. Detergents make the objects clean, they do not sterilize them.

Emulsifiers that are added to many foods to achieve a desired consistency may also have effects on the intestinal mucus.

The recent data on Il-10 gen-deficient mice support this hypothesis. Beate Sydora (Alberta University, Canada) has treated IL-10 gen-deficient mice with 2% carboxymethyl cellulose (CMC) dissolved in water. Normally IL-10 knock-out mice develop colitis in adult age, the small intestine is not involved. This pattern of distribution of inflammation is in accordance with murine bacterial colonization. IL-10 knock-out mice have usually no bacteria in the small intestine and high bacterial concentrations in the large intestine. In the CMC experiments of B.Sydora, the controls which were mice treated with water, had no inflammation and no bacteria between villi in the small intestine.

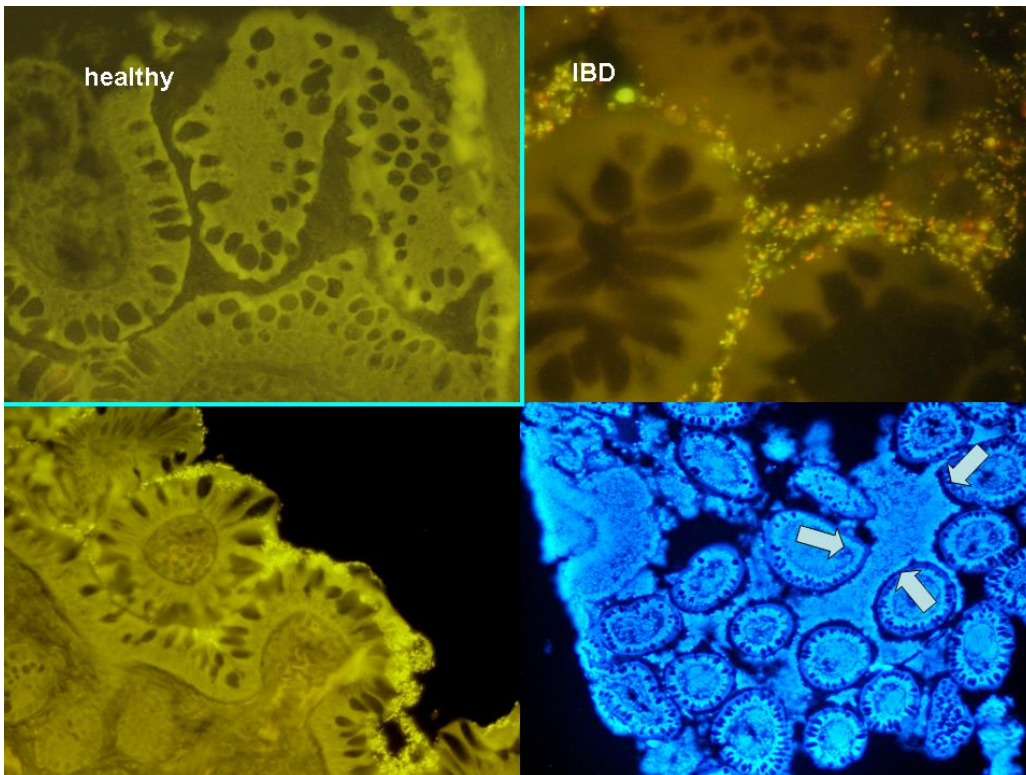
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IL-10

However bacteria and leukocytes were found between villi in the proximal parts of the small intestine in half of the CMC treated mice. The intensity of changes increased in the distal direction. In the ileum of all CMC treated IL-10 knock-out mice high bacterial concentrations were found within crypts of Lieberkuhn and these finding resembled visually the situation, which can be observed in the ileum of Crohn's disease patients.



CMC is extensively used in the food industry, because cellulose is so abundant and cheap and the emulsifying and thickening properties of CMC are remotely useful. The substance is added to food to stabilize emulsions, for instance in ice cream, to dissolve ingredients such as cacao in order to make perfect chocolate and sugar icing, to boost the flavor of the natural aroma and to keep bread fresh and soft. It can be found in toothpaste, chewing gum, a variety of baked goods, candies, sausages, ketchup and other sources. It is a filling and stabilizing component of most pills and it is a main substitute for gluten in manufactured gluten free products. Actually CMC is *everywhere* in quantities which are larger than those administered to the mice with the drinking water in our experiment. The annual amount of the CMC utilized by the food industry is tremendously increasing. Since CMC like natural fibers can not be absorbed and is chemically inert, and since it is broadly used worldwide since nearly one hundred years without apparent negative effects, its health impact as a food additive is thought to be purely related to the water and viscosity household within the intestine. Presently there are no quantitative restrictions on its use, and its addition to food does not even require to be declared.

CMC is however not the only emulsifier broadly used by food industry.

EU admitted emulsifiers

Factors affecting mucus barrier

Exogenic:

Detergents:

Bacterial virulence:

Glutens as natural emulsifiers need bacteria to be pathogenetic

Smoking

Endogenic:

Bile acids are normally fully resorbed in ileum but lead to diarrhea if arrive in large intestine

Defensins, Antibodies draining

Probiotics, Prebiotics,

Oligonucleotids Nucleinacidsderivates

Inflammatory response

Genetic

NOD 2 Mutation

E422 , Konjak
E422 bis E426 , Polysorbat
- E432, Polyoxyethylen-sorbitan-monolaurat (Polysorbat 20)
- E433, Polyoxyethylen-sorbitan-monoleat (Polysorbat 80)
- E434, Polyoxyethylen-sorbitan-monopalmitat (Polysorbat 40)
- E435, Polyoxyethylen-sorbitan-monostearat (Polysorbat 60)
- E436, Polyoxyethylen-sorbitan-tristearat (Polysorbat 65)
E441 , Polymere, Amidiertes Pektin
E442 , Ammoniumsalze von Phosphatidsäuren
E444 , Saccharose-acetat-isobutylrat
E445 , Glycerinester aus WurzelharzKokylkmonester
E430 bis E452 , Phosphate
E469 , Beta-Cyclodextrin
E460 bis E469 Cellulose und Celluloseverbindungen
- E460, Cellulose, Mikrokristalline Cellulose, Cellulosepulver
- E461, Methylcellulose
- E463, Hydroxypropylcellulose
- E464, Hydroxypropylmethylcellulose
- E465, Ethylmethylcellulose
- E466, Carboxymethylcellulose, Natriumcarboxymethylcellulose
- E468, Vernetzte Natrium-Carboxymethylcellulose
- E469, Enzymatisch hydrolysierte-Carboxymethylcellulose
E470a und E470b , Salze von Speisefettsäuren
- E470a, Natrium-, Kalium- und Calciumsalze von Speisefettsäuren
- E470b, Magnesiumsalze von Speisefettsäuren
E471 bis E472f , Mono- und Diglyceride von Speisefettsäuren
- E471, Mono- und Diglyceride von Speisefettsäuren, Monoglycerid
- E472a, Essigsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472b, Milchsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472c, Citronensäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472d, Weinsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472e, Mono- und Diacetylweinsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472f, Gemischte Essig- und Weinsäureester von Mono- und Diglyceriden von Speisefettsäuren
E473 , Zuckerester von Speisefettsäuren
E474 , Zuckerglyceride
E475 , Polyglycerinester von Speisefettsäuren, Polyglycerinester
E476 , Polyglycerin-Polyzinoleat
E477 , Propylenglycolester von Speisefetten
E482 , Thermocoagulierendes Sojaöl mit Mono- und Diglyceriden von Speisefettsäuren
E481 bis E483 , Natriumstearoyl-2-lactylat, Calciumstearoyl-2-lactylat, Stearylalcatrat
E491 bis E495 , Stearin- und Palmatverbindungen
E491 bis E495 , Stearin- und Palmatverbindungen

The table lists some of the emulsifiers which are permitted in EU. They are practically everywhere starting with Konjak.

Many other factors can influence mucus barrier. Bile acids for example are natural emulsifiers. Normally they are completely resorbed in ileum and do not reach colon. In cases of ileum resection, the resorption is disturbed, the bile acids reach large colon and induce diarrhoea.

Celiac disease is commonly regarded as an allergic response although until now it was impossible to define the exact structure within gluten molecules which could be allergic. We do know that symptomatic celiac disease is always ongoing with bacterial overgrowth in small bowel. The link between bacteria and glutens is poorly understood. Glutens are however naturally occurring emulsifiers. It could be that first bacteria make glutens harmful.

Smoking simulates mucus secretion but does not increase (probably diminishes) the mucus viscosity. The epidemiologic studies indicate that smoke is beneficial for ulcerative colitis but detrimental for Crohns disease. Thicker mucus barrier could indeed explain why mucus production could be protective for large intestine in UC patients but have no effect in Crohns disease, where bacterial suppression is more important, than bacterial separation.

Stress interferes both with mucus production and regulation of the mucus viscosity. It is an old known fact that IBD patients under stress incline to acute exacerbations of the disease.

Multiple other factors including defensins, probiotics, enteral pathogens, the inflammation itself, genetic background etc. interfere with the mucus barrier function. It is not the purpose of the presentation to discuss all of them.

As long as the mucus barrier is comprised, the conflict between the organism and the pathogens inhabiting our colon in large numbers and diversity is inevitable.

So what can be done to improve the mucus barrier? Actually we have already now multiple mechanisms to do so.

Possible ways to remodel the mucus barrier

- **Eradication of occasional pathogens comprising mucus barrier (Enterococcus, Fusobacterium nucleatum, Serpulina)**
- **Selective control of mucus secretion and dehydration (analogues of cortisol)**
- **Induction of a higher differentiation of epithelial cells, which leads to switch from mainly secretory to adsorptive function (analogues of anti TNF suppressing apoptosis)**
- **Reduction of the burden of detergents and emulsifiers in our foods**
- **Suppression of adherent bacterial biofilms (a possible effect of 5-ASA)**
- **Simulation of innate immunity (substances like GM-CSF, probiotics as living vaccines)**

Prednisolone is a very potent drug. As a glucocorticoid it stimulates the mucus secretion. Its mineralocorticoid activity increases the water resorption increasing the viscosity gradient within the intestinal mucus layer. A development of substances which can selectively control mucus barrier functions and deprived of typical for prednisone side effects could be of extreme advantage for IBD treatment.

We have previously mentioned that the columnar epithelial cells are differentiated and mainly absorptive, while crypt cells are immature stem cells and mainly secretory. A balance between

both is under TNF control. In cases of inflammation the cell turnover is increased. Anti TNF reduces the apoptosis of differentiated epithelial cells and may explain why of many know mediators of the inflammation only anti TNF antibodies have a clinically proven significance. A development of drugs with an effect on apoptosis regulation of the epithelial turnover should be considered in future.

Antibiotics can effectively reduce the number of pathogens contacting mucosa. They have however no direct influence on the mucus barrier and they can not sterilize the polymicrobial colonic microbiota. As soon as antibiotics are withdrawn for growing microbial resistance, mounting side effects or dysbiosis, the situation gets reverse. In long term antibiotics are therefore generally ineffective in IBD. The mucus barrier, however, can be comprised not only by environmental or genetic factors but also by specific pathogens like *Serpulina*, *Fusobacteria*, *Enterobacteriaceae*, or *Gardnerella*. These bacteria can specifically form adherent biofilms on the epithelial surface comprising mucus barrier and allowing a migration of other indigenous bacteria to the mucosa. A specific diagnose of such colonization and eradication of the last by aimed antibiotic treatment could be advantageous.

Mesalazin suppresses bacterial biofilms in vivo by mechanism, which are presently not clear. Different to antibiotic therapy the mesalazin suppression does not seem to induce bacterial resistance. It is possible that the biofilm suppressive effects of the mesalazin can be further expanded, when the mode of action is decoded.

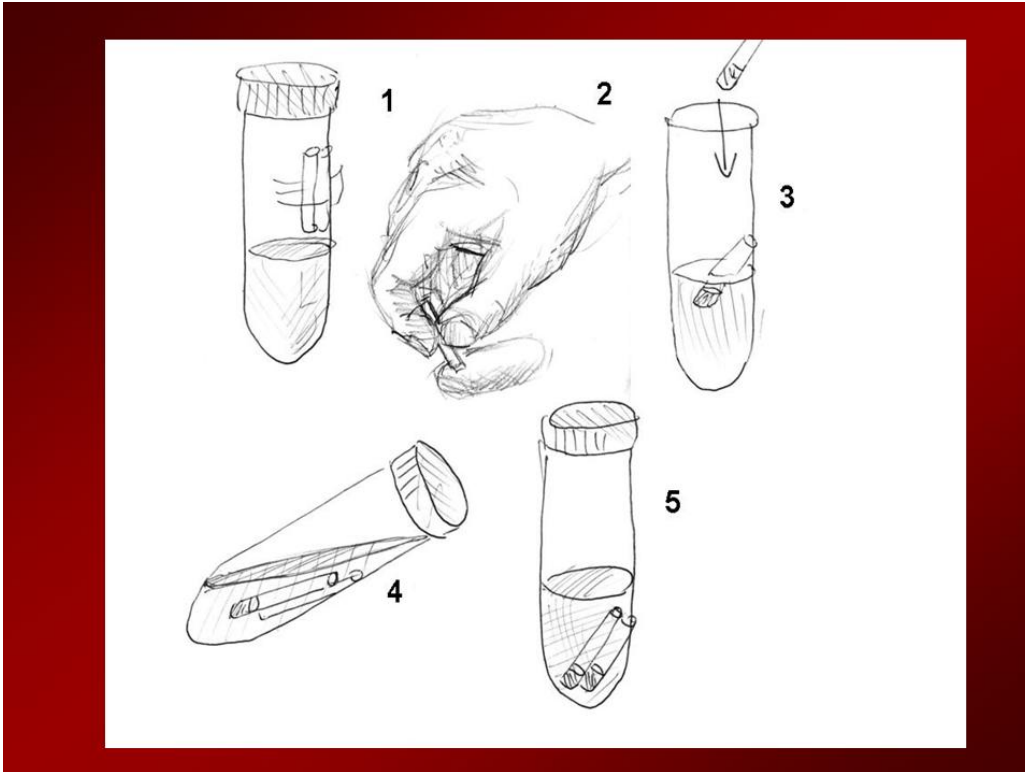
The reduction of the detergents and emulsifiers burden in our foods was mentioned. We do not know at present which of the substances may reach colon and accumulate in the human body. These questions are still to be investigated before exact recommendations can be made.

The stimulation of the immune response is an eligible aim. Previous trials on Interferon, GM-CSF were half hearted and inconsequent. PEG interferon was for example not tested at all. The therapeutic potential hidden here could be however enormous. After all probiotics may be some kind of living vaccines using attenuated strains and stimulating mucosal immunity.

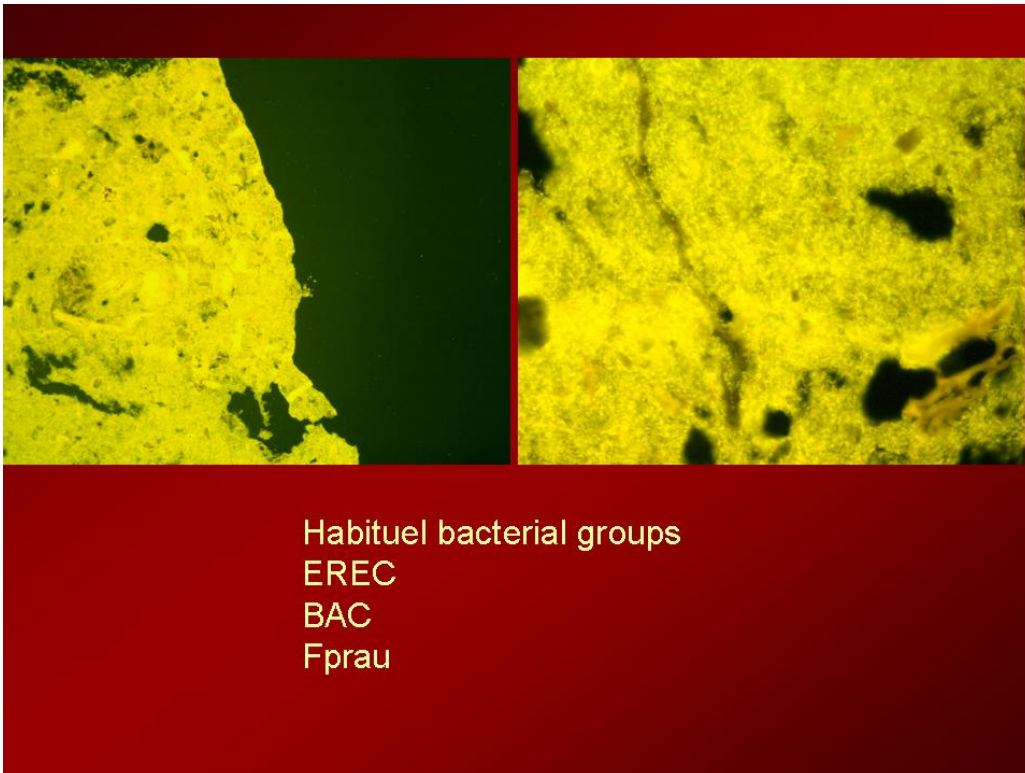
Actually we do not know how probiotics work. However, since the influence of antibiotics on polymicrobial microbiota is limited, the use of biologicals by the control of indigenous microbiota is intriguing. We must however admit, that all presently available probiotics use bacterial strains, which are marginal in human large intestine. They were selected mainly for ease in culture, storage, transport and stability within food products. The probiotic potential of anaerobes, which constitute the mass of the indigenous flora of the large intestine were not studied.

The evaluation of therapies remodelling mucus barrier affords simple and effective criteria of efficacy, which are independent of subjective complaints. FISH investigation of bioptic material is an important method, however it can not be performed at will for control of therapy. However the disturbance of the mucus layer leads to changes in biostructure of fecal microbiota, which can be also investigated. Previously the fecal microbiota were investigated based on homogenized samples. The feces are inhomogeneous. In analogy to core boring used for investigation of geologic formations we developed a method investigating the biostructure of fecal microbiota based on fixation of fecal cylinder.

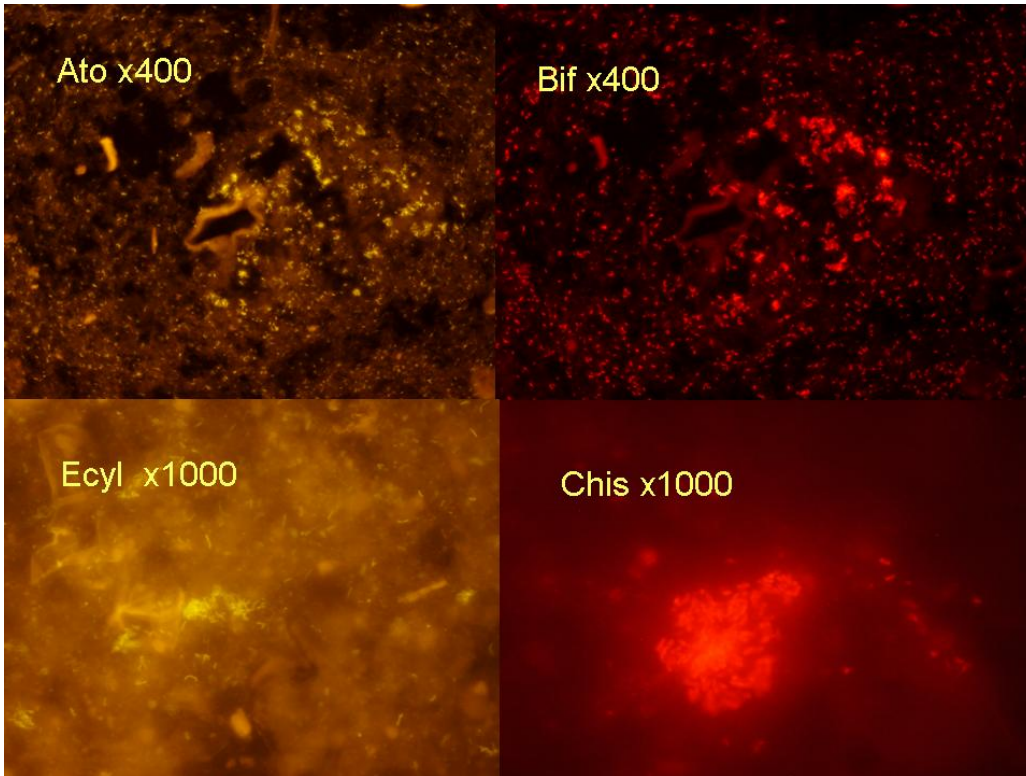
The cylinders are taken using drinking straws, fixated, embedded in paraffin, cut to slices and hybridized with FISH probes representing 86 different bacterial groups.



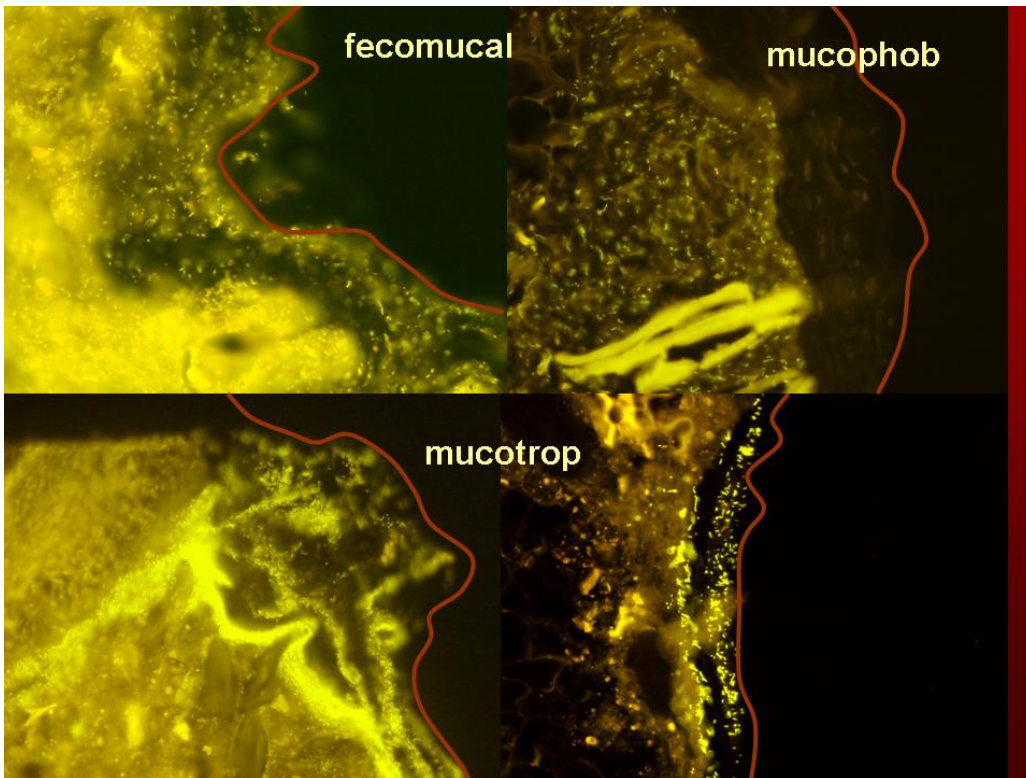
Feces proved to be highly organized spatially.



Healthy fecal microbiota can be divided in habitual composing the feces bacterial groups and occasional bacterial present only in subgroups of patients diffusely or locally condensed.



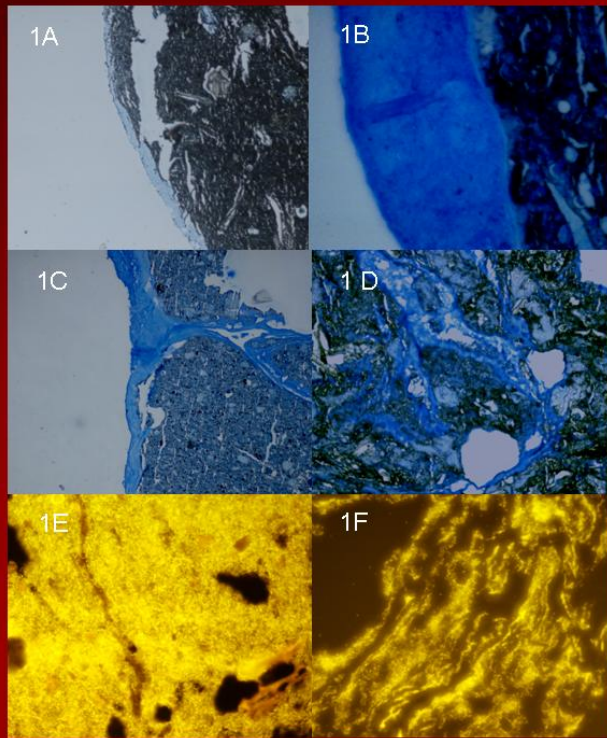
With regard to the fecal mucus bacteria could be divided in fecomucous, mucophob and mucotrop.



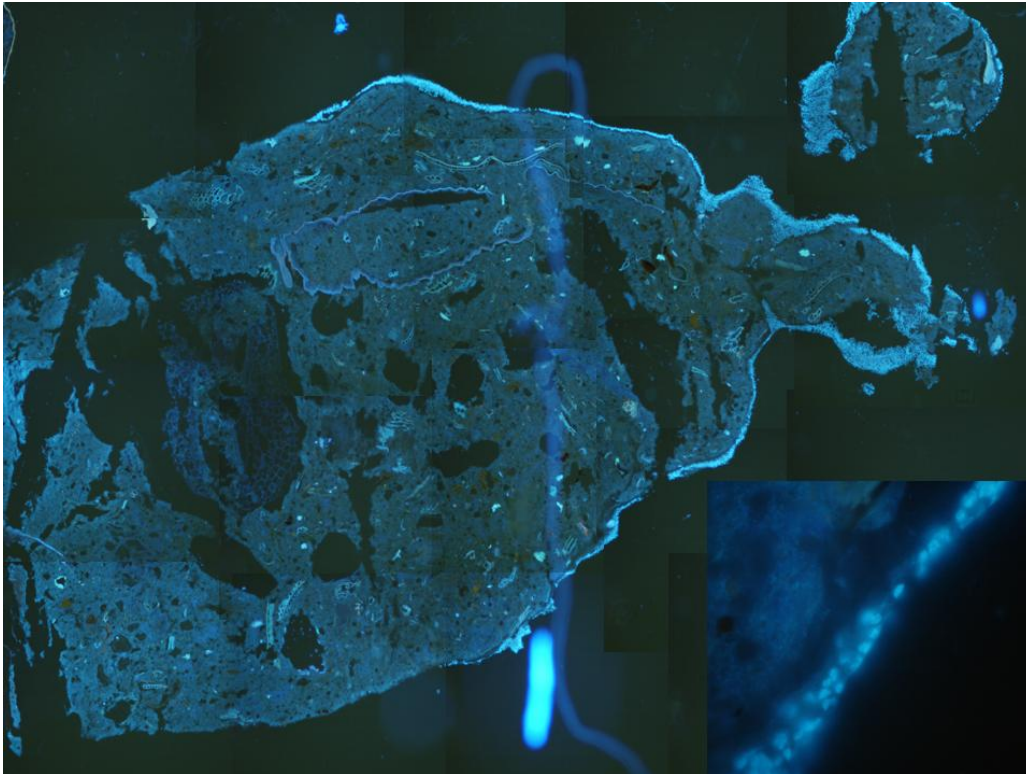
We were astonished to find that in healthy persons even the stool is covered with mucus which is free of bacteria.



In diarrheal patients the mucus secretion was increased. The superficial mucus layer grew thicker; mucus could be also found within feces enclosed in from of broad septer or multiple stria.



In ulcerative colitis the mucus was significantly reduced compared to all disease control groups and to healthy. The surface of the feces was covered with a layer of leukocytes instead. The distribution of leucocytes stresses the advantages of stool cylinder compared to fecal homogenitates, since no leukocytes are located within the fecal masses.



Up to day we have investigated more than 5000 fecal cylinder. The evaluation of 12 most representative bacterial groups in healthy, none-inflammatory disease controls, UC, and CD reveals many characteristic details, which enable discrimination between these conditions. The most prominent features in IBD were: reduction of mucus thickness especially in UC, progressive decrease in the concentrations of the habitual bacteria and disintegration of their web structure, spheroid precipitation of *Bacteroides* to isolated island in patients with UC, increased concentrations of leukocytes in the mucus and on the surface of feces in UC, reduction and loss of *Faecalibacterium prausnitzii* in CD, high concentrations by excellent fluorescence of *Faecalibacterium prausnitzii* in UC, increased concentrations and occurrence of mucotrop *Enterobacteriaceae* with decreased concentrations of mucotrop *Verucomicrobiaceae* (Hel274) in both CD and UC patients, increased concentrations of fecal *Enterobacteriaceae* in CD with low concentrations of fecal *Enterobacteriaceae* in patients with UC, reduced occurrence of *Eubacterium hallii* and *E. cylindroids* bacteria in CD, and elevated concentrations of *Bifidobacteriaceae* and *Atopobium* in patients with UC. The dynamics in concentrations and/or occurrence of *Faecalibacterium prausnitzii*, fecal *Enterobacteriaceae*, *Bifidobacteria*, *Atopobium*, *Eubacterium cylindroides*, *E. hallii*, and leukocytes were strikingly opposite in UC and CD, allowing differentiation between both and indicating that these diseases are distinctly different entities and not just different expressions of the same inflammatory process.

However, the quantitative assessment of 2 parameters: leukocytes at the feces/mucus border and *Faecalibacterium prausnitzii* concentrations, were sufficient to diagnose active CD and UC with a 79/80% sensitivity and 98/100% specificity.

	Ulcerative colitis				Crohn				IC	CD celiac	Sle	Healthy	other
	CAI >9	3< CAI ≤9	Rem. ≤1 year	Rem >1 year	CDAI >300	150< CDAI ≤300	Rem ≤1 year	Rem >1 year					
85 82	N=27	N=32	N=14	N=32	N=19	N=23	N=18	N=22	N=17	N=12	N=9	N=32	N=165
Fprau <1 Leuko ≤30 Crohnsease pattern		3%	7%	8%	84%	74%	50%	18%	18%	58%			
Fprau >1 Leuko >30 UC pattern	93%	69%	50%	14%	11%	9%	17%	10%	29%				
Fprau <1 Leuko >30 Intermediate IBD pattern	8%	7%	14%	4%	5%	9%			24%				
Leuko ≤30 Fprau >1 Non-inflammatory pattern	0	21%	29%	78%	0	9%	33%	72%	29%	42%	100%	100%	100%

The lack of sensitivity was due to overlap between CD and UC and IC, and the lack of specificity was due to overlap between CD and celiac disease/karzinoid of the small bowel. No overlap occurred between IBD and healthy controls, self-limiting colitis, and none-inflammatory disease subjects. In fact, none of the subjects from the healthy or the none-inflammatory control groups matched criteria for IBD.

Conclusions:

The intestinal wall is effectively protected from direct contact with potentially harmful bacterial groups such as *Bacteroides*, *Enterobacteriaceae*, *Enterococci*, and *Clostridium histolyticum*, which are indigeneous and high concentrated in colon. A well-developed mucus barrier and not the epithelial cell layer is the first line of defence against a variety of enteral pathogens. Before bacteria can adhere and invade mucosa, they must first traverse the mucus. When pathogens penetrate mucus and adhere to epithelial cells, inflammation clears mucosa from bacterial contact and mucus from bacteria, thus re-establishing the status quo.

The rising incidence of IBD over the last century may result from disturbed mucus barrier function caused by excessive use of detergents and emulsifiers and from changes in the types and numbers of bacteria in our surroundings.

Against this background, inflammatory bowel disease can be viewed as a polymicrobial infection, that is characterized by a sustained broken mucus barrier with subsequent bacterial migration toward mucosa and proliferation of complex bacterial biofilms on the epithelial surface.

As long as the mucus barrier function is impaired, the inflammatory process cannot successfully clear bacteria from the mucosal surface and the immunosuppressive therapy remains the main therapeutic option. Other therapy principals including regulation of the mucus secretion and viscosity, suppression of bacterial biofilms, eradication of occasional pathogens, probiotics and immunostimulation are however also possible and should increasingly considered and evaluated in future.

As consequence of inflammatory response the composition and structure of fecal microbiota is lasting changed. The structural changes can be exactly quantified and used to monitor the disease activity. Based on biostructure of fecal cylinder Crohns disease and ulcerative colitis can be distinguished from each other and other disease controls.

Ulcerative colitis and Crohn's disease are curable. The rising possibilities to monitor the disease activity in fecal samples will allow us to intensify the search for alternative therapeutic strategies aimed on curation of the disease instead on symptom control.