1347 Different Anastomosis Techniques in Colorectal Surgery

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Clinical study was performed since 1st April 1990 till 10th January 1994. The goal of this study was to compare the perioperative data of three groups of patients with colorectal resection because of malignancy. The operator and the preoperative preparation were the same.

Data of 92 patients (52 female and 40 male) were analysed. The patients were divided into three groups according to three types of anastomosis techniques. Group I contained 30 patients with "hand made" anastomosis (12 end to end; 6 end to side; 12 side to side). In Group II "end to end" anastomosis was made by EEA circular stapler (COMESA) in 27 patients. In Group III 35 "side to side" anastomoses were made by linear stapler (PLC-ETHICON or GIA-Autosuture C.). The groups were comparable. Distribution of sex, mean age and mean body weights were similar.

Only one patient of 92 died after the surgical procedure. It means 1% mortality rate. This case was in the Group II. Septic complication rate was also the highest in the Group II. The operative time was significantly shorter in the Group III in which linear stapler were used for the resection the bowel loops and making anastomosis. Bowel movement and first stool emission was earlier in this Group III compared to the other two groups.

Authors suggest using linear staplers because of their benefits in the colorectal surgery. The shorter operative time and less septic complication rate are very important factors in the gastrointestinal surgery.

1348 Protection Against Immune Complex-Induced Colitis in Rabbits by OR-1364

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OR-1364 (3-((3-cyanophenyl)methylene)-2,4-pentanedione) is a locally acting thiol modulating compound, which has been shown to markedly protect against experimental colitis mediated by acetic acid, hapten or free radicals. The aim of the present study was to test the effect of OR-1364 against immune complex-induced colitis in rabbits. Since interleukin-1 (IL-1) has been suggested to play an important role in the initiation of immune complex colitis, we assessed the effect of OR-1364 on IL-1 β release from human mononuclear cells in vitro.

Methods: 4 ml of 1% formalin was instilled into the distal colon of NZW rabbits and two hours later immune complexes were injected intravenously. OR-1364 (0.3, 1 and 3 mg/kg) and 5-ASA (100 mg/kg) were administered intracolically 24 h and 1 h before, and 24 h and 48 h after formalin application. The animals were sacrificed at 72 hours and the colonic lesions were scored macroscopically and histologically. Leukotriene B₄ (LTB₄) and prostaglandin E₂ (PGE₂) release from the colon was determined by RIA. IL-1 β production was determined in vitro by ELISA from culture media of lipopolysaccharide activated human mononuclear cells.

Results: OR-1364 inhibited dose-dependently the development of the colitis. The mean macroscopic score of the control group was 6.9 ± 0.6 and it was reduced to 2.3 ± 0.8 by 3 mg/kg of OR-1364. The histologic evaluation paralleled the macroscopic findings and a marked inhibition of neutrophil infiltration was observed. The release of LTB₄ was reduced by OR-1364 up to 73 % while the release of PGE₂ was not affected. 5-ASA affected neither the colitis score nor the eicosanoid release. OR-1364 decreased IL-1 β production in vitro dose-dependently at the range of 2–8 μ M.

Conclusion: Locally administered OR-1364 exerted a marked protection against immune complex mediated ulcerative colitis. In vitro OR-1364 inhibited IL-1 β production in human mononuclear cells at low micromolar concentrations. Thus, the observed inhibition of neutrophil infiltration into the rabbit colon may be a consequence of decreased IL-1 release by mucosal cells.

1349 Effect of Interleukin-8 on Leukocyte-Endothelial Cell Adhesion in a Model of Chronic Intestinal Inflammation

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Leukocyte endothelial cell adhesion (LECA) is affected by a variety of inflammatory mediators. Although one of them, interleukin-8 (IL-8), is known to stimulate neutrophil granulocytes in vitro there is little in vivo data to support this hypothesis. The objective of this study was to assess the role of IL-8 in mediating the LECA elicited in postcapillary venules during chronic intestinal inflammation in Sprague Dawley rats. **Methods:** Indomethacin (INDO, 7.5 mg/kg, s.c.) was injected 48 and 24 hrs. prior to the experiment. The mesenteric microcirculation was observed by intravital microscopy in animals treated with a monoclonal antibody (Mab) against IL-8 (DM/C7, 3 mg/kg i.v.) or a non-blocking control Mab. Leukocyte rolling velocity, the number of adher-

ent and emigrated leukocytes, vessel diameter, and erythrocyte velocity were monitored on ~30 μ m diameter postcapillary venules. **Results:** INDO treatment resulted in mucosal ulcerations, granulocyte infiltration, an increase in the number of adherent (8-fold) and emigrated (6-fold) leukocytes, and a reduction (80%) in leukocyte rolling velocity. While the non-binding Mab had no effect administration of Mab against IL-8 reduced the INDO-induced increase in leukocyte adherence and emigration by 60% each, while rolling velocity was increased to 37% as compared with controls. Granulocyte infiltration of the bowel wall was significantly reduced by 50% vs. the INDO-treated group. **Conclusion:** In chronic intestinal inflammation induced by INDO IL-8 is one of the mediators inducing leukocyte endothelial cell interaction – probably by activating adhesion molecules on granulocytes and/or the release of reactive oxygen metabolites.

1350 In Vivo Quantification of Intracolonic Release of Interleukin-1 β in Chronic Ulcerative Colitis

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Interleukin-1 β (IL-1 β) is a polypeptide cytokine with a well defined proinflammatory activity attributable to stimulation of eicosanoid generation. IL-8 production and immunocompetent cells. To investigate the role of IL-1 β in chronic ulcerative colitis (CUC), we studied 26 patients (17 men and 9 women, range 18–71 years) with untreated CUC and 7 patients with irritable bowel syndrome (IBS) (2 men and 5 women, range 18–46 years) who served as disease controls. In 7 CUC patients the disease was inactive and in 19 was mild to moderately active according to clinical and colonoscopic criteria. 7 patients with active CUC were studied before and after 4 weeks on oral treatment with 5-ASA (1 g b.i.d.). Colonic perfusion was performed by a double lumen tube placed into the descending/sigmoid colon. An isotonic solution was continuously infused 50 cm from the anal verge at 5 ml/min, and recovered 30 cm distally by siphonage. After 30 min washout and 30 min equilibration periods, 10 min effluent collections were obtained for 40 min. Aspirates were analyzed for IL-1 β by ELISA, polymorphonuclear elastase by IMAC and LTB₄ by specific RIA. **Results:** None of the IBS patients and 5 out of 7 inactive CUC patients had undetectable IL-1 β release. In active CUC, the release of IL-1 β was markedly increased in 17/19 (median 500, interquartiles 270–1582 pg/min, $p < 0.01$ vs IBS and inactive CUC). Elastase and LTB₄ release were significantly increased in active CUC (9 (5–23) μ g/min and 1.8 (0.9–3) ng/min respectively) as compared to inactive CUC (1.2 (1–2.5) and 0.4 (0.5–0.7), $p < 0.01$ for both) and IBS (0.1 (0–0.2) and 0.7 (0.5–1), $p < 0.01$ for both). LTB₄ release was similar in inactive CUC and IBS, whereas elastase release was higher in inactive CUC than in IBS. 5/7 CUC patients improved after 5-ASA treatment. In the responder patients IL-1 β became undetectable or declined.

Conclusions: active CUC is associated with enhanced IL-1 β release into the colonic lumen whereas such release does not occur in remission. This finding supports the concept that CUC flare ups involve increased IL-1 β production and suggest that IL-1 β antagonist could be clinically useful.

1351 Urinary Nitrite Dipstick: A Reliable Disease Activity Marker in Inflammatory Bowel Disease (IBD)

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Nitric oxide (NO) is an endogenous mediator of smooth muscle relaxation. It is generated from L-Arginine by NO synthase. This enzyme can be induced by various cytokines and endotoxins. Hence in IBD, NO production is enhanced which may play a role in regulating vascular permeability and colonic smooth muscle tone. NO has a short half life and is metabolised to its stable end products, nitrite and nitrate which are excreted in urine. Nitrite dipstick is based on Griess's test and is specific to nitrite. It's sensitivity limit is 0.05 mg/100 ml and reveals the presence of nitrites by a pink to red discoloration of the test patch.

The aim of this study was to test the urine of IBD patients for nitrites and to correlate the results with the clinical and biochemical parameters of disease activity. We used the Nephur test + Leuco dipstick for this study. 42 urine samples were tested randomly and were interpreted to be positive or negative. Urinary infection was excluded when these urine samples were collected previously, before they were stored at –20°C. They were thawed to room temperature prior to the dipstick test. 18 samples were positive for nitrites and 24 were negative. Clinical disease activity index (Harvey Bradshaw), ESR and C-Reactive protein were compared between the nitrite positive and negative groups using the student t test. For all the three indices there was a significant difference between the two groups (P value < 0.01) which also correlated well with the patient's relapses and remissions.

Our study shows that the nitrite dipstick is an inexpensive, rapid and reliable disease activity marker in IBD. We feel that a quantitative nitrite dipstick

may be more useful in grading the disease activity and for the follow up of these patients.

1352 The Role of Respiratory Allergy in the Precipitation of Ulcerative Colitis Episodes and Crohn's Disease

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The role of allergy in the aetiology of chronic inflammatory bowel disease (IBD) has often been mentioned. The demonstration of a seasonal factor in the precipitation of outbreaks of these diseases could suggest a link with environmental factors such as allergies. The aim of this study was to find a link between respiratory allergy and IBD.

Patients and methods: 54 patients with ulcerative colitis (UC), 40 patients with Crohn's disease (CD) and 33 healthy controls (HC) were questioned on the history of their illness, their personal and family allergic antecedents (asthma, hayfever, allergic conjunctivitis, atopic dermatitis and urticaria), had their total serum IgE level measured, underwent a Phadiatop® test, which detects the presence of specific IgE to pneumallergens common to the environment, and a test to demonstrate the presence of specific anti-pollen IgE.

Results: Allergy antecedents were significantly more frequent in IBD than in HC (personal antecedents IBD: 60%, HC:33%, $p < 0.01$; family antecedents IBD: 68%, HC: 48.5%, $p < 0.04$). There was no significant difference in the total serum IgE level between UC, CD and HC. There was a significantly higher percentage of patients with positive Phadiatop® results in UC (27.8%) than in HC (9.1%) ($p < 0.04$). Among the 15 patients with UC who gave a positive Phadiatop® result, 11 (73%) thought the exacerbation of their disease was related to the seasonal rhythm of sensitizing allergens.

Conclusions: Respiratory allergy seems more common in patients with ulcerative colitis and could play a role of co-factor in the onset of some outbreaks of the disease.

1353 Reactivity of Lamina Propria T-Lymphocytes in Crohn's Disease

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Human intestinal lamina propria lymphocytes exhibit minimal proliferation in response to antigen receptor stimulation in vitro. This study aimed at investigating, whether proliferative responses of intestinal lamina propria T cells (LPTL) to CD3 stimulation and their T cell receptor (TcR) repertoire are altered in patients with Crohn's disease (CD).

We examined the proliferative response of lamina propria T lymphocytes in patients with CD to stimulation with CD3 monoclonal antibody and interleukin 2 and compared it with controls. The TcR diversity of LPTL and peripheral blood lymphocytes (PBTL) were determined by direct immunofluorescence using a panel of TcR V β .

In CD purified LPTL showed an enhanced responsiveness to CD3 and Interleukin 2 stimulations compared to controls. Moreover, perhaps as a consequence, an enhanced frequency of in vivo activated T cells was seen. LPTL and PBTL exhibit similar TcR V β gene usage both in controls and patients with CD. The results are discussed in regard to the antigen-specific unresponsiveness, superantigen selective stimulation of T cells and impairment at the mucosal barrier in advanced and early lesions.

Polyclonal activation of lamina propria T lymphocytes due to impairment of local adjustment i.e. insufficient downregulation of TcR/CD3 dependent signalling process may contribute to the pathogenesis of Crohn's disease.

1354 The Source of Superoxide Anion in Ulcerative Colitis is not Xanthine Oxidase

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Reactive oxygen metabolites (ROMs) including superoxide anion are believed to be important in the pathogenesis of ulcerative colitis (UC). Xanthine oxidase (XO) metabolises xanthine to uric acid with the production of superoxide anion which may combine with nitric oxide to give peroxynitrite, a powerful oxidant which produces colonic inflammation in rats and which may oxidise sulphhydryl groups, thus converting cysteine to cystine. The content of xanthine, uric acid, cysteine and cystine was determined by HPLC in snap frozen mucosal biopsies taken at colonoscopy in 21 patients with UC or normal mucosa.

	Controls n = 10	Ulcerative colitis n = 11
Cystine/Cysteine Ratio ($\pm$ SEM)	0.18 ($\pm$ 0.08)*	1.79 ($\pm$ 0.25)* $p < 0.01$
Uric Acid mmol/mg protein ($\pm$ SEM)	7.45 ($\pm$ 0.68)	7.23 ($\pm$ 0.86) NS
Urate/Xanthine Ratio ($\pm$ SEM)	3.17 ($\pm$ 0.29)	3.31 ($\pm$ 0.31) NS

* $p < 0.01$ Wilcoxon rank sum test.

The increased cystine to cysteine ratio confirms the production of ROMs in active UC but, as no corresponding increase in XO activity was detectable, these must come from other sources. XO inhibitors such as allopurinol are unlikely to be effective in the treatment of UC.

1355 Protective Effect of Smoking in Ulcerative Colitis – An Effect of Increased Catalase Activity?

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Reactive oxygen metabolites (ROMs) which are believed to be important in the pathogenesis of ulcerative colitis (UC) are broken down by the enzymes superoxide dismutase and catalase. Catalase is increased in monocytes from smokers. The activity of catalase was determined in snap frozen mucosal biopsies taken at colonoscopy from patients with active or quiescent UC (n = 18) who were known to be non-smokers, and from patients found to have normal mucosa whose smoking habits were known.

Biopsies were homogenised and incubated with 4.2 mM hydrogen peroxide and methanol to form formaldehyde the concentration of which was determined after addition of Purpald (Aldrich Chemical Co.) and fixation with potassium periodate in a spectrophotometer at 550 nm.

The mean catalase activity in non-smoking controls was 3.6 ± 0.63 (SEM) μ g/mg protein/mg Hb and 4.85 ± 1.07 in active UC. In quiescent UC however, the activity was significantly increased at 10.6 ± 2.17 ($p = 0.004$, Wilcoxon Rank Sum Test). In mucosal biopsies from smokers the mean activity was similarly raised 17.3 ± 4.7 (n = 5).

Catalase activity is increased in quiescent UC and also in smokers. Increased protection of the mucosa by catalase from ROMs may be important in the protection smoking provides in UC, and induction of catalase activity may prove to be of therapeutic benefit.

1356 Glutathione is Depleted in Active Crohn's Disease

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Inflammatory bowel disease (IBD) is associated with an increase in reactive oxygen metabolites (ROMs). Glutathione (GSH) is the main intracellular molecule protecting against oxidative stress. ROMs may oxidise GSH reducing its concentration in tissues and whole blood.

GSH was measured in whole blood from 5 healthy controls and in 19 patients with Crohn's Disease (CD), classified as being active or quiescent on the basis of the Harvey and Bradshaw Index (HBI).

The formation of 5-lactyl GSH from GSH and methyl glyoxal in the presence of the enzyme glyoxylase I was determined in a spectrophotometer at 240 nm.

Results (Mean $\pm$ SEM)

	Controls n = 5	Crohn's Disease	
		Quiescent HBI < 6 n = 11	Active HBI > 6 n = 8
GSH μ M	867 $\pm$ 33	765 $\pm$ 26	471 $\pm$ 25*
GSH μ M/gHb	6.13 $\pm$ 0.44	5.999 $\pm$ 0.28	3.97 $\pm$ 0.2*

* $p < 0.001$ (t test between Quiescent and Active CD).

There was a significant negative correlation between the fall in GSH concentration and HBI ($r = -0.85$; $p < 0.0001$) and C reactive protein (CRP) ($r = -0.59$; $p < 0.008$), but not with ESR or alpha-1-antichymotrypsin.

4 untreated patients with acute CD had GSH concentrations of 465 ± 9.6 μ M, which increased to 792.4 ± 13 μ M after 7 days therapy with elemental diet. Depletion of GSH in acute CD may reflect its consumption by ROMs.

1357 Soluble ICAM-1: An Inflammatory Marker in Crohn's Disease

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Intercellular adhesion molecule 1 (ICAM-1) is presented on colonic endothelium and lymphocytes in association with active inflammation in patients with Crohn's disease (CD). It is important in the adhesion of inflammatory cells to target tissue. ICAM-1 is also found in soluble form in serum but its patho-

physiological role is unclear. Our aims were to determine whether soluble ICAM-1 is elevated in CD, and to assess its relationship with disease activity, inflammation and immune activation. Serum soluble ICAM-1 was measured by ELISA in 31 CD patients and in 24 healthy controls. In all disease patients, corresponding CD activity index (CDAI) and Harvey-Bradshaw index were calculated. Routine markers of inflammation – ESR, C-reactive protein (CRP), platelets, haemoglobin, packed cell volume (PCV) and albumin were determined. Immune activation was assessed by measuring peripheral lymphocyte interleukin-2 receptor expression by flow cytometry. Serum soluble ICAM-1 levels, mean [SE], were higher in CD in comparison to controls [313[13] vs 242[16], $p < 0.0005$). Although no significant correlation was found with clinical indices of disease activity, serum soluble ICAM-1 correlated closely with objective markers of inflammation.

Rs	P<		Rs	P<	
CRP	0.6	0.001	PCV	0.38	0.05
ESR	0.5	0.005	haemoglobin	-0.36	0.05
albumin	-0.4	0.02	Platelets	0.36	0.05

No correlation was found with lymphocyte activation.

We conclude that serum soluble ICAM-1 is elevated in CD and is a marker of inflammation. The close correlation with inflammation and the lack of correlation with lymphocyte activation suggests its source may be inflamed and necrotic colonic endothelium, stimulated by inflammatory cytokines rather than activated lymphocytes.

1358 Mucosal Antibodies Against HSP70/72 in Patients with Chronic Inflammatory Bowel Disease (IBD)

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Serological studies have provided evidence of an increased prevalence of HSP-antibodies in Crohn's disease (CD) and ulcerative colitis (UC). In the current study we developed a method to detect specific antibodies against HSP at the site of inflammation, i.e. the intestinal mucosa.

Methods: Tissue biopsies were obtained during colonoscopy. The specimens were homogenized and subjected to iso-electrofocusing. After protein separation a reverse blot was performed with biotin- and fluorescein-labelled antigen. HSP- antigen was examined by a western blot procedure.

Results: Mucosal HSP-antigen was detected in all patients with Crohn's disease (5/5) and ulcerative colitis (6/6). Just as frequently, HSP-antigen could be found in the control group (5/6). HSP-70/72 antibodies were detected in all patients with CD (8/8) and most of the control patients (13/15), although less frequently in patients with UC (2/4).

Conclusions: Both HSP 70/72 antigen as well as corresponding antibodies were similarly prevalent in both normal and diseased human intestinal mucosa. Therefore, it appears unlikely that an induction of a local immune response to bacterial HSP cross reacting with human HSP is the autoimmune basis of IBD.

1359 Refractory Interferon-Gamma (IFN γ) Activities in Inflammatory Bowel Disease (IBD)

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Cytokine exerts fundamental roles in the intestinal immune system. Although the pathogenesis of IBD still remains unknown, recent evidences suggest that abnormal activities of cytokines released from lamina propria mononuclear cells (LPMC) in IBD-affected intestine may be involved, as demonstrated by decreased production of interleukin-2 (IL2) and increased levels of IL1, IL6 and IL8. In the present study, we investigated the activities of IFN γ , an important immunoregulatory cytokine, as well as modulatory effects of IL4, one of inhibitory cytokines, on IL2-induced IFN γ expressions in IBD at cellular and molecular levels.

LPMC isolated from 18 control, 12 Crohn's disease (CD) and 11 ulcerative colitis (UC) patients were cultured with and without PHA, or in the absence and presence of IL2 and IL4 (10 units/ml). IFN γ activities (ng/ml) in culture supernatants were measured by ELISA. In addition, total cellular RNA was extracted from cultured LPMC and hybridized with cDNA probes specific for IFN γ by Northern blotting. Relative differences in IFN γ mRNA expression were measured by densitometry.

In unstimulated cultures, variable low levels of IFN γ were obtained although no significant differences were observed between IBD and control LPMC. When cells were stimulated with PHA, levels of IFN γ in the supernatants were substantially augmented, and both CD (26 ± 8) and UC (11 ± 3) LPMC could produce significantly ($p < 0.01$) less cytokine than control LPMC (60 ± 10). In agreement with protein values, IBD LPMC displayed consistently lower levels of IFN γ mRNA than control LPMC in PHA-stimulated cultures,

while no remarkable differences were observed in spontaneous cultures. In addition, when the inhibition of IL4 on IL2-induced IFN γ mRNA of LPMC were investigated, IL4 suppressed IFN γ mRNA expression in both control and IBD LPMC. However, IL4 was able to induce significant ($p < 0.005$) less inhibition of IFN γ transcripts in CD LPMC than those of control, so that relatively higher levels of cytokine mRNA were maintained in CD LPMC.

Our results demonstrated decreased IFN γ activities of IBD LPMC, as determined by protein and mRNA levels. Furthermore, CD LPMC responded relatively less to IL4 for regulation of IFN γ expression as compared to control LPMC, indicating that refractoriness to IL4 might be responsible for the continuation and persistence of inflammation in CD even with impaired capacity of IFN γ production.

1360 Suppression of Neutrophil-Derived Oxygen Radical Production by the Reversible Thiol Reagent OR-1364

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Inflammatory bowel disease (IBD) is characterized by an extensive mucosal infiltration of activated neutrophils that produce large quantities of inflammatory mediators and oxygen radicals. OR-1364 [3-[(3-cyanophenyl)methylene]-2,4-pentanedione] is a locally acting agent, which is protective in various animal colitis models at doses from 0.3 to 10 mg/kg. OR-1364 is known to reversibly conjugate with glutathione (GSH). Since protein thiols are essential in the neutrophil superoxide production, we assessed the effect of OR-1364 on human and rat neutrophils *in vitro* and *in vivo*, respectively. Furthermore, the reversibility of OR-1364 binding to protein thiols was evaluated *in vitro* with a thiol reagent activatable enzyme, microsomal glutathione-S-transferase (ms-GST).

Methods: Isolated human neutrophils were activated with phorbol ester (PMA) or chemotactic peptide (FMLP) and superoxide production was evaluated as cytochrome c reduction. Crude NADPH-oxidase was prepared from activated human neutrophils and assayed for as luminol enhanced chemiluminescence. The effect of OR-1364 (10 or 100 mg/kg, intracolonic) on rat peripheral neutrophils 1 h and 49 h after dosing was evaluated in whole blood by luminol enhanced chemiluminescence. Activity of msGST in rat liver microsomes was determined spectrophotometrically with chloro-dinitrobenzene as the substrate after preincubation with OR-1364 and GSH.

Results: OR-1364 inhibited PMA- and FMLP-stimulated superoxide production in human neutrophils with IC₅₀-values of 6.5 μ M and 1.5 μ M, respectively. The OR-1364 analogue without thiol reactivity was ineffective. However, when already activated, the human neutrophil NADPH oxidase was not inhibited by OR-1364. Intracolonic dosed OR-1364 had no effect on neutrophil oxygen radical production measured in blood at 1 h and 49 h after the treatment. OR-1364 caused a concentration-dependent activation of msGST that was reversed by subsequent GSH addition, which suggests reversible binding of OR-1364 to cysteinyl thiol of msGST.

Conclusion: The protective effect of OR-1364 in various animal colitis models is suggested to be due to local reversible modulation of critical (protein) thiols involved in activation of neutrophils and other inflammatory cells, while the host defense system remains functional elsewhere in the body.

1361 Indomethacin Enteropathy in Rats; Assessment Using a Novel Granulocytic Marker Protein, (GMP)

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Indomethacin (Indo) causes intestinal damage in both humans and experimental animals which results in bleeding, ulceration and biochemical alterations via a multistage pathogenic mechanism. Assessment of mucosal damage in animals has previously relied on histology, which is particularly demanding because damage can be wide-spread and patchy. Recently have calprotectin, a novel marker protein from granulocytes, been shown useful in measuring leucocyte sequestration into the gastrointestinal tract in both IBD and NSAID enteropathy. We have isolated a protein from rat-granulocytes (GMP), which seems to behave similarly to calprotectin in humans. We used GMP to study NSAID induced mucosal damage in rats. Male Sprague-Dawley rats (5/experiment) received 5 mg/kg Indo dissolved in Sodium Bicarbonate daily for seven days. Control animals received the buffer only, and all animals were allowed continuous access to food and water throughout the experiment (except prior to permeability determination). Sequential changes in permeability were assessed with 51 CrEDTA. Faecal GMP levels were determined daily using an ELISA technique, and the results are shown below.

	Faecal GMP (in mg/l)	Intest. permeability (% 51 CrEDTA)
Controls	4.29 ± 4.01	2.12 ± 0.80
Indo day 1	8.39 ± 5.61	4.80 ± 0.60**
Indo day 2	6.19 ± 3.07	-
Indo day 3	56.2 ± 49.7*	-
Indo day 4	116 ± 25.0*	-
Indo day 5	115 ± 41.8*	-
Indo day 6	128 ± 13.6*	-
Indo day 7	119 ± 23.2*	10.3 ± 4.63***

*p < 0.0001 **p < 0.0002 ***p < 0.005.

The results show that increased permeability precede the intestinal inflammatory changes caused by Indomethacin. Furthermore, faecal GMP appears to be suitable for assessing and quantitating intestinal inflammation.

1362 Neuroendocrine Differentiation in Colon Carcinomas

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Neuroendocrine (NE) differentiation was evaluated in 91 patients with colon carcinomas by immunostaining with antibodies against human chromogranin A (CgA) and neuron specific enolase (NSE). NE differentiation was demonstrated in 25% of the colon carcinomas. In patients who died during the study, NE expression in tumor was found in 42%, while the corresponding percentage in survivors was 15% (p = 0.006). CgA and pancreastatin-like immunoreactivity (PST-LI) were determined by radioimmunoassay methods in sera from 56 patients of these patients. Elevated serum levels of CgA and PST-LI were found in 36% and 43%, respectively, and when combined, in 61% of the patients. Of the patients with serum elevation of CgA/PST-LI, 28% had positive immunohistochemistry for either NSE or CgA, versus 5% in those with normal serum levels of CgA/PST-LI (p < 0.04). In patients with colon tumors of midgut origin, CgA concentrations were increased in 50% (8/16), versus 30% (12/40) in those with hindgut derived tumors (p = 0.1). For PST-LI we found the inverse relationship, respectively 25% (4/16) and 55% (22/40) (p = 0.07). In patients who died during the study, 53% had raised serum values of CgA, versus 29% in those who survived (p = 0.13). The corresponding figures for PST-LI elevation were 59% and 37%, respectively (p = 0.15). In conclusion, we have demonstrated NE differentiation in 25% of the colon carcinomas. Furthermore, we have for the first time found elevated serum levels of CgA and PST-LI in patients with colon carcinomas. In accordance with previous studies, our data confirm that NE differentiation is an indicator of poor prognosis.

1363 Exploring the Immunogenetic Susceptibility of Crohn's Diseases at the HLA-DPB Locus

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Introduction: Although the etiology of Crohn's disease (CD) is not known there is an increasing body of evidence for a genetic predisposition. Considering the data for immunogenetic events in the pathogenesis of the disease, likely candidate genes are those involved in the immunological process. It has been claimed, that genetic markers for CD might be located on chromosome 6 in the human lymphocytes antigen class II region genes. Therefore, we determined the allele frequencies of the HLA-DPB1 gene in CD and normal controls. **Methods:** 37 unrelated patients with the diagnosis of CD based on standard clinical, histological and immunological criteria and 47 unrelated normal control patients among a German population were included in this study. The alleles of the HLA-DPB1 gene were determined according to a modified protocol of the recommended PCR oligotyping procedure of the XIth HLA workshop. The polymorphic second exon of the DPB1 gene was amplified by PCR and hybridized with 25 sequence specific oligonucleotide probes to assign the HLA-DPB1 alleles on the basis of known sequence variations. **Results:** Out of the 36 different HLA-DPB1 alleles so far known, the HLA-DPB1*0101 allele was found to be positive in 19% (7/37) of patients with CD and 9% (4/47) in the control population, resulting in a relative risk estimate of 2.51 (95% confidence limits: 0.674-9.334). A slightly higher frequency of the allele HLA-DPB1*0301 was observed in patients with CD (11/37) compared to normal control patients (6/47) with a relative risk estimate of 2.89 (CI: 0.953-8.765). No major differences were observed comparing frequencies of other DPB1 alleles in our study group. **Conclusions:** These data demonstrate a minor role for the HLA-DPB1 alleles contributing to the genetic susceptibility of Crohn's disease.

1364 Expression of Vascular Adhesion Molecules and Early Mucosal Changes in Crohn's Disease

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To identify early mucosal changes in Crohn's disease, distribution and localization of cellular adhesion molecules on vascular endothelial cells and mononuclear cells were studied immunohistochemically using frozen sections from colonic mucosa in patients with Crohn's disease. Specimens were obtained from macroscopically normal areas and inflamed mucosa of 18 patients with Crohn's disease. Disease control and normal control specimens were obtained from 20 patients with ulcerative colitis and 14 patients with colonic cancer or polyp, respectively. The specimens were fixed in PLP solution, frozen in dry ice ethanol and sectioned on microtome. An indirect immunoperoxidase method was applied using a series of monoclonal antibodies of intercellular adhesion molecules-1 (ICAM-1), endothelial leukocyte adhesion molecule-1 (ELAM-1), vascular cell adhesion molecule-1 (VCAM-1), lymphocyte function associated antigen-1, sialyl Lewis X, and very late antigen-4. To determine the phenotypic characteristics of vascular endothelial cells and mononuclear cells, an immunoperoxidase double staining method was also performed.

In the mucosa of normal controls, ICAM-1 was expressed on vascular endothelial cells within the lamina propria, while ELAM-1 staining was weakly found on vascular endothelial cells and VCAM-1 on follicular dendritic cells in the lamina propria. In the macroscopically normal areas of Crohn's disease, ICAM-1 and ELAM-1 were expressed on vascular endothelial cells. In contrast to normal control mucosa, expression of ICAM-1 and ELAM-1 was strongly found on vascular endothelial cells in areas without aggregation of inflammatory mononuclear cells in several cases of Crohn's disease. Double staining method revealed that most of ICAM-1 positive vascular endothelial cells and ELAM-1 positive vascular endothelial cells were HLA-DR positive. In ulcerated lesions of Crohn's disease, ICAM-1 and ELAM-1 were expressed on vascular endothelial cells, and ICAM-1 positive mononuclear cells were markedly increased in the lamina propria, which was similar to the findings of ulcerative colitis.

In conclusion, these findings suggest that the expression of cellular adhesion molecules on vascular endothelial cells may play an important role in facilitating leukocyte migration into sites of early inflamed mucosa and maintaining chronic inflammation in Crohn's disease.

1365 The cAMP and Therapy Results Correlation in Ulcerative Colitis

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The cyclic nucleotide concentration (cAMP and cGMP) were determined in rectal mucosal biopsates (RMB) in 67 patients with Ulcerative colitis (UC) to research the correlation between effectiveness of treatment and intercellular regulation state. The cAMP and cGMP concentration were measured by radioimmune assay. The healthy persons were used as a control. Accordingly to cAMP level two groups of patients were examined: 31 patients with cAMP rectal concentration not more than in controls (1500 pmol/g) and 36 patients with high cAMP concentration (more than 2000 pmol/g). In group with high cAMP level the treatment was unsatisfactory in 13 persons (41.9%), but in group with normal cAMP level only 3 (8.3%) cases without therapeutic effect have been noticed. Thus, the correlation between the initial cAMP concentration in RMB and effect of treatment have been observed. Perhaps, patients with high cAMP content are more resistant to the treatment than persons with its normal level. We believe that intercellular regulation may influence on the results of therapy in UC and had to be taken into consideration in the drug therapy choice.

1366 Liver Histopathology and Liver Function Tests in Ulcerative Colitis

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Hepatobiliary disorders are well known complications in patients with Ulcerative Colitis. In this study we reevaluated the liver biopsies from 31 patients (12 female and 19 male patients) with Ulcerative Colitis who admitted to our clinic during 1991-1993. None of the patients showed clinical or biochemical signs of hepatobiliary disease. 15 patients (48.4%) had completely normal biopsy results. 7 patients (22.6%) showed fatty infiltration of the liver, 4 patients had focal necrosis (12.9%) and in 1 patient cholangitis (3.2%) was detected. In the remaining group minimal lesions were seen. The histopathological findings were unrelated to either activity and age of colitis. As a result, in the absence of clinical and biochemical parameters, changes in liver histology

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