

Literaturservice I-GAP

Bedeutung des Leaky guts in der Immunmodulation

1: Clin Rev Allergy Immunol 2008 Jul;

Atypical p-ANCA in PSC and AIH: A Hint Toward a "leaky gut"?

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Primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH) are enigmatic chronic inflammatory diseases of the liver, which are frequently associated with chronic inflammatory bowel diseases. Both types of liver disease share various distinct autoantibodies such as atypical perinuclear antineutrophil cytoplasmic antibodies (p-ANCA), and thus are considered autoimmune disorders with atypical features. The discovery that atypical p-ANCA recognize both tubulin beta isoform 5 in human neutrophils and the bacterial cell division protein FtsZ has renewed the discussion on the potential role of microorganisms in the pathogenesis of both diseases. In this paper, we review the evidence for microbial infection in PSC and AIH and discuss new concepts how cross-recognition between microbial antigens in the gut and host components by the immune system along with stimulation of pattern recognition receptors might give rise to chronic hepatic inflammatory disorders with features of autoimmunity.

2: Neuro Endocrinol Lett 2008 Jun;29(3)

The cytokine hypothesis of depression: inflammation, oxidative & nitrosative stress (IO&NS) and leaky gut as new targets for adjunctive treatments in depression. Minireview.

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This paper hypothesizes that inflammatory, oxidative and nitrosative (IO&NS) pathways, and an increased translocation of LPS from gram-negative bacteria are causally related to depression following external (psychological) and internal (organic) stressors and that IO&NS pathways are novel targets for antidepressant development. We review that depression is accompanied by an inflammatory reaction as indicated by an increased production of pro-inflammatory cytokines, such as interleukin-1beta (IL-1beta), IL-6, tumour necrosis factor-alpha (TNF-alpha) and interferon-gamma (IFN)-gamma. These cytokines are stress-sensitive and may cause depressive behaviors. The latter may be induced by an increased catabolism of tryptophan, the precursor of serotonin, to neurotoxic TRYCATs (tryptophan catabolites along the indoleamine oxidase pathway). Inflammatory biomarkers are detected in animal models of depression. Newly developed animal models of depression are based on induced inflammation. Most if not all antidepressants have specific anti-inflammatory effects. Anti-inflammatory

compounds may augment the clinical efficacy of antidepressants. Depression is also accompanied by an IgM-related (auto)immune response directed against disrupted lipid membrane components, such as phosphatidyl-inositol, by-products of lipid peroxidation, e.g. azelaic acid and malondialdehyde, and NO-modified amino-acids, which are normally not detected by the immune system but due to damage caused by O&NS have become immunogenic. Increased translocation of lipopolysaccharide from gram-negative bacteria, which may be induced by internal and external stressors, may further aggravate the induced IO&NS pathways. Future research to disentangle the complex pathophysiology of depression calls for a powerful paradigm shift, i.e. using a high throughput screening according to the translational medicine methodology.

3: Top HIV Med 2007;15(4):114-7

HIV Disease Progression: Immune Activation, Microbes, and a Leaky Gut.

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Recent findings indicate that the majority of all CD4+ T lymphocytes are lost during acute HIV infection, with mucosal compartments being most severely affected. **The frequency of infection is very high in gut CD4+ T cells, and depletion of these cells persists into the chronic phase of infection. Infection is associated with increased gut permeability,** with microbial translocation being evidenced by increased circulating lipopolysaccharide (LPS) levels. Plasma LPS levels correlate with systemic immune activation, which drives chronic HIV infection. Antiretroviral therapy reduces plasma LPS, and greater CD4+ T cell reconstitution is associated with lower LPS levels. These findings have a number of implications for therapeutic strategies. This article summarizes a presentation on HIV disease progression made by Daniel Douek, MD, PhD, at an International AIDS Society-USA Continuing Medical Education course in San Francisco in May 2007.

4: Neuro Endocrinol Lett 2008 Feb;29(1)

The gut-brain barrier in major depression: Intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression.

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There is now evidence that major depression (MDD) is accompanied by an activation of the inflammatory response system (IRS) and that pro-inflammatory cytokines and lipopolysaccharide (LPS) may induce depressive symptoms. The aim of the present study was to examine whether an increased gastrointestinal permeability with an increased translocation of LPS from gram negative bacteria may play a role in the pathophysiology of MDD. Toward this end, the present study examines the serum concentrations of IgM and IgA against LPS of the gram-negative enterobacteria, *Hafnia Alvei*, *Pseudomonas Aeruginosa*, *Morganella Morganii*, *Pseudomonas Putida*, *Citrobacter Koseri*, and *Klebsiella Pneumoniae* in MDD patients and normal

controls. We found that the prevalences and median values for serum IgM and IgA against LPS of enterobacteria are significantly greater in patients with MDD than in normal volunteers. These differences are significant to the extent that a significant diagnostic performance is obtained, i.e. the area under the ROC curve is 90.1%. The symptom profiles of increased IgM and IgA levels are fatigue, autonomic and gastro-intestinal symptoms and a subjective feeling of infection. **The results show that intestinal mucosal dysfunction characterized by an increased translocation of gram-negative bacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. It is suggested that the increased LPS translocation may mount an immune response and thus IRS activation in some patients with MDD and may induce specific "sickness behaviour" symptoms.** It is suggested that patients with MDD should be checked for leaky gut by means of the IgM and IgA panel used in the present study and accordingly should be treated for leaky gut.

5: Hepatology 2006 Apr;43(4):715-22

Is a leaky gut involved in the pathogenesis of intrahepatic cholestasis of pregnancy?

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Increased gastrointestinal permeability has been demonstrated in several liver diseases. It may facilitate the absorption of gut-derived endotoxin-stimulating Kupffer cells to release proinflammatory cytokines or other potentially hepatotoxic compounds. We examined gastrointestinal permeability, plasma levels of anti-lipopolysaccharides (anti-LPS), and four proinflammatory cytokines in 20 patients with intrahepatic cholestasis of pregnancy (ICP) compared with 22 normal pregnant and 29 non-pregnant women. Urinary excretion of sucrose and the urinary lactulose/mannitol (L/M) ratio after a standard oral load were used to assess gastrointestinal permeability. Anti-LPS (IgA, IgM, and IgG) were measured in peripheral blood by Human EndoCAb test kit; TNF-alpha, IL-1beta, IL-6, and IL-10 by Quantikine HS human immunoassays. Sucrose urinary excretion was similar in the three groups, indicating normal gastric permeability. The urinary L/M ratio was significantly higher in ICP than in the other groups [median (interquartile range): 0.018% (0.011-0.023) in ICP, 0.012% (0.009-0.016) in normal pregnancies, and 0.009% (0.008-0.012) in non-pregnant women, $P < .01$]. No significant differences were found in anti-LPS or cytokines plasma levels except slightly higher levels of IL-6 in ICP patients than in non-pregnant women ($P < .05$). Four of five women with abnormal urinary L/M ratio during ICP continued to show abnormalities in tests up to 2 years after delivery. **In conclusion, an increased intestinal permeability was detected in ICP patients during and after pregnancy. A "leaky gut" may participate in the pathogenesis of ICP by enhancing the absorption of bacterial endotoxin and the enterohepatic circulation of cholestatic metabolites of sex hormones and bile salts.**

6: Eur J Immunol 1995 Jun;25(6):1643-53

A gut-homing, oligoclonal CD4+ T cell population in severe-combined immunodeficient mice expressing a rearranged, transgenic class I-restricted alpha beta T cell receptor.

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We studied the peripheral T cell compartment of H-2b severe combined immunodeficient (scid) mice that express a transgenic (tg) alpha beta T cell receptor (TcR) specific for the H-Y (male) epitope presented by the H-2 class I Db molecule. Large populations of CD3+ NK1.1-TcR beta T+ T cells were present in spleen, mesenteric lymph nodes, peritoneal cavity, lamina propria and epithelial layer of the small and large intestine of 6- to 10-month-old, male and female tg scid mice. Only low numbers of CD3+ T cells were recovered from inguinal, popliteal, or axillary lymph nodes. We studied CD4+ T cells in these tg scid mice. CD4+ T cells were found in the peritoneal cavity, in the mesenteric lymph nodes and in the intraepithelial layer and lamina propria of the gut. All CD4+ T cells were CD44+ (i.e. showed evidence of antigen-driven differentiation) and expressed the tg V beta 8.2 TcR beta-chain (TcR beta T+). Only few CD4+ T cells expressed the tg V alpha 3+ TcR alpha-chain (TcR alpha T). cDNA was prepared from CD4+ T cells from spleen or mesenteric lymph nodes of individual male and female tg scid mice; sequence analyses of polymerase chain reaction-amplified, endogenous TcR alpha-chain (TcR alpha E) transcripts indicated that > 90% of the TcR alpha E-chain transcripts were in-frame, that the TcR alpha E repertoire in CD4+ T cell populations was oligoclonal, and that the TcR alpha E repertoire was different in individual tg scid mice. Hence, an oligoclonal, leaky CD4+ T cell population is selected in tg scid mice that apparently responds to gut-derived antigens. No inflammatory bowel disease (IBD) was evident in the small or large intestine of 6- to 10-month old tg scid mice. After adoptive transfer of purified CD4+ T cells (10⁵ cells per mouse) from tg scid mice into non-tg H-2b scid mice, CD4+ TcR alpha T-beta T+ cells were found in gut tissues of the immunodeficient host. Transplanted scid mice developed clinical and histological signs of IBD. An oligoclonal, gut-homing, memory/effector CD4+ CD44+ TcR beta T+ TcR alpha T-T cell subset from leaky tg scid mice thus has a **pathogenic potential when released from the control of TcR beta T+ TcR alpha T+ T cells.**

7: Rev Esp Enferm Dig 2006 Jun;98(6):460-72

Kupffer cells and alcoholic liver disease.

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Liver disease is a major cause of illness and death worldwide. A central component in the complex network leading to the development of alcoholic liver disease is the activation of Kupffer cells by endotoxin and other soluble mediators. Alcohol consumption induces a state of "leaky gut" increasing plasma and liver endotoxin levels. When Kupffer cells become activated, they interact with a complex of proteins located on the extracellular membrane signaling to produce a wide array of soluble factors, including cytokines, chemokines, growth factors, cyclooxygenase and lipoxygenase metabolites, and reactive oxygen species such as superoxide anion, hydrogen peroxide, and nitric oxide, all of which provide physiologically diverse and pivotal paracrine effects on all other liver cell types and, ultimately, liver injury. Kupffer cells are also central to the liver homeostatic response to injury as upon cellular degenerative changes, they immediately respond to the insult and release mediators to orchestrate inflammatory and reparative responses. Thus, the homeostatic

responses are initiated by Kupffer cell-derived mediators at the cellular level and underlie the liver's defense and reparative mechanisms against injury. In order to understand better the role of Kupffer cells in the onset of liver injury, animal models in which Kupffer cells are inactivated, and cell culture settings (e.g. co-cultures) are being used with promising results that advance our understanding of alcoholic liver disease.

8: Chir Ital 1993;45(1-6):73-6

[Propedeutic to general problems of the hypothesis of a new "open" interpretation of firearm wounds of soft tissues]

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On the basis of a review of the literature and their own personal knowledge and experience, the authors define the state of the art regarding a point of considerable importance, **namely the leaky gut hypothesis**. Taking gunshot wounds in soft tissues as their starting point, they believe that such lesions are among the most suitable for illustrating the chain of events which translates an entirely local pathology--admittedly serious--into a systemic pathology carrying a very severe prognosis, if the physician is unable to interrupt this clinical course.

Immunomodulation mit Mutaflor

1: Vnitr Lek 2006 Mar;52(3):215-9

[Effect of administration of Escherichia coli Nissle (Mutaflor) on intestinal colonisation, endo-toxemia, liver function and minimal hepatic encephalopathy in patients with liver cirrhosis]

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The purpose of the study was to verify effects of Escherichia coli Nissle (Mutaflor) on intestinal colonisation, endotoxin levels, hepatic encephalopathy and liver function in patients with liver cirrhosis. The study involved 39 patients (22 taking Mutaflor and 17 taking placebo). Even though the number combination test showed extended reaction time in patients with described minimal hepatic encephalopathy the drop was not significant in the trend evaluation. However, the treated group displayed significant improvement of intestinal colonisation ($p < 0.001$) and a trend towards significant reduction of endotoxin levels on day 42 ($p = 0.07$) and improvement of liver function assessed with the Child-Pugh classification on days 42 and 84 ($p = 0.06$). **Probiotic preparations can therefore represent a significant contribution to this group therapy.**

2: Fortschr Med 1989 Sep;107(28):599-602

[The treatment of intestinal diseases with Mutaflor. A multicenter retrospective study]

E Schütz

The preparation Mutaflor contains a defined strain of physiological E. coli bacteria. One hundred and sixty-seven physicians documented the treatment of 1,074 patients suffering from functional or chronic inflammatory bowel disease. For all indications the tolerance of Mutaflor was judged to be good to very good in over 90% of the cases. Adverse reactions which needed treatment or led to termination of the therapy, were reported in 1.5% of the cases. Initially occurring side effects, which did not need treatment and disappeared spontaneously, were observed in 2.8% of the cases. Interactions with additional or concomitant drugs did not occur. As a parameter for the evaluation of efficacy, the subjective assessment of the results by physicians and patients was used. In 84% of the patients with functional intestinal disorders and 78% of the patients with chronic inflammatory bowel disease results of treatment were judged to be good to very good.

4: Med Klin (Munich) 1994 Nov;89(11):587-93

[Treatment of chronic constipation with physiologic Escherichia coli bacteria. Results of a clinical study of the effectiveness and tolerance of microbiological therapy with the E. coli Nissle 1917 strain (Mutaflor)]

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AIM: A randomized, double-blind clinical trial including a change-over of medication was carried out for 9 weeks to investigate the efficacy of an E. coli preparation. The study's main objective was to prove that patients of the verum group had 1.5 stools/week more than placebo patients after a therapeutic period of just 4 weeks. Stool consistency as well as efficacy and compatibility of the medication as judged by doctor and patient were additional criteria. PATIENTS AND METHOD: For a 7-day run-in phase 134 patients were recruited who had suffered from constipation for 18.8 years in average. In this initial phase 64 patients evacuated more than 2 stools per week and were excluded from the study. The remaining 70 patients entered the therapeutic phase being randomly distributed amongst verum and placebo medication. After 4 weeks of therapy patients who delivered 2 or less stools/week obtained the alternative medication (change-over). RESULTS: Within the 4th week of therapy the average number of stools per week from patients treated with the E. coli preparation (4.9) was already significantly higher than from placebo-treated patients (2.6; $p < 0.001$). At the end of the 8th week of therapy the number of stools/week rose to 6.0 for verum-treated patients, whereas for the placebo-treated control group a decrease in stool frequency was observed (1.9 stools/week). The results of change-over patients confirmed the data of the therapy weeks 1 to 4. CONCLUSION: The E. coli preparation proved to be successful in the therapy of the idiopathic chronic constipation almost free of side effects.

8: Eur J Gastroenterol Hepatol 2007 Dec;19(12):1111-3

The effect of probiotics on gut flora, level of endotoxin and Child-Pugh score in cirrhotic patients: results of a double-blind randomized study.

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OBJECTIVE: To determine the effect of Escherichia coli Nissle (Mutaflor, Ardeypharm GmbH, Herdecke, Germany) on the intestinal colonization, level of endotoxin and liver functions in patients with liver cirrhosis. METHODS: Thirty-nine patients with liver cirrhosis diagnosed by means of biopsy and clinical examinations were randomly allocated to treatment with E. coli Nissle or placebo for 42 days. Standard clinical examination, biochemical and hematological examinations, level of endotoxin and microbiological examination of the stool were performed before and after the treatment. RESULTS: In comparing the treatment of E. coli Nissle and placebo, significant improvement of the intestinal colonization ($P < 0.001$) in the E. coli Nissle group was described. We found a trend of significant lowering of the endotoxemia ($P = 0.07$) and improvement of liver functions evaluated by Child-Pugh score ($P = 0.06$). CONCLUSION: E. coli Nissle seems to be effective in the restoration of normal colonic colonization and can probably lower endotoxemia in cirrhotic patients.

9: Med Klin (Munich) 2007 Nov;102(11):888-892

[Treatment of Irritable Bowel Syndrome with Escherichia coli Strain Nissle 1917 (EcN). A Retrospective Survey.]

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BACKGROUND AND PURPOSE: In many patients with irritable bowel syndrome (IBS), one symptom is predominant (e.g., diarrhea, constipation, meteorism, or alternating stool consistency). For IBS therapy, probiotic drugs such as Mutaflor((R)) (active ingredient: Escherichia coli strain Nissle 1917 [EcN]) are also being used. A systematic survey on the efficacy and safety of EcN in different IBS subgroups is still missing. PATIENTS AND METHODS: In a gastroenterologic outpatient practice, results of 150 IBS patients treated with EcN were evaluated retrospectively. Most IBS patients enrolled belonged to the subgroups "diarrhea", "meteorism", and "alternating stool consistency". RESULTS: Regarding the diarrhea subgroup, not only a statistically significant improvement in stool frequency was observed, but also a marked improvement in concomitant symptoms such as abnormal urge, flatulence, and abdominal fullness. These complaints improved under EcN therapy in the other IBS subgroups as well, so that efficacy was assessed as "good to very good" in 73.4% of all cases. Tolerance to treatment was "good to very good" in 97.9% of the cases. CONCLUSION: The results point to the possibility of EcN being a therapeutic option for patients of various IBS subgroups which is almost free of side effects.

10: Wien Klin Wochenschr 2007 Aug;119(15-16):456-462

Are probiotics detectable in human feces after oral uptake by healthy volunteers?

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GOALS: Assessment of the presence of probiotic bacteria in feces after oral ingestion. BACKGROUND: Probiotic bacteria are said to have beneficial effects on the host. As a precondition for any effect, probiotic strains must survive passage through the gastrointestinal tract. STUDY: The feces of seven volunteers were analyzed for the presence of probiotic strains after one week's oral ingestion of each of six commercially available products: *E. coli* Nissle 0.5-5 x 10⁹ cells (Mutaflor((R))), *Enterococcus faecium* SF 68 7.5 x 10⁷ cells (Bioflorin((R))), *Lactobacillus acidophilus* and *Bifidobacterium infantis* both 1 x 10⁹ cells (Infloran((R))), *Lactobacillus gasseri* and *Bifidobacterium longum* both 1 x 10⁸ cells (Omniflora((R))), *Lactobacillus casei rhamnosus* 1 x 10⁹ cells (Antibiophilus((R))), and yoghurt enriched with *Lactobacillus casei* Immunitas 1 x 10¹⁰ cells (Actimel((R))). Ten colonies were selected from each stool sample, and DNA was extracted and typed using random amplification of polymorphic DNA (RAPD). Typing patterns of the ingested probiotics and the fecal isolates were compared. RESULTS: Fingerprints identical to the ingested probiotic strains were recovered from fecal samples of 4/7 volunteers after one week of Mutaflor((R)), from 4/6 after taking Bioflorin((R)), and from 1/6 after Infloran((R)). Cultivation of strains of the same species from fecal specimens was negative after consumption of *Antibiophilus*((R)), *Omniflora*((R)) and *Actimel*((R)). CONCLUSIONS: After oral consumption of probiotics, *E. coli* and enterococci could be detected in stool samples (57% and 67%, respectively). In contrast, with only one exception, ingested lactobacilli and bifidobacteria could not be detected in human feces.

11: MMW Fortschr Med 2006 Aug;148(35-36):30-4

[Probiotics in chronic inflammatory bowel disease]

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Current data show that probiotics are more effective in preventing the recrudescence of an inflammatory process than in suppressing active disease. This is reflected in the solid evidence for the effect of *E. coli* Nissle 1917 (Mutaflor) in the maintenance of remission of ulcerative colitis, and of VSL#3 in preventing the recurrence of pouchitis. These indications have since been incorporated in valid guidelines. Initial clinical studies have also provided promising results regarding the efficacy of VSL#3 in preventing pouchitis immediately following proctocolectomy.

12: Infect Immun 2004 May;72(5):2879-88

Functional transfer of Salmonella pathogenicity island 2 to Salmonella bongori and Escherichia coli.

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The type III secretion system (T3SS) encoded by the Salmonella pathogenicity island 2 (SPI2) has a central role in systemic infections by Salmonella enterica and for the intracellular phenotype. Intracellular S. enterica uses the SPI2-encoded T3SS to translocate a set of effector proteins into the host cell, which modify host cell functions, enabling intracellular survival and replication of the bacteria. We sought to determine whether specific functions of the SPI2-encoded T3SS can be transferred to heterologous hosts Salmonella bongori and Escherichia coli Mutaflor, species that lack the SPI2 locus and loci encoding effector proteins. The SPI2 virulence locus was cloned and functionally expressed in S. bongori and E. coli. Here, we demonstrate that S. bongori harboring the SPI2 locus is capable of secretion of SPI2 substrate proteins under culture conditions, as well as of translocation of effector proteins under intracellular conditions. An SPI2-mediated cellular phenotype was induced by S. bongori harboring the SPI2 if the sifA locus was cotransferred. An interference with the host cell microtubule cytoskeleton, a novel SPI2-dependent phenotype, was observed in epithelial cells infected with S. bongori harboring SPI2 without additional effector genes. S. bongori harboring SPI2 showed increased intracellular persistence in a cell culture model, but SPI2 transfer was not sufficient to confer to S. bongori systemic pathogenicity in a murine model. Transfer of SPI2 to heterologous hosts offers a new tool for the study of SPI2 functions and the phenotypes of individual effectors.

13: FEMS Immunol Med Microbiol 2004 Apr;40(3):223-9

The probiotic Escherichia coli strain Nissle 1917 interferes with invasion of human intestinal epithelial cells by different enteroinvasive bacterial pathogens.

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The probiotic Escherichia coli strain Nissle 1917 (Mutaflor) of serotype O6:K5:H1 was reported to protect gnotobiotic piglets from infection with Salmonella enterica serovar Typhimurium. An important virulence property of Salmonella is invasion of host epithelial cells. Therefore, we tested for interference of E. coli strain Nissle 1917 with Salmonella invasion of INT407 cells. Simultaneous administration of E. coli strain Nissle 1917 and Salmonella resulted in up to 70% reduction of Salmonella invasion efficiency. Furthermore, invasion of Yersinia enterocolitica, Shigella flexneri, Legionella pneumophila and even of Listeria monocytogenes were inhibited by the probiotic E. coli strain Nissle 1917 without affecting the viability of the invasive bacteria. The observed inhibition of invasion was not due to the production of microcins by the Nissle 1917 strain because its isogenic microcin-negative mutant SK22D was as effective as the parent strain. Reduced invasion rates were also achieved if strain Nissle 1917 was separated from the invasive bacteria as well as from the INT407 monolayer by a membrane non-permeable for bacteria. **We conclude E. coli Nissle 1917 to interfere with bacterial invasion of INT407 cells via a secreted**

component and not relying on direct physical contact with either the invasive bacteria or the epithelial cells.

14: Aliment Pharmacol Ther 2003 Jul;18(1):45-56

Inhibitory effect of probiotic Escherichia coli strain Nissle 1917 on adhesion to and invasion of intestinal epithelial cells by adherent-invasive E. coli strains isolated from patients with Crohn's disease.

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BACKGROUND: Pathogenic adherent-invasive Escherichia coli have been isolated from ileal lesions of Crohn's disease. AIM: : To investigate the non-pathogenic E. coli strain Nissle 1917 (Mutaflor) as possible maintenance therapy in inflammatory bowel disease by testing its ability to prevent adherent-invasive E. coli strains from adhering to and invading human intestinal epithelial cells in vitro. METHODS: Bacterial adhesion to and invasion of intestinal epithelial cells (Intestine-407) were assessed by counting the colony-forming units. The inhibitory effect of E. coli Nissle 1917 was determined after co-incubation with adherent-invasive E. coli strains or after pre-incubation of the intestinal epithelial cells with this probiotic strain prior to infection with adherent-invasive E. coli strains. RESULTS: Strain Nissle 1917 exhibited dose- and time-dependent adherence to intestinal epithelial cells and inhibited the adhesion and invasion of various adherent-invasive E. coli strains. In co-infection experiments, the inhibitory effect on adherent-invasive E. coli adhesion reached 78-99.9%. Pre-incubation of intestinal epithelial cells with strain Nissle 1917 reduced adherent-invasive E. coli adhesion by 97.2-99.9%. The inhibitory effect on adherent-invasive E. coli invasion paralleled that on adhesion. CONCLUSION: **As strong and significant inhibitory effects on adherent-invasive E. coli adhesion and invasion were observed in co-infection and pre-infection experiments,** E. coli Nissle 1917 could be efficient for preventive or curative probiotic therapy in patients with Crohn's disease.

15: Eur J Gastroenterol Hepatol 2003 Mar;15(3):313-5

The effect of non-pathogenic Escherichia coli in symptomatic uncomplicated diverticular disease of the colon.

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BACKGROUND: The effect of probiotics in symptomatic uncomplicated diverticular disease of the colon has not been followed. DESIGN: Treatment (T1) with an intestinal antimicrobial (dichlorochinolol) and absorbent (active coal tablets) was compared with the same set-up supplemented with non-pathogenic Escherichia coli(T2) in a prospective open trial. SETTING: The study was performed at the outpatient department of a tertiary centre. PARTICIPANTS: Fifteen subjects (5 males, 10 females) aged 68-91 years (average 74.8 years) presented with abdominal pain, irregular defecation, bloating and excessive flatulence. Diagnosis was established with

colonoscopy, double-contrast barium enema, or both. INTERVENTIONS: The T1 regimen was administered for 1 week. In the T2 regimen, the application of *E. coli* strain Nissle (Mutaflor capsules, 2.5×10^{10} viable bacteria/capsule) followed immediately after T1 for an average of 5.2 weeks. MAIN OUTCOME MEASURES: The lengths of two successive remissions with the T1 set-up were compared with the length of remission after T2. The intensity of symptoms before and after administration of the probiotic was also evaluated. RESULTS The lengths of two successive remissions after T1 amounted to 2.66 and 2.20 months (average 2.43 months). The average length of remission after T2 was 14.1 months ($P < 0.001$). All symptoms after T2 decreased significantly ($P < 0.001$). CONCLUSIONS: Non-pathogenic strain Nissle significantly prolonged the remission period and improved the abdominal syndrome in symptomatic uncomplicated diverticular disease. A randomized, placebo-controlled study is recommended.

16: Vet Q 1998;20 Suppl 3:S78-81

Probiotics and *E. coli* infections in man.

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After oral administration of live oral vaccines COLINFANT and MUTAFLOR prepared from non-enteropathogenic *E. coli* strains, both strains colonized effectively the intestine in full-term and preterm infants and remained for many weeks showing, that they were capable to establish themselves as a resident strain in the infant's gut. The presence of *E. coli* stimulated significantly antibody production in gut, saliva and serum of colonized infants. An early induction of secretory IgA production is important particularly in formula-fed infants, where it partly replaces the lacking immunoglobulin supplied with mother milk. In full-term and premature infants the early presence of non-pathogenic *E. coli* strains in the intestine decreased significantly the presence of pathogenic bacterial strains in the intestine but also other mucosal surfaces of the body. The COLINFANT strain decreased the number of nosocomial infections, mortality rate in connection with infection, and the need for antibiotics. Both strains replaced successfully pathogenic strains in carriers after treatment with antibiotics.

17: Arzneimittelforschung 1997 Jun;47(6):793-6

Augmentation of host defence against bacterial and fungal infections of mice pretreated with the non-pathogenic *Escherichia coli* strain Nissle 1917.

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Escherichia coli strain Nissle 1917 (DSM 6601, Mutaflor) was investigated for its ability to enhance the immune response against bacterial or fungal infections in vivo. Mice were infected intravenously with either 6×10^3 colony forming units (cfu) of *Listeria monocytogenes* bacteria or 5×10^5 *Candida albicans* cells. One day prior to infection, mice were treated

orally with four different concentrations of *E. coli* strain Nissle 1917 (10^6 , 10^7 , 10^8 , and 10^9 viable cells). Three days after infection with *L. monocytogenes* or one day after infection with *C. albicans*, mice were sacrificed and the parasite burden of the main target organs of the respective infection model were examined. The protective effect of *E. coli* strain Nissle 1917, compared to placebo-treated controls and to mice treated with a dose of 10^4 . Units interferon gamma, is shown as the reduction of viable bacteria in spleen and liver or viable fungi in the kidneys of infected animals, respectively. Orally administered *E. coli* strain Nissle 1917 reduced *Listeria monocytogenes* and *Candida albicans* in a dose-dependent manner. Treatment with 10^9 cfu of *E. coli* bacteria led to a reduction of *Listeria* counts to 7.4% in spleen and 2.4% in liver. A more than 10-fold decrease of viable *Candida albicans* (residual parasitaemia 6.8%) in the kidneys of the infected animals was also achieved by this *E. coli* concentration. These results suggest that *E. coli* strain Nissle 1917 is a potent immunostimulator of bacterial origin with highly protective efficacy against pathogenic bacterial of fungal infections.

18: Biol Neonate 1997;71(4):224-32

Effect of preventive administration of a nonpathogenic *Escherichia coli* strain on the colonization of the intestine with microbial pathogens in newborn infants.

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Institute for Care of Mother and Child, Prague, Czech Republic.

In a randomized, double-blind study, 27 healthy newborn infants were colonized with the nonpathogenic *Escherichia coli* strain Nissle 1917 (*E. coli* DSM 6601, Mutaflor) during the first 5 days of life by daily oral inoculation of 1 ml of a suspension with 10^8 living cells. A second group of 27 newborns, used as controls, received a placebo suspension (1 ml of phosphate-buffered saline) instead. Stool samples were taken on days 1, 2, 3, 5, and 21, and 6 months after birth. All samples were examined for the presence of the nonpathogenic *E. coli* strain and of pathogenic and potentially pathogenic microorganisms. The administered *E. coli* strain was detected in the stools of the colonized newborns from day 2 and remained present throughout the study in more than 90% of these infants.

Colonization with true and potential bacterial pathogens was significantly reduced in infants receiving *E. coli* strain Nissle 1917 compared to the placebo group--both with respect to numbers of pathogens and to the spectrum of species.

Immunomodulation Symbioflor

1: Int J Med Microbiol 2007 Apr;

Comparative genomic analysis for the presence of potential enterococcal virulence factors in the probiotic *Enterococcus faecalis* strain Symbioflor 1.

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Enterococci are members of the natural microbiota of animal and human intestinal tracts and are capable of causing opportunistic infections. They are also used as starter cultures in the food industry as well as in health supplements and probiotics by the pharmaceutical industry. This Janus-faced status requires a careful evaluation on the basis of pathogenic traits to ensure the safety of the strain used to produce food and pharmaceuticals. We performed gapped-genome sequencing of a probiotic strain *Enterococcus faecalis* Symbioflor 1 and present initial results deriving from comparative genome analysis with that of the previously sequenced pathogenic clinical isolate *E. faecalis* V583. There was strong overall conservation of synteny between both strains and a detailed analysis revealed the absence of large genomic regions from the chromosome of the probiotic strain, indicating gene loss. Genes absent from the Symbioflor 1 strain included those encoding the enterococcal cytolysin, enterococcal surface protein, and gelatinase (coccolysin) as well as hyaluronidase and the peptide antibiotic AS-48. This data was confirmed using PCR primers specific for the respective genes. However, other enterococcal determinants such as aggregation substance, collagen adhesion protein, the ability to resist oxygen anions as well as capsule formation were detected. The presence of these traits may be advantageous for the strain Symbioflor 1 since they potentially enable colonization and proliferation of the bacterium on mucosal surfaces thereby conferring on it probiotic traits.

2: *Arzneimittelforschung* 2003;53(5):385-91

Functional characterization of pro-biotic pharmaceuticals by quantitative analysis of gene expression.

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Functional characterization and quality control of complex biological pharmaceuticals are currently difficult to achieve. Classical analytical methods are not suitable due to the heterogeneity of such substances. Conventional biological assays based on the detection of proteins and functional physiologic responses are highly variable in sensitivity and therefore, difficult to standardize. The quantification of expressed genes in contrast, does not require the accumulation of measurable protein and hence is a more sensitive and dynamic method for the functional characterization of complex biological substances. This report describes a standardized system based on real-time polymerase chain reaction (PCR), which allows a reliable stability monitoring and quality control of such preparations, as exemplified by three pro-biotic pharmaceuticals that contain living bacteria of *Enterococcus faecalis* (Symbioflor-1), *Escherichia coli* (Symbioflor-2) and a preparation of sterile autolysate of both species (Pro-Symbioflor). This system might be universally applicable to characterize complex biological pharmaceuticals by selecting appropriate sets of target cells and regulated genes.

3: *Arzneimittelforschung* 2002;52(8):622-7

[Reduction of acute recurrence in patients with chronic recurrent hypertrophic sinusitis by treatment with a bacterial immunostimulant (*Enterococcus faecalis* Bacteriae of human origin)]

Habermann W, Zimmermann K, Skarabis H, Kunze R, Rusch V

Institut für Mikroökologie, Herborn-Hörsbach, Germany.

A double-blind, placebo-controlled multicenter study in 157 patients with chronic recurrent sinusitis investigated the occurrence of acute relapses during treatment of patients with a bacterial immunostimulant (3 x 30 drops/day), comprised of cells and autolysate of human *Enterococcus faecalis* bacteria (Symbioflor 1, n = 78) in comparison to placebo (n = 79). The study included a treatment period of 6 months and a follow-up period of 8 months. Under verum the occurrence of relapses (50 incidents) was about half (56%) the number observed under placebo (90 incidents). In the Kaplan-Meier test the verum preparation emerged as significantly superior (p = 0.045, log rank test) compared to placebo. This superiority of verum was found during the treatment period with 17 vs. 33 relapses (p = 0.019) as well as during the follow-up observation with 33 vs. 57 relapses (p = 0.013). The time interval to the first relapse was clearly longer under verum (513 days) than under placebo (311 days). **The relative risk for a relapse under the test preparation compared to placebo was 49.0% during the treatment and 55.8% during the follow-up period. Severity of the acute relapses was comparable in both groups. However, antibiotic therapy was only required in 2 patients treated with verum compared to 6 patients in the placebo group.** Both preparations were well tolerated and serious side effects did not occur in either group. No changes in laboratory tests--hematology and clinical chemistry--were observed. Potential immunomodifying effects of the test preparation in view of the significant reduction in relapses were discussed.

4: Arzneimittelforschung 2001 Nov;51(11):931-7

[The effect of a bacterial immunostimulant (human *Enterococcus faecalis* bacteria) on the occurrence of relapse in patients with]

Habermann W, Zimmermann K, Skarabis H, Kunze R, Rusch V

Institute für Mikroökologie, Herborn-Hörsbach, Berlin.

The following double-blind, placebo-controlled, multicenter study investigated the influence of a bacterial immunostimulant (Symbioflor 1, cells and autolysate of human *Enterococcus faecalis*) on the occurrence of relapses in patients with chronic recurrent bronchitis (n = 136; placebo n = 66, verum n = 70) in a 6 months treatment period and a follow-up period of 8 months, compared to placebo. Under verum 39 incidents of relapses were recorded, which was about 60% the number observed among the patients treated with placebo (66 incidents). The verum preparation exhibited superior clinical efficacy compared to placebo (p = 0.001) in the Kaplan-Meier test. This better clinical efficiency of the test preparation was particularly observed during the treatment period, with 12 vs. 27 relapses (p = 0.013), but less during the follow-up observation period, with 27 vs. 39 relapses (p = 0.127). In addition, the time span until occurrence of the first relapse was clearly longer under verum (699 days) than under placebo (334 days) and after the end of the observation period 91% of patients under verum experienced only one relapse compared to 62% in the placebo group (p = 0.01). **Severity of relapses under verum was also reduced significantly (chi 2; p = 0.001. Only 4 patients under verum required antibiotic therapy compared to 13 patients under placebo.** Verum was equally well tolerated as placebo, with no serious side effects in either group. No changes in laboratory tests--haematology and clinical chemistry--were observed. It can be concluded, that previously demonstrated immunomodifying

effects of the test preparation have clinical relevance for the treatment of chronic recurrent bronchitis because not only the number but also the severity of acute relapses could be clearly reduced. This is discussed in view of the current literature.

5: Infection 1998;26(4):232-3

Escherichia coli as a probiotic?

Jansen GJ, Wildeboer-Veloo AC, van der Waaij D, Degener JE

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The influence of oral treatment with a suspension of non-pathogenic Escherichia coli cells (commercially available as: Symbioflor II) on the morphological composition of the gut microflora and on the systemic humoral immune response (the IgG-, IgA- and IgM-isotype) against the bacterial cells in the Symbioflor II preparation was measured. After a pretreatment period of 21 days, ten healthy human volunteers ingested 1×10^8 cells of E. coli daily for 14 days. Thereafter a follow-up period of 28 days completed the study. The results of this study indicated that no effect of the treatment on the composition of the gut microflora could be observed. However, the immune-fluorescence measurements revealed a significant increase in circulating amounts of IgG directed against the administered E. coli cells. It is concluded that the treatment only resulted in a specific humoral immune response, while the gut microflora is not modulated.

6: Arzneimittelforschung 1994 May;44(5):691-5

[Immunomodulator action of living, nonpathogenic Enterococcus faecalis bacteria from humans]

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Immunomodulating Effect of Living Nonpathogenic Enterococcus faecalis Originated from Humans Symbioflor 1 is a pharmaceutical preparation, consisting of a suspension of living nonpathogenic Enterococcus faecalis (E. faecalis). The effect on the liberation of cytokines of E. faecalis was investigated in in-vitro experiments with human peripheral mononuclear blood cells revealing the following results: 1. E. faecalis stimulates the liberation of interleukin 1 (IL-1 beta) and interleukin-6 (IL-6) in a dose-dependent manner; the E. faecalis induced liberation of IL-1 beta and IL-6 is inhibited by dexamethasone (Dm) but not by cyclosporin A (CsA). 2. E. faecalis stimulates the liberation of gamma-interferon (IFN-gamma) in a dose-dependent manner, which is inhibited by both Dm and CsA. 3. Phytohemagglutinin (PHA)-induced liberation of gamma-IFN and interleukin-2 (IL-2) is inhibited by E. faecalis in a dose-dependent manner. The relevancy to clinical trials of the in vitro results is discussed.

7: Versicherungsmedizin 1997 Oct;49(5):162-6

["Control of symbiosis" or "microbiological therapy"--an immunological therapy principle?]

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"Flora modulation" or "microbiologic therapy"-- an immunologic method of treatment? "Microbiologic therapy" includes the methods of quantitative measurement of the intestinal "microbial flora", the oral or parenteral application of microbial pharmaceuticals and the preparation of autovaccines from excretions of the treated persons. Initially a "flora modulation" was supposed to be the mechanism of "microbiologic therapy". After the failure of this hypothesis, some physicians claim the "microbiologic therapy" to be a special form of immunomodulation or -stimulation. Most influential in this immunologic foundation of "microbiologic therapy" was the "Institut für Mikroökologie" in Herborn, Germany. A detailed analysis of the available publications however reveals, that all methods of "microbiological therapy" are based on theoretically untenable presumptions. **Furthermore, up to now there is no scientific evidence for the effectiveness of this form of therapy.**

Immunmodulation durch Colibiogen

1: Br J Dermatol 1989 Aug;121(2):229-33

Preventive effect of an E. coli-filtrate (Colibiogen) in polymorphous light eruption.

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During the administration of Colibiogen, a commercially available E. coli-filtrate, there was a significant (P less than 0.01) increase of the test dose of UVA necessary to elicit skin lesions in nine patients with polymorphous light eruptions. Control tests performed in the same manner without the drug indicate that this was due to treatment. Colibiogen appears to be of **value in the treatment of polymorphous light eruption (PLE)**.

2: Fortschr Med 1985 Dec;103(46):1076-80

[Treatment of Crohn's disease using Colibiogen. Cellular reactions of the immune system in patients with Crohn's disease]

Auer IO, Röder A, Mittelstaedt A

4: Arzneimittelforschung 2001;51(4):332-8

Double-blind randomised placebo-controlled phase III study of an E. coli extract plus 5-fluorouracil versus 5-fluorouracil in patients with advanced colorectal cancer.

Unger C, Häring B, Kruse A, Thumann A, Schneider B, Clemm C, Weber B, Clevert HD, Hockertz S, Kalousek MB

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The primary aim of this study was to evaluate the toxicity (mucositis, diarrhea and leucopenia) of a therapy with 5-fluorouracil (CAS 51-21-8; 5-FU) plus an E. coli extract (LC-Extract, Laves coli extract, Colibiogen inject, cell-free soluble fraction from lysed E. coli, Laves strain) in comparison with 5-FU plus placebo. Secondary endpoints included general toxicity, response rate according to WHO, survival time and quality of life. 164 patients with advanced colorectal cancer were enrolled in this randomised, placebo-controlled, double-blind, multicenter phase III study. The treatment consisted of 0.167 ml/kg/d LC-Extract or placebo followed by 500-750 mg/m²/d 5-FU on five consecutive days, repeated every three weeks for up to six treatment cycles. 158 (77 verum, 81 placebo) patients were evaluable for toxicity, 144 (72 verum, 72 placebo) evaluable for response. The therapy with LC-Extract was well tolerated. Adverse events that occurred during the study were mainly judged as 5-FU- or tumor-related. Toxicity from treatment with 600 mg/m²/d 5-FU in both treatment groups was very low. After treatment with 750 mg/m²/d 5-FU patients in the placebo-group experienced a higher CTC toxicity than in the LC-Extract groups. **Remission rate and survival time showed a slight trend in favour of LC-Extract. These results suggest a positive benefit-risk ratio of the additional application of LC-Extract to 5-FU in the treatment of advanced colorectal cancer especially for administration of high doses of 5-FU.**

5: Arzneimittelforschung 1995 May;45(5):657-61

Cytokine production by mononuclear cells following stimulation with a peptide-containing, endotoxin-free Escherichia coli extract.

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Borstel Research Institute, Department of Immunology and Cell Biology, Germany.

The beneficial effects of the E. coli extract Colibiogen inj. N (Cb) observed in therapy of inflammatory bowel diseases, allergies, or gastrointestinal tumors are possibly mediated by the induction of cytokines in human leukocytes or vascular cells. Thus, the induction of the cytokines interleukin 1 (IL1), IL6 and tumor necrosis factor (TNF) in human mononuclear cells (MNC) and vascular cells was investigated in vitro. Various administration forms of the extract (including Cb-inj. N, Cb-oral, and Cb-infantibus N) induced the release of IL1 and IL6 from MNC. The compounds stimulated TNF production less potently, possibly due to a lower sensitivity of the TNF assay system, as compared to the IL1 and IL6 detection system. The MNC produced the cytokines with a kinetics similar to that observed with other stimuli. Monospecific antibodies abolished the respective cytokine activity in the biological assays. Addition of submaximal amounts of endotoxin potently enhanced the IL1- and IL6-inducing activity of the bacterial extract, indicating synergism of the extract and

endotoxin. These results provide evidence that cytokines produced by MNC following administration of the tested bacterial extract may contribute to the regulation of the immune response during therapy of gastrointestinal tumors. At present the *in vivo* production of cytokines following treatment with the bacterial extract tested is under investigation in a phase III study.

6: Arzneimittelforschung 1986 Aug;36(8):1216-20

[Existence of an anabolically acting principle in an extract of E. coli]

Schole J, Sallmann HP, Sonnenschein B

Colibiogen, extracted from E. coli (in the following called coli extract) was examined for factors with anabolic efficiency, especially for anabolically efficient bases of nucleic acids and for peptides. The results obtained are the following: Tests for nucleotides, nucleosides and bases of nucleic acids by thin-layer chromatography technique turned out negative. To test anabolically efficient substances the so-called glutathione state test in the rat liver was used. In this test intraportal dosages of 200 micrograms coli extract and also 200 micrograms of the enzymatically decomposed muscle proteins (Pepton resp. Lab Lemco) gave rise to positive effects within 2 min. Contrary to peptides from the culture medium the efficiency of coli extract was considerably increased by previous tryptic fission (efficient concentration 6 micrograms). The quantities applied were related on microgram peptide. A coli extract preparation the phase of growth of which had been shortened to 12 h was separated into 4 fractions. The fourth fraction (lowest molecular weight) showed anabolic efficiency with 6 micrograms peptide in the state test. Before the denaturative extraction took place, the coli extract was separated by centrifugation in a third test series into coli extract bacteria mass and coli extract supernatant. Nothing but the supernatant showed anabolic properties. Two fractions, obtained by the separation of the bacteria mass, did not show any activity in the glutathione state test. It is discussed that E. coli-specific peptides with anabolic efficiency are candidates for the coli extract effects.

Immunmodulation mit Glutamin

1: Zhongguo Wei Zhong Bing Ji Jiu Yi Xue 2006 Oct;18(10):595-8

[Influence of glutamine and growth hormone intensified nutrition support on immunomodulation in critically ill elderly patients]

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OBJECTIVE: To evaluate the impacts of glutamine (Gln) and recombinant human growth hormone (rhGH) intensified nutrition support on critically ill elderly patients. **METHODS:** Ninety critically ill aged patients were included in a prospective, randomized and controlled clinical study, and randomly divided into three groups: group A (standard nutrition support), group B (standard nutrition support+10% Gln 100 ml/d), group C (standard nutrition support+ Gln 100 ml/d+rhGH 10 U/d). Before treatment and then 7 and 14 days after treatment, blood samples were collected for analysis of

serum proteins including albumin (ALB), pre-albumin (PAB), C-reactive protein (CRP), immunoglobulin G (IgG). Meanwhile, the variables including T-cell subsets, CD14 human leukocyte antigen (locus) DR (CD14 HLA-DR), and total lymphocytes were measured. The changes in acute physiology and chronic health evaluation II (APACHE II) and multiple organ dysfunction syndrome (MODS) scores, the durations of intensive care unit (ICU) stay and mechanical ventilation, and 28-day survival rate were recorded. RESULTS: Comparing with group A and B, the levels of serum ALB, PAB and IgG were significantly elevated in group C. The T-cell subsets, CD14 HLA-DR and the number of total lymphocytes were markedly higher in group C ($P < 0.01$), and the APACHE II and MODS scores were decreased significantly in group C ($P < 0.05$ or $P < 0.01$). The levels of serum CRP were lowered significantly in group C ($P < 0.01$). There were no significant differences in the durations of ICU stay, mechanical ventilation and 28-day survival rate among three groups (all $P > 0.05$). CONCLUSION: Gln and rhGH intensified nutrition support can improve nutritional condition and immune function, downregulate the inflammatory response in the critically ill elderly patients.

2: Langenbecks Arch Chir Suppl Kongressbd 1996;113:342-4

[Immunomodulation after parenteral glutamine administration in colorectal surgery]

Morlion BJ, Siedhoff HP, Joosten U, Köller M, König W, Fürst P, Puchstein C

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The influence of parenteral L-alanyl-L-glutamine dipeptide on the cysteinyl-leukotriene (cys-LT) synthesizing capacity from neutrophils was studied in patients undergoing colonic surgery. The decrease in cys-LT, observed postoperatively, could be normalized with parenteral glutamine, while the cys-LT decrease persisted in controls. We conclude that the provision of glutamine in the postoperative state improves normalization of neutrophil functions (e.g., generation of cys-LT), which is an essential prerequisite for host defences.

3: Nutrition 1998;14(7-8):618-26

Role of glutamine in immunologic responses.

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Glutamine has traditionally been thought of as a nonessential amino acid, but laboratory and clinical data suggests that it may be essential during certain inflammatory conditions, such as infection and injury. Glutamine is a necessary nutrient for cell proliferation, serves as a specific fuel for inflammatory cells and enterocytes, and, when present in appropriate concentrations, enhances cell function. During inflammatory states, glutamine consumption may outstrip endogenous production and a relative glutamine deficiency state may exist. Animal and clinical studies suggest that improved outcome may be possible by providing the appropriate dose of this nutrient by the appropriate route to achieve adequate tissue concentrations. Such an approach prevents patients from being exposed to

some of the inadequacies of present day conventional nutrition. The overall benefit of providing an appropriate glutamine-supplemented diet to all metabolically compromised patients arises from the multiple anabolic and host protective effects of this amino acid, of which immunomodulation is only one important facet of glutamine's essential nature.

4: Int J Sports Med 1997 Mar;18 Suppl 1:S2-7

Exercise-induced immunomodulation--possible roles of neuroendocrine and metabolic factors.

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Acute muscular exercise induces an increased neutrophil count concomitant with recruitment of natural killer (NK), B and T cells to the blood as reflected by an elevation in the total lymphocyte count. Meanwhile, following intense exercise of long duration the lymphocyte count declines, non-MHC-restricted cytotoxicity is suppressed, but the neutrophil concentration increases. In relation to eccentric exercise involving muscle damage, the plasma concentrations of interleukin-1, interleukin-6 and the tumor necrosis factor are elevated. In this review we will propose a model based on the possible roles that stress hormones play in mediating the exercise-related immunological changes: adrenaline and to a lesser degree noradrenaline are responsible for the immediate effects of exercise on lymphocyte subpopulations and cytotoxic activities. The increase in catecholamines and growth hormone mediate the acute effects of exercise on neutrophils, whereas cortisol may be responsible for maintaining lymphopenia and neutrocytosis after exercise of long duration. Lastly, the role of beta-endorphin is less clear, but the cytokine response is closely related to muscle damage and stress hormones do not seem to be directly involved in the elevated cytokine level. Other possible mechanisms of exercise-induced immunomodulation may include the so-called glutamine hypothesis, which is based on the fact that skeletal muscle is an important source of glutamine production and that lymphocytes are dependent on glutamine for optimal growth. Furthermore, physiological changes during exercise, e.g. increased body temperature and decreased oxygen saturation may also in theory contribute to the exercise-induced immunological changes.

5: Curr Gastroenterol Rep 2007 Apr;9(2):99-106

Nutritional immunomodulation of acute pancreatitis.

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Despite the great advances in our understanding of the pathophysiology of acute pancreatitis, no specific therapy has emerged, and treatment remains supportive. In patients with the severe form of the disease, in which mortality remains high at 20% to 30%, the function of the upper gastrointestinal tract is disturbed due to extrinsic compression by the

inflamed and swollen pancreas, and normal eating is impossible. Such patients often develop multiple organ failure, necessitating intensive-care management and artificial ventilation for weeks on end. In this setting, protein catabolism will rapidly result in protein deficiency and further complications unless nutritional support is commenced. Recent studies have shown that, despite the risk of disease exacerbation through pancreatic stimulation, enteral feeding is more effective than parenteral feeding in improving outcome. Experimental studies suggest that this can be attributed to its content of specific immunomodulating nutrients, such as glutamine, arginine, and n-3 fatty acids, and by its stabilizing effect on the gut flora through the provision of prebiotics. Further studies are indicated to examine whether dietary enrichment with these substrates, along with regulation of the gut bacteria with probiotics, can improve outcome further.

6: Neonatal Netw 2007;26(1):57-60

Immunomodulation, Part V: probiotics.

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The five-part "Pointers in Practical Pharmacology" immunomodulation series has presented some of the agents researchers are investigating in hopes of finding the means to effectively prevent and treat infectious processes in neonates. The phosphodiesterase inhibitor pentoxifylline appears promising, but large, randomized, clinical trials are still lacking. So far, there is no clear evidence to support the use of G-CSF for either the prevention or the treatment of sepsis. The results of a large, randomized, clinical trial of G-CSF in the United Kingdom are pending. Although intravenous immunoglobulin (IVIG) therapy does not appear to be useful in the prevention of sepsis, its effectiveness in the treatment of sepsis is uncertain. It is hoped that the results of the International Neonatal Immunotherapy Study will provide definitive answers regarding treatment of sepsis with IVIG. The "conditionally essential" amino acid glutamine administered either enterally or parenterally does not make a difference in the rate of systemic infection or NEC in very low birth weight infants. Finally, probiotics appear promising as documented by at least two of the three randomized, clinical trials described here. As the search continues for agents to enhance the neonate's immune system and prevent and treat infectious diseases, remember that our best prevention tool is excellent and consistent hand hygiene.

10: J Physiol 2004 Aug;559(Pt 1):35-40

A novel physiological mechanism of glycine-induced immunomodulation: Na⁺-coupled amino acid transporter currents in cultured brain macrophages.

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Glycine is known to modulate immune cell responses. However, the physiological mechanisms underlying inhibitory effects of glycine on

macrophages are not well understood. Here we show that glycine is capable of inducing inward currents in brain macrophages (microglia). In contrast to glycine, the glycine receptor agonist taurine failed to elicit currents. Glycine-evoked currents of brain macrophages were unaffected by strychnine, Cl(-)-free extracellular solution, N-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine (NFPS) and amoxapine, but were abolished upon omission of extracellular Na(+). Furthermore, glycine caused increases in the intracellular Na(+) concentration and pronounced membrane depolarization. Glycine-evoked depolarization was Na(+) dependent and occurred independently of the intracellular Cl(-) concentration. Similarly to glycine, glutamine and alpha-(methylamino)isobutyric acid (MeAIB) elicited inward currents in brain macrophages. In the presence of either glutamine or MeAIB, glycine-induced currents were inhibited. It is concluded that neither functional glycine receptors nor glycine transporters are expressed in brain macrophages. We suggest that glycine mediates its effects by activation of system A Na(+)-coupled neutral amino acid transporters.

11: JPEN J Parenter Enteral Nutr 1999;23(5 Suppl):S52-8

Glutamine-enriched enteral feeding in trauma patients: reduced infectious morbidity is not related to changes in endocrine and metabolic responses.

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BACKGROUND: Recently we have shown that glutamine-enriched enteral nutrition in trauma patients reduced the occurrence of pneumonia, bacteremia, and sepsis. In that study, no clear explanation for these results was found except for lower tumor necrosis factor (TNF)-soluble receptors, suggesting immunomodulation. Here we present data on the course of endocrine and metabolic plasma mediators that were analyzed to provide more insight into the working mechanism of glutamine. METHODS: Endocrine and metabolic mediators were measured in plasma samples taken on admission (day 0) and on days 1, 2, 3, 7, and 10. Glucose, prealbumin, albumin, alanine, C-reactive protein, alfa1-antitrypsin, complement factors, cortisol, glucagon, insulin, and growth hormone were assessed by standard techniques. RESULTS: The rate of feeding, demography, and injury severity did not differ between the glutamine and control group. There was a sustained hyperglycemic response in both groups. Insulin levels rose in the second phase of the period of observation. A moderate cortisol and glucagon response was seen in both groups. There was no alteration in growth hormone levels in either group. C-reactive protein, alfa1-antitrypsin, and complement factors showed similar increases in both groups but levels remained in the normal range. The course of alanine, albumin, and prealbumin also showed no difference between the groups. CONCLUSIONS: Glutamine-enriched enteral nutrition had no influence on the endocrine and metabolic response in trauma patients. Therefore, the reduction in infectious morbidity seen in glutamine-supplemented trauma patients is most likely not explained by a modulation of the humoral stress response and its metabolic consequences.

12: Rev Hosp Clin Fac Med Sao Paulo 1999;54(1):21-4

Pilot study with a glutamine-supplemented enteral formula in critically ill infants.

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Seriously ill infants often display protein-calorie malnutrition due to the metabolic demands of sepsis and respiratory failure. Glutamine has been classified as a conditionally essential amino acid, with special usefulness in critical patients. Immunomodulation, gut protection, and prevention of protein depletion are mentioned among its positive effects in such circumstances. With the intent of evaluating the tolerance and clinical impact of a glutamine supplement in seriously ill infants, a prospective randomized study was done with nine patients. Anthropometric and biochemical determinations were made, and length of stay in the intensive care unit (ICU), in the hospital, and under artificial ventilation, and septic morbidity and mortality were tabulated. Infants in the treatment group (n = 5) were enterally administered 0.3 g/kg of glutamine, whereas controls received 0.3 g/kg of casein during a standard period of five days. Septic complications occurred in 75% of the controls (3/4) versus 20% of the glutamine-treated group (1/5, $p < \text{or} = 0.10$), and two patients in the control group died of bacterial infections (50% vs. 0%, $p < \text{or} = 0.10$). Days in the ICU, in the hospital, and with ventilation numerically favored glutamine therapy, although without statistical significance. The supplements were usually well tolerated, and no patient required discontinuation of the program. **The conclusion was that glutamine supplementation was safe and tended to be associated with less infectious morbidity and mortality in this high-risk population.**

14: Tokai J Exp Clin Med 1986;11 Suppl:207-13

Immunomodulation in autoimmune diseases.

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Systemic autoimmune diseases are generally treated with immunosuppressive agents. In some instances immunomodulatory agents have shown promise in the treatment of certain types of autoimmune disorders. The in vitro and/or in vivo effects of some of these agents (glutaurine, ketoconazole, gutimine and its derivative) are demonstrated. Glutaurine exerts moderate immunostimulating activity, but fails to influence the clinical course of systemic lupus erythematosus. Although ketoconazole suppresses immune responses in vitro, it does not influence cellular reactivity of patients in vivo. **The immunostimulatory activity of gutimine and its derivative (T 001) have been demonstrated in vitro, and need to be tested also in vivo.**

15: Ann Surg 2003 Jan;237(1):110-5

Perioperative GLY-GLN infusion diminishes the surgery-induced period of immunosuppression: accelerated restoration of the lipopolysaccharide-stimulated tumor necrosis factor- α response.

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OBJECTIVE: To investigate whether the administration of different glutamine-containing dipeptides, glycyl-l-glutamine (GLY-GLN) and l-alanyl-l-glutamine, has a differing impact on perioperative immunomodulation. **SUMMARY BACKGROUND DATA:** Surgery leads to transitory immunosuppression, which is associated with decreased plasma glutamine (GLN) levels and increased susceptibility to infection and sepsis. A useful tool to detect immunocompetence is the ex vivo lipopolysaccharide (LPS)-stimulated tumor necrosis factor alpha (TNF-alpha) secretion in whole blood. **METHODS:** Forty-five patients undergoing major abdominal surgery were randomized prospectively to receive 0.5 g/kg/24 h GLN dipeptides administered as GLY-GLN or as ALA-GLN or isonitrogenous Vamin (a GLN-free amino acid solution; control group) as a continuous infusion over 72 hours, starting 24 hours before surgery. Blood samples were collected before infusion, at the end of surgery, and 48 hours postoperatively to determine the TNF-alpha release into whole blood stimulated with LPS. Groups were compared by analysis of variance. **RESULTS:** The groups were comparable in age, gender distribution, and length of operative time. At the end of surgery a significant reduction in ex vivo LPS-stimulated TNF-alpha production was observed in all groups. In patients who received GLY-GLN, the induced TNF-alpha production was restored after 48 hours. **CONCLUSIONS:** In this study perioperative infusion of GLY-GLN reduced immunosuppression. The effect of GLN-containing dipeptides seems to be different when administered in glycine or alanine form.

16: Br J Nutr 2002 Jan;87 Suppl 1:S133-4

Immunonutrition in patients after multiple trauma.

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Severe trauma threatens the life of the victim, both directly and indirectly via immunological dysregulation during the subsequent clinical course. Inflammatory or infectious episodes may complicate the clinical course and ultimately result in sepsis and multiple organ failure, which have mortality rates of up to 80%. Immunomodulatory intervention aims to ameliorate the early hyperinflammatory phase (systemic inflammatory response syndrome, SIRS) to avoid the development of sepsis. One of the immunomodulation strategies is enteral feeding supplemented with specific nutrients, such as glutamine, n-3-polyunsaturated fatty acids, and nucleotides ('immunonutrition'), because changes in the GALT (gut-associated lymphoid tissue) immune response may contribute to intestinal dysfunction and increase susceptibility to post injury gut-derived sepsis. In a prospective, randomized, double-blind, controlled study in twenty-nine patients suffering severe trauma we were able to show that immunonutrition (arginine, n-3-fatty acids, and nucleotides) significantly reduces the number of SIRS days per patient, and also lowers the multiple organ failure (MOF) score on day 3 and days 8-11 ($P < 0.05$). Other studies have reported a reduction in septic complications and MOF rates, shortened hospital stay, and reduction in the use of antibiotics in patients randomized to the immune-enhancing diet. This improved clinical outcome was reflected in a reduction in hospital costs. In the recovery period after trauma (1-72 h after injury) a limitation of the inflammatory response of immunocompetent cells must be achieved as quickly as possible (<72 h). The only strategy available to clinicians caring for trauma patients is immunonutrition, and this should be strongly considered as a rational approach improving immune

function and reducing septic complications in critically ill or injured patients.

17: Ann Fr Anesth Reanim 1995;14 Suppl 2:102-6

[Role of new nitrogen substrates during peri-operative artificial nutrition in adults]

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Some amino acids and their derivatives, such as arginine (ARG), glutamine (GLN) in free form or as dipeptides, and ornithine-ketoglutarate (OKG), have specific pharmacological properties concerning namely immunomodulation, control of protein turn-over, maintenance of gut trophicity. In the context of the postoperative nutrition in the adult, supplementation of enteral nutrition with ARG and of parental nutrition with GLN and OKG has improved nutritional and biochemical markers such as nitrogen balance, muscle protein synthesis and glutamine content. However, only few studies have tried to demonstrate a clinical benefit with such a supplementation. At present only the beneficial effect of OKG on postoperative wound healing has been recognized.

18: J Proteome Res 2008 Jul;

NMR-Based Metabonomic Investigations into the Metabolic Profile of the Senescence-Accelerated Mouse.

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In this work, metabonomic methods utilizing ^1H NMR spectroscopy and multivariate statistical technique have been applied to investigate the metabolic profiles of SAM. The serum metabolome of senescence-prone 8 (SAMP8), a murine model of age-related learning and memory deficits and Alzheimer's disease (AD), was compared with that of control, senescence-resistant 1 (SAMR1), which shows normal aging process. Serum samples were collected for study from both male and female 12-month-old SAMP8 and age matched SAMR1 ($n = 5$). ^1H NMR spectra of serum were analyzed by pattern recognition using principal components analysis. The results showed that the serum metabolic patterns of SAMP8 and SAMR1 were significantly different due to strains and genders. Subtle differences in the endogenous metabolite profiles in serum between SAMP8 and SAMR1 were observed. The most important metabolite responsible for the strain separation was lack of inosine, which meant the protective function of anti-inflammation, immunomodulation and neuroprotection might be attenuated in SAMP8. Other differential metabolites observed between strains included decreased glucose, PUFA, choline, phosphocholine, HDL, LDL, D-3-hydroxybutyrate, citrate and pyruvate and increased lactate, SFA, alanine, methionine, glutamine and VLDL in serum of SAMP8 compared with those of SAMR1, suggesting perturbed glucose and lipid metabolisms in SAMP8. Besides the differences observed between the strains, an impact of gender on metabolism was also found. The females exhibited larger metabolic deviations than males and these gender differences in SAMP8 were much larger than in SAMR1. Higher levels of VLDL, lactate and amino acids and lower levels of HDL, LDL

and unsaturated lipids were detected in female than in male SAMP8. These facts indicated that the metabolism disequilibrium in female and male SAMP8 was different and this may partly explain that females were more prone to AD than males. The results of this work may provide valuable clues to the understanding of the mechanisms of the senile impairment and the pathological changes of AD, as well as show the potential power of the combination of the NMR technique and the pattern recognition method for the analysis of the biochemical changes of certain pathophysiologic conditions.

19: Khirurgiia (Mosk) 2004;(10):31-3

[Treatment of intestinal insufficiency syndrome in patients with peritonitis]

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Results of the treatment of 90 patients with generalized peritonitis and syndrome of intestinal insufficiency were analyzed. In the study group (45 patients) enteral administration of 1% pectin solution and glutamin solution (15-30 g/day) were included in combined therapy. Clinical and laboratory control, radiation monitoring and bacteriological studies carried out for evaluation of efficacy of therapy established. A decrease of endogenous intoxication and time of repair of functional activity of the gastrointestinal tract, immunomodulation, normalization of microbiocenosis of the small intestine. This therapy diminishes the number of complications and lethality.

20: Gut 2003 Feb;52(2):212-7

Investigation of the putative immunodominant T cell epitopes in coeliac disease.

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BACKGROUND: Coeliac disease (CD) is an enteropathy mediated by gluten specific T cells which secrete interferon gamma (IFN-gamma) when stimulated by gluten peptides presented by HLA-DQ2 or DQ8 molecules. Residues 62-75 of alpha(2) gliadin have been proposed as the immunodominant epitope in the majority of CD patients. Deamidation by tissue transglutaminase (tTG) of the glutamine (Q) at position 65 to glutamic acid (E) is essential for T cell stimulation. AIMS: To investigate the antigenicity of this peptide and to establish whether its T cell activating properties can be downregulated by the formation of altered peptide ligands. PATIENTS: Individuals with known CD. METHODS: Peptide G4 corresponding to alpha(2) gliadin residues 62-75, Q-E65 and analogues, substituting each amino acid, except E65, in turn for alanine residues, were synthesised. Small intestinal biopsies were obtained from patients. Biopsies were cultured overnight with a peptic/tryptic digest of gliadin (PTG). Lymphocytes were cultured and restimulated with tTG treated PTG. A T cell line was cloned and clones tested for stimulation and IFN-gamma production in response to G4 and its analogues. RESULTS: Some high activity clones were isolated with, for example, a stimulation index (SI) of 15 to G4 and secreting 327 pg/ml of IFN-gamma. Substitution of amino acids at several positions abolished or

downregulated stimulation and IFN-gamma production. CONCLUSIONS: Peptide G4 is highly immunogenic. Certain amino acid substitutions in peptide G4 abolish T cell reactivity while others are partial agonists which may have potential in immunomodulation in this condition.

21: Curr Opin Gen Surg 1993;:92-104

Malnutrition, injury, and the host immune response: nutrient substitution.

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Improvements in surgical management and intensive care therapy have enabled many patients to initially survive severe life-threatening trauma or major surgical procedures only to die after delayed bouts of sepsis. This paper reviews literature published within the past year on the effects of nutrient substitution on malnutrition, injury, and the host immune response. Topics discussed include immunodeficiencies in trauma and malnutrition, immunomodulation by nutrition, and parenteral versus enteral nutrition. We also discuss the roles of arginine, glutamine, omega-3 fatty acids, and dietary nucleotides in the host immune response.