

Probiotics and Antibiotics in IBD/IBS

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Dr. Scherl's current interests encompass investigational therapies for ulcerative colitis and Crohn's disease. She is a Fellow of the American College of Physicians and is a member of numerous professional societies, including the American Gastroenterological Association Institute and the American College of Gastroenterology. She lectures frequently on IBD at local, regional, and national meetings. Dr. Scherl is chairperson of the Medical Advisory Committee of the Crohn's and Colitis Foundation of America (New York chapter).

An editorial reviewer for the Journal of Clinical Gastroenterology and Gastrointestinal Endoscopy, she is the co-author of "Crohn's Disease of the Small Intestine in Gastroenterology and Hepatology: The Comprehensive Visual Reference." Dr. Scherl has extensive experience as an investigator in many clinical trials and is currently participating in several national multicenter trials focusing on both ulcerative colitis and Crohn's disease. She has recently established an IBD tissue bank at Weill Medical College of Cornell University / New York-Presbyterian Hospital.

I'm Ellen Scherl. This is the Focused Clinical Update on Probiotics and Antibiotics in Inflammatory Bowel Disease and Irritable Bowel Syndrome. The unifying theme today is going to be exploring the interaction between bacteria and the intestinal epithelial layer and how they can result in dysregulated immune responses in inflammatory bowel disease and irritable bowel syndrome. We will be looking at a similar pattern in *C. difficile*, traveler's diarrhea, post-traveler's diarrhea, IBS and IBD, and we'll mention celiac disease and how it may fit into this pattern as well. Hopefully, the discussion will lead the way to exploring and understanding the rationale for using antibiotics and probiotics in these disorders. We will discuss some of the newer work on engineering decoy molecules using molecular mimicry such that the molecules actually become bacterial toxin receptors. This has recently been described in the treatment of both cholera and *C. difficile*. We will touch on all of that.

For a long time there has been a so-called "antibiotic underground." The use of antibiotics in IBD is based on experienced-based observations rather than controlled trials. In fact, if we wish to be solely evidence-based, there are no good randomized controlled trials with antibiotics for the induction or maintenance of remission in Crohn's disease or closure of fistula in Crohn's disease. The randomized controlled trials are few and have small numbers of patients. Just because there are no randomized controlled trials doesn't mean that antibiotics don't work. When some patients with Crohn's disease are treated with antibiotics for bronchitis and other disorders, we know their bowels improve. Remember, the National Crohn's Cooperative Study concluded that there was no role for maintenance therapy. However, they under-dosed azathioprine and stopped it at 16 weeks just when it would start to kick in. There is often a discrepancy between evidence-based clinical trials and our experience, particularly in IBD/IBS. It would be a mistake, therefore, to focus solely on controlled trials when extensive clinical experience actually tells us that our observations are correct and useful. So the question today is, "Are antibiotics and probiotics useful in inflammatory and irritable bowel diseases?" If you consider inflammatory bowel disease as a dysregulated immune response to luminal bacteria, your therapeutic options are either to

decrease the bacteria or decrease the immune response. Thus, the importance of exploring the rationale of antibiotics in IBD is to decrease luminal bacterial concentrations and selectively inhibit or eliminate the pro-inflammatory bacteria thereby decreasing the tissue invasion.

How does the mucosal immune response control inflammation and tolerate the presence of luminal bacteria? This has been explored from a genetic standpoint. We think that in genetically predisposed hosts, these bacteria trigger an unregulated or uncontrolled inflammation. Through genome wide searches, we have found at least one gene (and actually more but today we are going to focus on NOD2 on chromosome 16) which is integrally linked to the innate immune system. The innate immune system resides in the epithelial layer of the gut and recognizes pathogen associated molecular patterns or PAMPS. I want to underscore that concept of molecular mimicry that we are going to come back to. NOD2 is expressed in the intestinal epithelial cells in response to bacterial stimuli. As a result of that stimuli, there is an upregulation of inflammatory cascade which ultimately results in increased NFkappa B activation. So, NOD2 is like having an endogenous antimicrobial system in place. When it functions, NOD2 is upregulated and clears the bacteria and then down regulates and that is controlled inflammation. In inflammatory bowel disease, there's a failure to turn down the NOD2 system. Thirty-five percent of patients with Crohn's disease actually carry some form of variation in NOD2 compared to 10-15% in the normal population and patients with ulcerative colitis. Three percent of Crohn's disease patients have a double mutation which codes for a very aggressive ileal fibrous stenosing disease that occurs in younger patients.

We have looked at the original animal studies and are all familiar with the importance of bacteria. When knockout mice that are genetically engineered to get or develop inflammatory bowel disease, are delivered into a germ-free environment, there's no colitis; no bacteria, no colitis. We've also learned from studies that different bacteria induce different forms of colitis. If you have cecal bacteria in these knockout mice, you end up with an aggressive colitis. If you have mice that are populated with *Bacteroides vulgarius*, there's moderate colitis. *E. coli* is protective so there is no colitis. If you take the cecal bacteria that are particularly aggressive and you co-administer lactobacillus GG, there is no colitis. So, here again we see the importance of *E. coli* and the importance of other probiotics as a protective effect. The IL-10 knockout mice and concomitant or commensal bacteria lead to no colitis, but *E. fetalis* leads to left-sided colitis and aggressive *E. coli* lead to right sided colitis.

Abstract 218829: "A one year, randomised, double-blind placebo controlled trial of a lactobacillus or Bifidobacterium probiotic for maintenance of steroid-induced remission of ulcerative colitis"

Probiotics have been shown in animal models to be effective in attenuating severity of IBD. The goal of this study was to compare two different probiotics versus placebo in maintaining remission that was steroid-induced in patients with ulcerative colitis. They looked at lactobacillus salivarius subspecies Salivarius UCC118, and Bifidobacterium infantis 35624 versus placebo. Each was administered as a rehydrated, blended yogurt powder. The patients were entered within one month of achieving clinical remission of their ulcerative colitis. This was documented and defined as less than three bowel movements a day out of seven without frank, gross blood while off steroids. Patients were allowed on stable doses of aminosalicylates. There were no immunomodulators allowed. A total of 157 patients were entered with similar numbers (about 50) per group. The distribution of disease was left-sided in about one-third, limited proctitis or 20 cm in about one-third, and pancolitis in the remaining third. There was no difference across the three study groups. The risk of relapse was not significantly different whether they received probiotics or not. The lack of difference might be related to timing; perhaps manipulating the enteric flora prior to onset of disease may be better. It may relate to differences in disease severity and the potential effect of probiotic dose and body weight.

Perhaps if we use probiotics early enough, we change the flora, and the immune response. Perhaps if probiotics are used instead of or in addition to mesalamines early in disease treatment, maybe it would not be necessary to escalate the mesalamines or go on to steroids. I think that is really the challenge for us; earlier use of both probiotics and antibiotics (if they are safe) perhaps as first line.

Let's go to a very exciting concept using *E. coli*.

Abstract 216629: "Treatment of mild to moderate acute attacks of distal ulcerative colitis with rectally-administered *E. coli* Nissle 1917: Dose-dependent efficacy"

This is from Berlin. As a background, there is a high prevalence of adherent and invasive *E. coli* in ileal mucosa in Crohn's disease. Oral *E. coli* Nissle 1917 (EcN) has been shown to have efficacy in maintenance of remission in ulcerative colitis. After a phase I study showed that enemas were well tolerated, this group looked at a rectal formulation of the probiotic, *E. coli* Nissle 1917 for maintenance remission of ulcerative colitis. This was a randomized controlled double blind study in 90 patients treated with either 40, 20, or 10 ml enemas containing 10×10^8 EcN/ml or placebo. The patients received the study medication once daily over a four-week period of time. The patients had ulcerative proctitis or proctosigmoiditis, mild to moderate. They used a Sutherland score of 4-9 without the endoscopic scoring. This is purely a clinical observation. That is a potential weakness of the study. Patients reaching a clinical disease activity index less than 2 with therapy were regarded as responders. This is not a classic endpoint. According to the study, 57 of the 90 per protocol patients were assessed. The responder rate significantly corresponded to the dose of the Nissle 1917. Only two out of 11 patients receiving placebo had a response. Three out of 11 (27%) receiving a 10 ml dose of Nissle 1917, 44% of patients receiving 20 mls of Nissle1917 and 52.9% responded in those receiving 40 ml ($p=.04$). The time to remission was the shortest in the 40 ml group followed by the 20 ml group. The assessment of tolerance by patients and by physicians as well as the number of patients with suspected side effects did not reveal significant differences between the treatment groups. Rectal application of 20 or 40 ml of Nissle 1917 was actually effective for mild to moderate distal colitis and was well tolerated. Again, this raises the question of using this as an alternative to first-line topical aminosalicylates or steroids.

Some patients were on aminosalicylates or steroids, and had active disease. The endpoints are not well-defined. A flexible sigmoidoscopy to find out the severity of proctosigmoiditis would have been helpful. The endoscopic score, which is critical, is not evaluated. Nonetheless, it is an intriguing study and again it underscores the importance of looking at the role of probiotics in the human setting. The concept of an enema warrants further study.

Abstract 217642: "Increasing incidence of *Clostridium difficile* infection in patients with inflammatory bowel disease"

We know by way of background that *C. difficile* is increasing. In fact it's the most common nosocomial infection. The incidence in inflammatory bowel disease has not really been looked at. The question is, "Are we seeing more of a *C. difficile* epidemic in IBD patients?" The authors analyzed a seven-year database looking at both Crohn's and ulcerative colitis. Because Crohn's disease in particular has been treated with antibiotics usually effective against *C. difficile* namely (metronidazole) it was hypothesized that *C. difficile* rates would be lower in Crohn's than in the ulcerative colitis patients. They hypothesized that *C. difficile* would be acquired as an outpatient so that *C. difficile* toxin positively would, on average, occur within 48 hours of admission. They looked at more than 300,000 adult inpatient admissions from 1998 to 2004 at a large, tertiary hospital. The presence of *C. difficile* infection and the time from admission to a positive *C. difficile* test was looked at. The incidence of *C. difficile* was calculated per year for all admissions combined and separately. What they showed was for all the years combined the rates of

C. difficile for Crohn's and ulcerative colitis were 16 and 39 cases per 1,000 admissions respectively, they compared to 13 cases for all admissions. So, the incidence of *C. difficile* is increased in all groups over time, but the IBD group was higher. The *C. difficile* incidence doubled for all admissions for Crohn's disease and tripled in ulcerative colitis in the time period studied. Compared to Crohn's disease, the odds ratio for *C. difficile* in ulcerative colitis was 2.3 and compared to non-IBD admissions, the odds ratio for *C. difficile* in Crohn's disease and ulcerative colitis were 1.5 and 3.5 respectively. The median times in days from admission to a positive *C. difficile* test was 0.8 days in Crohn's, 0.9 in ulcerative colitis, and 4.0 for other admissions.

In conclusion, over the seven-year period studied, the incidence of *C. difficile* in IBD increased and was higher in ulcerative colitis than Crohn's disease. In both Crohn's disease and ulcerative colitis, *C. difficile* toxin assays were usually positive within 48 hours of admission, suggesting that the infection was acquired prior to hospitalization.

Abstract 220397: "Viable bacteria versus DNA extracts as anti-inflammatory agents in Crohn's disease"

The concept of proinflammatory bacteria subsets such as bacteroides, enterococcus faecalis and enteroadherent *E. coli* versus the protective probiotics lactobacillus, bifidobacteria, non-pathogenic *E. coli* is what's driving this discussion. I just want to say that also when we look at DNA extracts, it raises the question of recombinant probiotics for treatment of various diseases and in this month's *Gastroenterology*, Patton looked at recombinant probiotics for the treatment and prevention of cholera. Again, this is developing based on a therapeutic strategy looking at molecular mimicry of the host receptors for bacterial toxins and these are circuits of benign coli. In this abstract, the investigators looked at viable lactobacillus casei versus bacterial DNA in inflamed ileal mucosa from Crohn's disease patients. The explants of ileal mucosa (20-30 mg) from four patients with Crohn's disease were incubated for 18 hours with viable DNA extracts from lactobacillus casei, Bifidobacterium longum, Bifidobacterium brevis or the medium alone (so that would be the placebo) or blank again. Each condition was run in triplicate so there were three times that this was checked. The tissue viability and pH were monitored. TNF- α levels and recovery of bacteria were assessed in the supernatant at the end of the incubation period and the following cytokines were analyzed in each one of these conditions: IL-1b, IL-2, IL-6, INF-g, IL-13, IL-4, IL-5, IL-10. The tissue incubation with viable L. casei and bifidobacteria longum DNA significantly decreased TNF- α concentration. The lactobacillus DNA alone and the bifidobacteria brevis DNA did not induce significant changes. Again, the DNA extract in the lactobacillus casei increased the level of antiinflammatory cytokines in the Crohn's mucosa but the inhibitory effect of the viable bacteria on TH1 cytokine was not produced by the DNA extract alone. It's an intriguing concept. If we could look at the effects of parts of probiotics we might be able to advance. What is important in this study is that they studied the proinflammatory and antiinflammatory cytokines. I think that's going to be critical in the studies going forward, if we are able to define the right sequence of DNA or amino acids that actually make these probiotics work well.

I think that until we are smart enough to recognize specific molecular patterns, we need to stay with live bacteria with probiotics. We still don't know enough about the secondary immune response or the bacteriology of the gut. The stakes are high because if we can use probiotics and antibiotics first line in the select patients, then we can eliminate or delay steroids. I would like to see us be able to go to probiotics, antibiotics, and then biologics. I would like to see studies that look at steroids in conjunction with bacteria to evaluate the effect of steroids on gut microecology.

Abstract 223558: “6 months treatment of symptomatic uncomplicated diverticular disease (SUDD) of the colon with lactobacillus paracaseii F19”

It has been suggested that abnormal breath testing is common in subjects with IBS (84% versus 20% in healthy controls). We know that there is no specific treatment for symptomatic or uncomplicated diverticular disease. We tend to recommend a high-fiber diet. Not surprisingly, some patients actually have difficulty as they increase their fiber. Different fibers might be helpful, but fiber is not necessarily the answer to uncomplicated diverticulosis. Some observations suggest a possible role for gut microflora. These authors looked at the efficacy of lactobacillus paracaseii F19 in patients with uncomplicated diverticular disease. This was a randomized controlled study looking at 43 patients aged 47 to 78, diagnosed by double-contrast barium enema and biochemistry to exclude acute inflammation. Patients had abdominal symptoms, pain less than 24 hours, and bloating. They had to have these symptoms for at least six months before enrollment so there was no chance this was acute diverticulitis. All patients had validated detailed questionnaires for abdominal symptoms, functional dyspepsia as well as a visual analog scale. The exclusion criteria were complicated diverticular disease, previous colonic surgery, antibiotics, laxatives taken within 30 days, and NSAIDs. A daily intake of 30 grams of fiber was recommended for all patients. It would be important to know how many patients actually took that high-dose of fiber. Patients were randomly assigned to Group A - 1 sachet bid of the lactobacillus for 14 days for six months. Group B was a fiber only group, or Group C – 1 sachet lactobacillus again for 14 days for six months. The endpoint in the study was to evaluate a decrease in abdominal pain and bloating after six months of treatment. The results were expressed in terms of standard deviation. Only 34 patients completed the study so there was a significant drop out (nine patients). One patient in Group A was withdrawn for diarrhea. Eight patients (three from Group A, four from Group B, and one from Group C) were lost to follow-up. The symptoms related to functional dyspepsia were minimal and not affected by any treatment. Notably, they did not look at lactose breath test. The patients with uncomplicated diverticulosis benefit from treatment with *L. paracaseii* since it significantly decreases the intensity of abdominal pain and bloating. The efficacy of higher dosage was similar to that of lower dosage. The fact that breath testing and the Rome criteria was not used makes it hard to know how to actually quantify the results. If they had used the Rome criteria they could call this IBS-associated diverticulosis. I think without any validated scoring system, it is very hard to know what this means.

Looking at the *C. difficile* data we do know that we are capable, at least with antibiotics, of changing the enteric flora. How and for how long and with what probiotic we don't know.

Abstract 220363: “Survival of *E. coli* strain Nissle 1917 given in combination with oral mesalamine to healthy volunteers”

This is a randomized placebo controlled trial in at 48 healthy volunteers looking at *E. coli* Nissle (EcN) two capsules once a day (Mutaflor[®] 2.5 – 25 x 10⁹ viable bacteria per capsule) plus mesalamine (Salofalk[®] 1500 mg bid) or (EcN) placebo. There was a run in period of 17 days with all volunteers taking only the Nissle 1917 preparation. The volunteers detected positive for *E. coli* received a combination treatment for 7 days. Fecal samples were then examined at two to three day intervals for *E. coli* by Multiplex PCR assay and cultivation. Diaries were completed for tolerability. During the run in period, fecal samples with positive results of the *E. coli* Nissle analysis increased and viable *E. coli* was detected in 83%. Eight volunteers were not eligible for treatment as no *E. coli* was detected in the run in period. From day 1 to 7 of combination treatment, the number of volunteers with positive *E. coli* detection was between 70% and 80% in the mesalamine group and between 85% and 95% in the placebo group. The differences between the groups were not statistically significant. One week after the follow-up when treatments were stopped, the proportion of positive Nissle 1917 detection amounted to 16 out of 20 in the mesalamine group and 15 out of 20 on placebo. It dropped continuously up to week 12 after

combination treatment. It decreased to seven out of 20 and 14 out of 20 in the mesalamine and placebo group respectively. There were no differences between both groups and tolerability and safety could be seen. In conclusion, the Nissle 1917 was detected in the intestines of 83% of healthy subjects after treatment with oral Nissle preparation for 17 days. Mesalamine showed no significant effect on the survival of the *E. coli* in the intestines of healthy subjects and therefore, a combination of both treatments seems to be possible. Further studies are warranted to estimate the benefit of combined therapy with the Nissle 1917 and mesalamine. The study doesn't really answer the question as to what happens with the Nissle alone without mesalamine, but certainly in this study, these are healthy volunteers and the mesalamine didn't seem to have any deleterious effect or diminished efficacy.

Abstract 212878: “Effect of nitazoxanide in treating rotavirus gastroenteritis in hospitalized pediatric patients”

Nitazoxanide is a new anti-infective that is effective against both protozoans and viruses as well as potentially *C. difficile*. In this study, nitazoxanide therapy reduced the duration of rotavirus diarrhea in hospitalized pediatric patients. It was true that both the nitazoxanide as well as its metabolite tizoxanide inhibited the replication of rotavirus. This is an important new therapy for rotavirus.

Abstract 221943: “Treatment of traveler’s diarrhea: Rifaximin, Rifaximin plus loperamide, or loperamide alone”

This was looked at in Mexico where the strains are mostly *E. coli*. Eighty percent of bacteria is, in fact, *E. coli* except in Southeast Asia. The trouble with antimotility agents alone is that they don't eradicate the cause of the bacteria. In the Rifaximin alone group, there was a better response than loperamide alone group. Although the loperamide alone conferred rapid symptomatic improvement, the effect was transient with a high rate of continuing diarrhea, which was not surprising. Rifaximin treatment resulted in a clinical cure and combination of Rifaximin and loperamide together conferred rapid initial symptomatic improvement coupled with long-term results. This may be optimal for the treatment of traveler's diarrhea in North and South America where enteroadherent *E. coli* is the cause.

Abstract 214539: “Successful therapy of unspecific prolonged diarrhoea in infants and toddlers with the probiotic *E. coli* Nissle 1917”

Prolonged diarrhea can be successfully treated and due to the excellent efficacy, the probiotic Nissle 1917 was a suitable remedy. These are infants, so the question is does this early probiotic therapy, which was actually dramatic, reduce post-infectious IBS or IBD? There is no way of knowing this, but it would be interesting to watch these patients long-term. I think the number that we should take away from this is that the response rate increased continuously. At day 14, 93% of patients had a response compared to placebo (65%) and at day 21, there was a 98% response with the Nissle 1917 compared to only 70% placebo. More studies are needed, but intriguing.

Abstract 226136: “Nitazoxanide in the treatment of *Clostridium difficile* associated disease”

Failure in treatment with metronidazole has increasingly been documented with these new hyper virulent strains and there is increasing concern about vancomycin resistant enterococcus fetalis and other organisms, therefore an alternative therapy in the hospital environment is warranted. After seven days of treatment, 82% of patients responded to metronidazole and 89% who received nitazoxanide also responded. All isolates of *C. difficile* collected during the study were susceptible to metronidazole and nitazoxanide as well as its metabolite tizoxanide. Sustained responses at 31 days were seen in 66-74% of those treated with nitazoxanide. In conclusion, nitazoxanide 500 mg twice daily for 7 or 10 days was at

least as effective as metronidazole 250 mg four times daily for 10 days in the treatment of *C. difficile*. Randomized controlled trials are warranted.

Thank you.

Abstracts Discussed

218829: A One Year, Randomised, Double-Blind, Placebo Controlled Trial of a Lactobacillus or a Bifidobacterium Probiotic for Maintenance of Steroid-Induced Remission of Ulcerative Colitis. *Fergus Shanahan, Francisco Guarner, Atte von Wright, Terttu Vilpponen-Salmela, Diarmuid O'Donoghue, Barry Kiely, Progid Investigators*

Background: Probiotics have been shown to have modest but consistently significant efficacy in attenuating disease severity in animal models of inflammatory bowel disease (IBD). However, the role of probiotics in human IBD is less clear. **Aim:** to compare two different probiotics vs. placebo in the maintenance of remission of ulcerative colitis following steroid-induced remission. **Design:** a prospective, balanced, randomised, parallel group, double blind, placebo-controlled trial of (10^9 daily for one year) *Lactobacillus salivarius* subsp. *Salivarius* UCC118 or *Bifidobacterium infantis* 35624 versus placebo, each administered as a rehydrated blended yoghurt powder. Patients were entered within one month of achieving clinical remission of ulcerative colitis following a documented relapse that required steroids to induce remission. Remission was defined as <3 bowel movements/day (without frank/gross blood) out of 7, while off all steroids. A stable dose of aminosalicylate was the only concomitant medication for colitis permitted. The number of patients in remission at study end was the primary efficacy variable with time to relapse being a secondary criterion. A total of 157 patients were recruited with similar numbers (52-53) per group. **Results:** Patients' demographic characteristics were similar across the three treatment groups. The extent of colitis which was similar across the groups was left-sided in about one third, limited (proctitis) in one third and pan-colitis in one third. About half of all patients were still in remission at end of the study with no difference across the three study groups. The risk of relapse was not significantly different across the groups. Patient age was the only prognostic factor with a significant ($p = 0.04$) influence on risk of relapse, with odds for an older patient decreasing over that of younger patients by 2.8%/year of age. Mean times to relapse were also similar for each study group. Adverse events were uncommon, unrelated to the treatment, and similar across the groups. **Conclusions:** Prolonged feeding with live probiotics is safe in patients with ulcerative colitis. However, in contrast to studies in animal models using the same probiotics, there was no significant benefit with either probiotic over placebo. Differences in probiotic efficacy between animal and human IBD may reflect the timing of administration and might indicate the importance of manipulating the enteric flora prior to the onset of disease, and/or may relate to differences in disease severity or effective probiotic dose/body weight.

216629: Treatment of Mild to Moderate Acute Attacks of Distal Ulcerative Colitis With Rectally-Administered E. coli Nissle 1917: Dose-Dependent Efficacy. *Harald Matthes, Thomas Krummenerl, Manfred Giensch, Corinna Wolff, Juergen Schulze*

Introduction: Distal ulcerative colitis (UC) is commonly treated with anti-inflammatory medication, with rectal formulations being particularly successful. Anecdotal clinical observation point to the successful use of enemas containing stool suspensions or probiotic suspensions. Efficacy of oral therapy with probiotic E. coli Nissle 1917 (EcN) for remission maintenance in UC patients has been shown in several studies. Subsequent to a documented phase-I study of EcN enemas which were well tolerated in 80 patients, clinical effectiveness as compared to a placebo was investigated at various dosages to test for efficacy and dose-dependency. Study design: A total of 90 patients were enrolled. In a double-blinded study patients were randomly treated with either 40, 20 or 10 ml enema containing 10×10^8 EcN per ml ($n = 24, 23$ or 23) or a placebo ($n = 20$). Patients received the study medication once daily over at least 4 weeks. Admission criterion was UC proctitis or proctosigmoiditis with mild to moderate activity (DAI according to Sutherland 4 - 9). After 2 and 4 weeks the clinical DAI (primary objective) as well as data on tolerance were determined. Patients reaching clinical DAI ≤ 2 within the therapy phase were regarded as responders. Results: According to the (Jonckheere-Terpstra) test for rank correlation evaluating dose-dependent efficacy, 57/90 per-protocol patients (no concomitant acute-phase therapy with aminosalicylates or glucocorticoids, no abrupt stop of acute-phase therapy prior to study commencement, maximal spread of inflammation 45 cm from anus) were assessed: the responder rates significantly correspond to the applied EcN doses by increase in ranking ($p = 0.04$). Remission was achieved by 2/11 patients (18.2%) receiving a placebo, 3/11 (27.3 %) receiving EcN 10 ml, 8/18 (44.4 %) receiving EcN 20 ml, and 9/17 (52.9 %) receiving EcN 40 ml. Time to remission was the shortest in the EcN-40-group, followed by the EcN-20-ml group. Assessment of tolerance by patients and by the physician as well as the number of patients with suspected side effects did not reveal significant differences between the treatment groups. Conclusion: Rectal application of 20 or 40 ml EcN enemas (10×10^8 EcN/ml) for the therapy of mild to moderate distal UC represents an effective and well tolerated alternative to topical use of aminosalicylates or glucocorticoids.

217642: Increasing Incidence of Clostridium Difficile Infection in Patients with Inflammatory Bowel Disease. *Joseph F Rodemann, Kimberly A Reske, Erik R Dubberke, Christian D Stone*

BACKGROUND: In the past decade, rates of nosocomial *Clostridium difficile*-associated disease (CDAD) have been increasing for all diagnoses. The incidence of CDAD in patients with inflammatory bowel disease (IBD) has not been

examined, however. We analyzed a seven-year database of inpatient admissions to determine if the incidence of CDAD has also been increasing in Crohn's disease (CD) and ulcerative colitis (UC). Because CD in particular is often treated with outpatient antibiotics effective against *C. difficile*, we hypothesized that the CDAD rate would be lower in CD than in UC. Based on the observation that CDAD is sometimes the cause for admission in IBD, we also hypothesized that *C. difficile* toxin positivity would, on average, occur within 48 hours of admission, which suggests the infection was acquired in the outpatient setting. METHODS: An informatics database of 345,808 adult inpatient admissions from 1998 to 2004 at a large tertiary hospital was analyzed for demographic data, length of stay (LOS), presence of a *C. difficile* infection, and time from admission to a positive *C. difficile* test. Incidence of CDAD was calculated per year for all admissions combined and separately for the primary ICD-9 discharge diagnoses of CD and UC. Cases with insufficient data or indeterminate colitis were excluded. Descriptive statistics and Chi-square testing were used to determine the risk for CDAD in IBD. RESULTS: For all years combined, the rates of CDAD for CD and UC were 16 and 39 cases/1000 admissions respectively, compared to 13 cases/1000 for all admissions. The incidence of CDAD increased in all three groups over the time period studied. Comparing 1998 and 2004 data, CDAD incidence doubled for all admissions (8.8 to 16.9/1000 admissions) and CD (9.5 to 22.2/1000 admissions), and tripled in UC (18.4 to 57.3/1000 admissions; Chi-square test for trend in UC = 3.8, p=0.05). LOS did not differ significantly between the three groups. Compared to CD, the odds ratio (OR) for CDAD in UC was 2.3 (95% CI: 1.5, 3.6). Compared to non-IBD admissions, the ORs for CDAD in CD and UC were 1.5 (1.1, 2.1) and 3.5 (2.5, 4.7) respectively. The median times in days from admission to a positive *C. difficile* test for CD, UC and all admissions were 0.8, 0.9 and 4.0 respectively. CONCLUSIONS: Over the seven years studied, the incidence of CDAD in IBD has increased and is higher in UC than CD. IBD patients are more likely to be admitted to the hospital with a *C. difficile* infection compared to non-IBD patients. In both CD and UC, *C. difficile* toxin assays were predominantly positive within 48 hours of admission, suggesting infection was acquired prior to hospitalization.

220397: Viable Bacteria Versus Dna Extracts As Anti-Inflammatory Agents In Crohn's Disease. *Monica Carol, Marta Llopis, Maria Antolin, Cristina Martinez, Natalia Borrue, Francesc Casellas, Francisco Guarner, Juan-Ramon Malagelada*

Background: Other investigators described an immunomodulatory effect of DNA extracts from probiotic bacteria via TLR-9 in animal models and cellular lines. Our group reported a potent anti-inflammatory effect of viable *Lactobacillus casei* DN-114 001 in ileal tissue from Crohn's disease patients. Aim: To compare the effect of viable *L. casei* versus bacterial DNA in inflamed ileal mucosa from Crohn's disease patients. Material and methods: Explants of ileal mucosa (20-30 mg) from 4 patients with Crohn's disease were incubated for 18h with viable *Lactobacillus casei* (*L. casei*, 10E6 CFU/mL), DNA extracts from specific bacterial strains (5 µg/mL), including *L. casei* (Lc-DNA), *Bifidobacterium longum* (BI-DNA), and *Bifidobacterium brevis* (Bb-DNA), or medium alone (blank). Each condition was run in triplicate (n=12). Tissue viability (LDH) and pH of the culture media were monitored; TNF-α levels and recovery of bacteria were assessed in the supernatant at the end of the incubation period. The following additional cytokines: IL-1b, IL-2, IL-6, IFN-γ, IL-13, IL-4, IL-5, IL-10 were analyzed in *L. casei*, Lc-DNA and blank conditions. Results: Tissue incubation with viable *L. casei* and BI-DNA significantly decreased TNF-α concentration in the supernatant, but Lc-DNA and Bb-DNA did not induce significant changes (blank: 194.7±43.5; *L. casei*: 27.9±15.7*; Lc-DNA: 69.4±16.1; BI-DNA: 32.6±8.2*; Bb-DNA: 71.8±40.6; results are mean ± sem in ng/mL, *p<0.05 v.s. blank). Likewise, levels of IL-1b, IL-2, IL-6, and IFN-γ were significantly decreased by viable *L. casei* but not by Lc-DNA. On the other hand, IL-13, IL-4, IL-5 and IL-10 were significantly stimulated by both viable *L. casei* and Lc-DNA. Conclusion: DNA extract from *Lactobacillus casei* increases the level of antiinflammatory cytokines in Crohn's disease mucosa, but the inhibitory effect of the viable bacteria on Th1 cytokines is not reproduced by the DNA extract.

223558: 6 Months-Treatment of Symptomatic Uncomplicated Diverticular Disease (SUDD) of the Colon with Lactobacillus Paracasei F19. *Rosario Cuomo, Giovanni Maconi, Francesco De Giorgi, Edith Lahner, Giovanni Sarnelli, Bruno Annibale*

Background: Abdominal pain and bloating are frequent symptoms in SUDD, which are generally accepted to be related to functional disorders. Albeit no specific standard treatment for SUDD is accepted, guidelines of the American College of Gastroenterology suggest a high-fibre diet in patients with SUDD. Some observations suggest a possible role of gut microflora in determining symptoms related to SUDD, thus these patients may potentially benefit from probiotics treatment. Aim: to assess the efficacy of *Lactobacillus paracasei* F19 in patients with SUDD. Materials and Methods: Multicentric randomized controlled study on 43 pts, aged 47-78 years (15 M, 28 F) with SUDD, diagnosed by double contrast enema and biochemistry to exclude acute inflammation. SUDD was defined as: presence of abdominal symptoms (pain <24h and bloating) for at least 6 months before enrollment. All pts had a validated detailed questionnaire for abdominal symptoms and functional dyspepsia (FD) including a visual analogic scale (VAS). Exclusion criteria were: complicated diverticular disease, previous colon surgery, antibiotics or laxatives in the 30 days before enrollment, use of NSAIDs. In all pts a daily intake of 30 g of dietary fibre (vegetables and fruit) was recommended. Pts were randomly assigned to receive: 1 sachet b.i.d of *L. paracasei* F19 (containing 12.5x10⁹ viable bacteria and 750 mg of gluco-oligosaccharides) for 14 days/month for 6 months (Group A, n=14), only fibre diet (Group B, n=13) or 1 sachet once daily of *L. paracasei* F19 for 14 days/month for 6 months (Group C, n=16). Endpoint of the study was to evaluate the decrease of abdominal pain and bloating after 6 months of treatment by VAS. Results are 228 [To claim CME credit you must have Internet access and allow pop-up windows. Please CLICK HERE to claim CME.](#)

expressed as mean±SD. Results: At baseline, 40 pts (93%) had both abdominal pain and bloating, whereas 3 pts had only bloating. 34 patients (14 M, 20 F, 79%) completed the study: 1 patient (Group A) was withdrawn for diarrhea and 8 patients (3 from Group A, 4 from Group B, 1 from Group C) were lost at follow-up. Symptoms related to FD were minimal and not affected by any treatment. Conclusion: Patients with SUDD benefit of treatment with *L. paracasei* F19 since it significantly decreases the intensity of abdominal pain and bloating. The efficacy of higher dosage of probiotics is similar to that of lower dosage.

VAS of bloating	Group A	Group B	Group C
Baseline	5.1±2.7	5.6±2.2	4.9±2.3
6 mos treatment	2.3±2.6*	3.8±2.9	2.9±2.3*
VAS of pain<24 h			
Baseline	4.6±2.3	4.1±1.4	4.8±2.9
6 mos treatment	1.9±2.4*	3.0±1.3	2.1±2.6*

*p<0.05

220363: Survival of *E. coli* Strain Nissle 1917 Given in Combination with Oral Mesalamine to Healthy Volunteers. *Thai Hoa Nguyen-Xuan, Gabriele Blum-Oehler, Barbara Plaschke, Joerg Hacker, Lars Joeres, Stefan Saar, Juergen Schulze, Wolfgang Kruijs*

Background: Mesalamine and the probiotic *E. coli* strain Nissle 1917 (EcN) are both therapeutically effective agents for the treatment of ulcerative colitis. In the interest of even better efficacy the effects of a combined therapy are highly worth to look for. However, for some theoretical reasons, there may exist antimicrobial effects of Mesalamine against EcN. **Methods:** In this prospective, randomized, double-blind, placebo-controlled study healthy volunteers (n=48) were treated with either EcN (2 capsules Mutaflor® od; 2.5 - 25 x 10⁹ viable bacteria per capsule) plus Mesalamine (Salofalk®; 1500 mg bid) or EcN plus placebo. After a run-in period of 17 days with all volunteers taking only EcN, volunteers detected positive for EcN received combination treatment for 7 days. Faecal samples were examined in 2- to 3- days intervals for EcN by Multiplex-PCR assay and cultivation. Diaries were completed for tolerability of the treatment and compliance. Blood samples and urine samples were additionally taken for safety and compliance. **Results:** During the run-in period faecal samples with positive results of the EcN analysis increased and viable EcN was detected in 83 % of the vols (40/48). 8 volunteers were not eligible for combination treatment as no EcN was detected during the run-in period. From day 1 to day 7 of combination treatment, the number of volunteers with a positive EcN detection varied between 70 % (14/20) and 80 % (16/20) in the Mesalamine group and between 85 % (17/20) and 95 % (19/20) in the placebo group. The differences between the groups were statistically not significant (normal approximation, day 3 p>0.15, day 5 p>0.25, day 7 p>0.076). After one week of the follow up when treatments were stopped, the proportion of positive EcN detection amounted to 16/20 in the Mesalamine group and 15/20 in the placebo group and dropped down continuously up to week 12 after combination treatment. It decreased to 7/20 and 4/20 in the Mesalamine and placebo group, respectively. No differences between both groups in tolerability and safety could be seen. **Conclusions:** EcN was detected in the intestine of 83 % of the healthy subjects after the treatment with EcN for 17 days. Mesalamine shows no significant effects on the survival of EcN in the intestine of healthy subjects. Therefore a combination of both treatments seems to be possible, further studies are necessary to estimate a benefit of a combined therapy with EcN and Mesalamine.

212878: Effect of Nitazoxanide in Treating Rotavirus Gastroenteritis in Hospitalized Pediatric Patients. *Jean-Francois Rossignol, Mona Abu*

Rotavirus is a leading cause of severe, dehydrating gastroenteritis among children worldwide. It is estimated to be responsible for 500,000 deaths each year accompanied by enormous morbidity, medical and social costs. There is no effective treatment for rotavirus infection. Renewed efforts to develop a safe and effective rotavirus vaccine are ongoing. Nitazoxanide is a gastrointestinal anti-infective approved in the United States for treating diarrhea caused by *Cryptosporidium parvum* and *Giardia lamblia* in patients down to 12 months of age. Nitazoxanide is active in vitro against a broad range of viruses. With this background, we evaluated the activity of nitazoxanide against rotavirus in vitro and in a randomized, double-blind, placebo-controlled clinical study. **Methods:** Nitazoxanide and its circulating active metabolite, tizoxanide, were evaluated for activity against rotavirus (Simian rotavirus SA-11) in MA104 cell cultures. A randomized, double-blind, placebo-controlled clinical study was conducted in 50 pediatric patients hospitalized with rotavirus gastroenteritis at the Cairo University Children's Hospital. Rotavirus was diagnosed by stool test using ELISA (Ridascreen®, R-Biopharm AG). Patients were randomized to receive nitazoxanide suspension (100 mg/5 mL) 7.5 mg/kg BID for three days or an equivalent quantity of a matching placebo suspension. All patients received optimal supportive care including oral or intravenous rehydration therapy. Administration of the study medication, food intake, adverse events, and number and consistency of stools were monitored and recorded daily. The time from first dose to resolution of illness was recorded for each patient and compared by treatment group using a survival analysis and Wilcoxon test. **Results:** In vitro, nitazoxanide and tizoxanide inhibited replication of Simian rotavirus SA-11 with

IC50s of 0.5 µg/mL and selectivity indices >100. Phase contrast microscopy images showed that the compounds were cytoprotective. Further mechanistic studies indicated that the drug has a selective effect on the synthesis of rotavirus protein VP7. The clinical trial enrolled 50 pediatric patients from 4 months to 7 years of age (median age = 10 months). The median time from first dose to resolution of illness was 31 hours for the nitazoxanide-treated patients compared to 75 hours for patients randomized to the placebo group (P=0.0137). There were no significant adverse events. *Conclusion:* Our study indicates that nitazoxanide therapy reduces the duration of rotavirus diarrhea in hospitalized pediatric patients. Further studies are warranted to confirm our findings.

221943: Treatment of Travelers' Diarrhea: Rifaximin, Rifaximin Plus Loperamide or Loperamide Alone. *Herbert L DuPont, Zhi-Dong Jiang, Jaime Belkind-Gerson, Pablo C Okhuysen, Charles D Ericsson, Shi Ke, David B Huang, Margaret W DuPont, Javier A Adachi, F. Javier de la Cabada, Francisco M Sandoval*

Antimotility agents such as loperamide can be useful for rapid symptomatic relief of travelers' diarrhea, but they do not eradicate causative bacteria. Antibacterial drugs can eradicate causative bacteria and effect a clinical cure. The combination of an antimotility agent and an antibiotic may confer better therapeutic benefit than treatment with either alone. This study was conducted to compare the efficacy of the combination of the nonabsorbed (<0.4%) antibiotic rifaximin and loperamide with that of each agent administered alone. Consenting adults (n=319) ≥18 years of age from the U.S. studying in Mexico during the summers of 2004 and 2005 with acute diarrhea (passage of ≥3 unformed stools in 24 h with ≥1 sign/symptom of enteric infection) lasting ≤72 hours were enrolled (1:1:1) in a double-blind study to receive one of three drug regimens: rifaximin 200 mg TID for 3 days (R); loperamide 4 mg initially followed by 2 mg after each unformed stool, not to exceed 8 mg/day for 48 h (L); or both drugs in the same dosage schedule (R/L). Placebos were used to blind the study. Patients completed a daily diary reviewed each day in the clinic for 5 days. The major cause of illness was enterotoxigenic *Escherichia coli* found in 112 patients (35%). Over the 5-day study period, the median time from first dose of study drug until passage of last unformed stool (TLUS) was shorter with both rifaximin-containing regimens: R 23 h; R/L 19.5 h; than with loperamide alone: 41.5 h (P=0.01). The incidence of treatment failure (not achieving wellness in 5 days) was lower with the rifaximin-containing regimens: R 8/107 (7.5%); R/L 7/108 (6.5%); vs. L 17/104 (16.3%) (P=0.032). The median/mean numbers of unformed stools passed for the duration of illness were lower with R/L 2.5/3.99 than with either treatment alone R 4/6.23; L 4/6.72 (P=0.002/0.004). In the first 10 h after dosing, results for TLUS favored loperamide; however, after the first 10 h, results favored rifaximin-containing regimens. An initial loperamide response was also observed for median number of stools during the first 0-24 h: R 2; L 1; R/L 1 (P=0.002). 48 subjects had a TLUS of 0 h: R10; L 15; R/L 23 (P=0.049). While loperamide alone conferred rapid symptomatic improvement in travelers' diarrhea, the effect was transient, with a high rate of continuing diarrhea. Rifaximin treatment resulted in clinical cure. The combination of rifaximin and loperamide, which together confer rapid initial symptomatic improvement coupled with clinical cure, may be the optimal treatment for non-dysenteric travelers' diarrhea.

214539: Successful Therapy of Unspecific Prolonged Diarrhoea in Infants and Toddlers with the Probiotic E. coli Nissle 1917. *Jobst R Henker, Boris Blokhin, Jury Bolbot, Vitaly Maydannik, Lars Joeres, Corinna Wolff, Juergen Schulze*

Background: Infants and toddlers with prolonged diarrhoea over several days are in danger of developing dehydration and an acute deterioration of their general state of health. So far no effective causal therapy exists. Therefore in a confirmatory, randomized, double-blind clinical trial the efficacy of the probiotic bacterial strain E. coli Nissle 1917 (EcN) as compared to a placebo was tested. Patients and Methods: In total, 151 children aged 1 month to 47 months (Ø 25 months) with unspecific prolonged diarrhoea (> 3 watery or loose stools without blood in 24 hours of a diarrhoeal episode, which has been persisting for more than 4 consecutive days but not longer than 14 days) were randomized in a double-blind design to receive either the probiotic EcN suspension (n = 75) or placebo (n = 76). All children were dehydrated to a medium extent (5 - 10% loss of body weight) and received 1 to 3 ml verum or placebo suspension orally per day depending on the age (1 ml suspension contained 108 viable EcN bacteria) for 21 days. At study commencement, rehydration (ORL according to WHO) was performed once. Results: The number of patients showing a reduction of stool frequency to less than 3 watery or loose stools in 24 hours over a period of at least 4 consecutive days (response rate) was higher in the EcN group than in the placebo group already on day 7 (EcN 78.7%, placebo 59.2%) The response rate increased continuously as was measured on days 14 (EcN 93.3%, placebo 65.8%) and 21 (EcN 98.7%, placebo 71.1%). The two-sample test of rates following a group sequential test design showed statistically significant superiority for EcN on both days 14 (p = 0.0017) and 21 (p<0.0001). Conclusion: Prolonged diarrhoea can be successfully treated. Due to its excellent efficacy the probiotic EcN is a suitable remedy. Keywords: prolonged diarrhoea, infants, probiotic, Escherichia coli Nissle 1917

226136: Nitazoxanide in the Treatment of Clostridium difficile Associated Disease. *Daniel M Musher, Nancy Logan, Richard Hamill, Herbert L DuPont, Arnold Lentnek, Arvind K Gupta, Jean-Francois Rossignol*

Clostridium difficile associated disease (CDAD) has emerged as a major nosocomial infection in the developed world, having increased in incidence and prevalence, as well as in severity. Failure of treatment with metronidazole has increasingly been

documented. Concern over vancomycin-resistant organisms in the hospital environment has motivated the search for an alternative therapy. *Methods.* We carried out a prospective, randomized, double-blind study comparing metronidazole with nitazoxanide in treating hospitalized patients with CDAD. Inpatients from 7 hospitals in the United States were eligible for inclusion if they had diarrhea (≥ 3 unformed stools in 24 hr), a positive EIA for *C. difficile*, and fever, abdominal pain or leukocytosis. Patients were excluded if they had other known causes of diarrhea, were currently taking intravenous metronidazole or had been treated with more than 2 doses of oral metronidazole or vancomycin within the preceding 7 days. Patients were randomized to receive metronidazole 250 mg 4 times daily for 10 days, nitazoxanide 500 mg twice daily for 7 days or nitazoxanide twice daily for 10 days in double-blinded fashion. Response was evaluated after 7 days of treatment as either “well” (resolution of all symptoms of CDAD at baseline) or “failure”. Secondary efficacy endpoints included sustained response at study day 31, time from first dose to passage of last unformed stool and time from first dose to resolution of symptoms. *Results.* After 7 days of treatment, 28 of 34 (82.4%) patients had responded to metronidazole vs. 68 of 76 (89.5%) who had received nitazoxanide (difference = +7.1%, 95% CI: -7.0%, +25.5%). Thirty-one days after the beginning of treatment, sustained responses were observed in 19 of 33 (57.6%), 25 of 38 (65.8%) and 26 of 35 (74.3%) of patients who had received metronidazole for 10 days, nitazoxanide for 7 days and nitazoxanide for 10 days, respectively ($P=0.34$ for comparison of all groups, $P=0.20$ for comparison of nitazoxanide 10-day and metronidazole groups). The median time to last unformed stool and resolution of symptoms was 3 days for the nitazoxanide and metronidazole treatment groups. All isolates of *C. difficile* collected during the study were susceptible to metronidazole, nitazoxanide and its metabolite, tizoxanide. Median MICs ($\mu\text{g/mL}$) were 0.5 (range 0.25 to 2) for metronidazole, 0.5 (range 0.25 to 1) for both nitazoxanide and tizoxanide, and 4 (range 1 to 8) for vancomycin. *Conclusions.* Nitazoxanide is at least as effective as metronidazole in treating CDAD.