**15** Lymphocyte Subset, Cellular Immune Function and HLA Studies in Chronic Hepatitis C. Predictors of Response to Interferon

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In order to clear the role of immune system in the pathogenesis of hepatitis C virus (HCV)-positive liver disease, various cellular immune parameters have been studied in 24 patients with chronic hepatitis C. Lymphocyte subset distribution in peripheral blood (total B, T, CD4+, CD8+, CD16 + cell counts), as well as mitogen-induced lymphoproliferative response and natural killer (NK) cell activity, furthermore HLA A, B, C and DR phenotypes were determined.

In addition, we have searched for predictors of response to interferon (IFN) treatment.

**Results:** there was no marked difference from the normal values concerning the lymphocyte subsets and mitogen-induced proliferative response, but the NK-cell activity proved to be significantly decreased (p 0.02) in HCV-patients. The prevalence of HLA B6 was 33% vs 15.2% of controls, HLA DR3 occurred in 42.8% vs 12%, HLA DR4 28.5% vs 12.7%, and HLA DQw8 50% vs 18.1%, respectively. The good response to IFN was not influenced by either the pretreatment lymphocyte findings or HLA phenotype. However, during the IFN treatment, both CD4+ and CD8+ cell counts were significantly lower in responders than others, at the end of the first month of therapy, while B cell count was significantly higher in these responders after three months. Recombinant IFN-alfa given at a dose of 3 MU thrice weekly resulted in a sustained complete remission in 28% of patients, and enhanced both mitogen-induced proliferative response and NK-cell activity in 50-75%.

**Predictors of response were:** 1. a female predominance, 2. a shorter duration of the disease, (more exactly: HCV-positive blood transfusion given within 3 years), and 3. an absence of anti-HBC antibody in the sera.

**Conclusion:** our findings suggest the importance of immune reaction and even possibly of HLA factors in the development of chronic hepatitis C. Further studies are needed to clarify the exact nature of immune mechanisms in this disease.

**16** Prognosis of Hepatitis C: 15-Year-Analysis of Cellular-Molecular Virological Findings in a Special Group with an Identical Perenteral Infection

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The results of several studies concerning the prognosis of the hepatitis C virus (HCV) infection vary widely. They differed in the histological types of chronic hepatitis as well as in the frequency of cirrhosis and the majority of the studies did not include PCR-data. The aim of this study was to obtain reliable results by means of a long-term analysis of a well-defined group of young patients with an identical source of HCV-infection (virus-contaminated anti-D-immunoglobuline).

**Methods:** In 1978, in various centres of the former GDR, several breaks of hepatitis C had been occurred in women treated with anti-D-immunoglobuline for preventing Rh-incompatibility. Out of 2533 women who had fallen ill with hepatitis C the clinical, biochemical, serological and histological data of 412 patients of two hospitals in Leipzig and Dresden were correlated with the PCR-data of the 5’ noncoding region. For the determination of the chronicity rate and the type of chronic hepatitis 345 liver biopsies were performed and all patients were examined sonographically.

**Results:** in the acute stage of hepatitis C half of the patients were clinically asymptomatic. The incubation period was on average 45 days. 65% of the patients showed a polyphasic ALT-pattern. In 1993, current results were obtained from 80% of the 412 patients. The chronicity rate of 62% after a 10-year-period dropped to 51%. All cases of chronic hepatitis C were of the histological type of chronic persistent or lobular hepatitis. In 82% of all patients the anti-HCV-test and in 56% the HCV-PCR were reactive.

**Conclusions:** In a homogeneous group of HCV-infected women especially suited for the prognostic assessment of hepatitis C a chronicity rate of 51% was registered after 15 years. There was a uniform histological pattern of chronic persistent hepatitis and in most of these patients further viral replication was observed. In contrast to other authors we found no chronic active hepatitis or cirrhosis.

**17** Soluble Intercellular Adhesion Molecule-1: Significance in Chronic Liver Disease

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Intercellular adhesion molecule-1 (ICAM-1) is important in the migration of inflammatory cells from the circulation and their adhesion to target tissue. A soluble form, sICAM-1 has been described in chronic liver disease, but its function remains unclear. Our aims were to determine whether sICAM-1 has relative disease specificity, and whether it correlates with pathogenic mechanisms. sICAM-1 was measured by ELISA in 41 patients with primary biliary cirrhosis (PBC) (stage 1 = 10; stage 2 = 9; stage 3 = 8; stage 4 = 14), 9 with primary sclerosing cholangitis (PSC), 15 with alcoholic liver disease (ALD) and in 17 healthy controls. Liver function tests were determined by routine methods. Liver fibrosis by using interluekin-2 receptor expression using two colour flow cytometry. Kinetic assessment of cholestasis was performed in a sub-group of PBC patients (n = 9) by direct measurements of rates of hepatic uptake and excretion of 75SeHCAT. sICAM-1 was elevated in all three disease groups compared to controls. PBC (median 602 ng/ml, range 226-3276, p < 0.0001), PSC (1077, 393-219, p < 0.0001) and ALD (393, 96-408; p < 0.05). The levels in PBC and PSC were also significantly higher than ALD. In PBC, sICAM-1 was higher in late compared to early disease (p < 0.0002), and correlated significantly with histological progression, and markers of cholestasis (alkaline phosphatase, g-glutamyl transpeptidase and conjugated bilirubin). However, it did not correlate as well with markers of hepatocellular damage (alanine transaminase and albumin). There was a trend towards an inverse correlation with hepatic excretory rate (p = 0.07) but with hepatic uptake rate of 75SeHCAT. These latter parameters are direct measurements of cholestasis and hepatocellular function respectively. No correlation was detected with lymphocyte interluekin-2 receptor expression. We conclude that serum sICAM-1 is specifically elevated in autoimmune cholestatic liver diseases. In PBC it is related to progression of disease and is a marker of bile duct damage and cholestasis.

**18** Expression of ICAM-1 (CD54) in Human Hepatocellular Cell Lines is Regulated via Protein Kinase C

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**Background:** Expression of the ICAM-1 adhesion molecule is required for the interaction with and activation of specific T cells bearing the β2-integrin LFA-1. ICAM-1 expression varies considerably between different cell types; insights into its regulation may therefore help to understand tissue-specific immune responses. ICAM-1 expression on hepatocytes correlates with immune activation within the liver including rejection of human liver allografts.

**Aim:** Study the hepatocytic intracellular signal systems involved in regulation of constitutive and cytokine-mediated ICAM-1 expression.

**Methods and Results:** ICAM-1 protein was measured by quantitative cellular ELISA using fixed, adherent Hep G2 and SK-Hep1 cells. Total mRNA was prepared for Northern and slot blots that were hybridized with an anti-sense ICAM-1-sDNA probe. Hep G2 and SK-Hep1 were stimulated with agonists and antagonists of the protein kinase C, calmodulin, protein kinase A, and protaglandin system, in addition to the Ca2+ ionophore A23187. Only the protein kinase C stimulator PMA induced a significant increase in ICAM-1 protein (from 3 h, max. at 12 h) in a dose-dependent manner (from 10 nM, max. at 100 nM). ICAM-1 mRNA accumulated to a peak concentration at 3 h; this increase was completely dependent on protein kinase D. Cycloheximide, a protein synthesis inhibitor, synergistically enhanced ICAM-1 mRNA levels. Moreover, cytokine-mediated increase in ICAM-1 caused by the interferon-γ, tumour necrosis factor-α, and interleukin-1, was blocked by staurosporine, a PKC inhibitor.

**Conclusion:** Constitutive and cytokine-mediated ICAM-1 gene and protein expression in two human hepatocytic cell lines mainly involve intracellular signaling via protein kinase C. Our observation indicate that hepatocytes share at least some of the immunoregulatory pathways described for other cell types.

**19** Protective Effect of Zinc Sulfate (ZS) on Mortality, Narcosis and Hepatic Cell Necrosis Due to Acute Ethanol Intoxication

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**Aims:** This study pretends to observe if ZS has an effect on: a) mortality, b) narcosis and c) ethanol blood clearance and hepatic necrosis due to acute ethanol intoxication.

**Methods:** A mortality: 70 rats were intoxicated with an L500-IP dose of 30% ethanol (4.5 g/kg). Saline or ZS were intraperitoneally injected thirty minutes afterwards. Mortality was observed during 24 hr. B. Narcosis: 30 rats were IP intoxicated with a narcotic dose of 30% ethanol (4 g/kg). Saline or 50 mcg/kg of ZS were IP administered thirty minutes afterwards. Changes in behaviour were hourly rated using the behavioural rating scale of Majchrzicz who was unaware of
the animal's treatment status. C. Ethanol blood clearance and hepatocellular necrosis: 20 rats were administered an LD50-IP dose of ethanol and thirty minutes afterwards CS (60 mg/kg) were IP injected. Two animal cells from each group were hourly sacrificed in order to measure blood ethanol levels by gas chromatography and the extent of hepatocellular necrosis by a pathologist who was unaware of the animal's treatment status. Results: A. A reduction in mortality was observed in rats receiving CS. The strongest protective effect was observed with a dose of 50 mcg/kg (36% vs 83%, p < 0.05). B. CS shortened the necrotic period. The strongest effect was observed 4 hrs after intoxication (2.4 vs 1.9 Majchrzowicz Index, p < 0.05). C. Finally CS significantly decreased the extent of hepatocellular necrosis 3 and 5 hrs after intoxication (1% and 5% in CS vs 20% and 15% in controls). Blood ethanol levels were lower with CS than with saline. Conclusions: CS in physiological dose was shown to have a protective effect on acute ethanol intoxication. The precise mechanism of action remains to be elucidated.

20 HLA-DPB and Susceptibility to Primary Biliary Cirrhosis
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Introduction: While the precise etiology of primary biliary cirrhosis (PBC) is unknown, several studies suggest that immune abnormalities and a genetic susceptibility participate in the disease pathogenesis. The search for candidate genes has been centered on the HLA class II region located on chromosome 6. HLA genotyping techniques enabled the recognition of a large series of DPB1 alleles and the association of several diseases with particular DPB1 alleles. Recently, an increased frequency of the allele HLA-DPB1*0301 in patients with PBC has been described in a Japanese population. Considering the known differences among allele frequencies of HLA class II genes between different ethnic groups, we determined the allele frequencies of the HLA-DPB1 gene among Caucasians with PBC and normal controls. Methods: 28 unrelated patients with the diagnosis of PBC based on standard clinical, histological and immunological criteria and 47 unrelated normal controls among a German population were included. Genomic DNA was extracted, the second exon of the DPB1 gene amplified by the polymerase chain reaction and hybridized with 25 sequence specific oligonucleotide probes (PCR-SSO method) to assign the HLA-DPB1 alleles on the basis of known sequence variations, according to the protocols of the Xth HLA workshop. Results: The HLA-DPB1*0301 allele was found to be positive in 50% (13/26) of patients with PBC and 13% (6/47) in the control population (p < 0.001), resulting in a relative risk estimate of 6.8 (95% confidence limits: 2.2-21.6). A higher frequency of the allele HLA-DPB1*0301 in patients with PBC (15% vs 2%) was observed, but did not result in a significant relative risk estimate, probably due to the low number of patients. No differences were observed comparing frequencies of other DPB1 alleles in our study group. Conclusions: These data demonstrate a significant association between PBC and the allele HLA-DPB1*0301 in a Caucasian population. This supports a strong contribution of HLA-DPB1 alleles to the genetic susceptibility to primary biliary cirrhosis.

21 Fructose Stimulates Liver Growth After Partial Hepatectomy
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Graft function and ATP synthesis are impaired in transplanted livers. Since fructose may be a better source for ATP synthesis than glucose, the effect of a 20% sucrose diet on liver regeneration was determined in 60 rats. A diet of 59% starch, 20% protein, 10% fat, 3% fiber, and vitamins and salts was fed for 1 week. 15 rats had partial heptectomies and were fed the control diet; 15 had sham operations and this diet. 30 rats were fed a diet of 20% fructose, 39% starch, 20% protein, fiber, vitamins and salts for 1 day, and then 15 had partial heptectomies, and 15 had sham operations. Liver samples were obtained 1, 2, 3, 4 and 7 days after operation, and were assayed for ATP, total lipid, triglyceride, protein, and DNA synthesis. Mitotic index and fat vacuoles were determined by histology. Mitotic index increased after partial hepatectomy, and fructose further increased mitosis by 3-fold 1 day after hepatectomy. Fructose decreased triglyceridelevels and fat vacuoles in both hepatectomy and hepatectomy groups, but total lipid increased. ATP levels decreased slightly, but DNA synthesis and protein content was unchanged. Since 20% sucrose stimulated cell division after partial hepatectomy, fructose metabolism increased ATP utilization for energy requiring functions of liver regeneration. Low concentrations of fructose in enteral or parenteral nutrition may stimulate recovery of the transplanted liver.

22 Effects of Endothelin-1 on Systemic and Regional Hemodynamics After Intraperitoneal and Centralvenous Injection
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Lung and liver are important in clearing endothelin-1 from the circulation. To compare the hemodynamic effects of endothelin-1 (ET-1) after first pass through the liver with first pass through the lungs, six piglets received 10 μg 10^{-6} ET-1 in the portal vein and centralvenously in randomized order. Cardiac output with thermodilution technique and blood gas analysis was performed to confirm baseline conditions prior to injections. Flow was measured continuously in the hepatic and renal arteries and in the portal vein with transvascular ultrasonic transit-time probes. Pressure was measured continuously in the aorta, in the pulmonary artery, in the portal and the superior caval veins. Heart rate, oxygen consumption and carbon dioxide production were determined every minute.

Central venous injection of ET-1 caused a larger reduction in portal vein flow (a difference of 295 μl/min ± 66, p < 0.01), a larger increase in aortic pressure (45 mm Hg ± 7, p < 0.01), a larger increase in heart rate (23 ± 7, p < 0.05) and a smaller increase in portal vein pressure (3.5 mm Hg ± 0.6, p < 0.01) than intraportal injection. The effects on hepatic and renal artery flows seemed to depend upon the site of injection. The animal received the central venous or the intraportal injection first. Given prior to the intraportal injection, the central venous injection caused a loss of the hepatic arterial buffer response. This reaction was intact when the intraportal injection preceded the central venous injection. Thus, the hemodynamic effects of ET-1 given centralvenously is different from ET-1 given intraportally.

23 Symptoms and Quality of Life in Patients with Uncomplicated Duodenal Ulcer Disease

Patients treated for duodenal ulcer (DU) disease in primary care have been suggested to have a milder "uncomplicated" disease compared to hospital outpatients. In cooperation with local primary care units we investigated untreated primary care patients with possible ulcer disease regarding endoscopic findings, symptoms, history and Quality of Life before endoscopy, during treatment and follow up. Patients were treated with effective acid inhibition during 2-4 weeks and at symptomatic relapse (2-4-w) for 12 months.

Quality of Life (QOL) was investigated with two earlier well established and validated questionnaires Psychological General Well Being Index (PGWB) and Gastrointestinal Symptom Rating Scale (GSRS). These questionnaires were filled out before endoscopy, day 15, 14 days after healing and at 8 and 12 months follow up.

1526 patients were investigated with endoscopy. A total of 393 DU were included, 57% males, 56% smokers, mean age 50 years. History of ulcer symptoms was <5 years in 35% and >20 yrs in 24%. During the last year 64% had 1-2 and 27% 3-5 symptomatic periods. In 16% this was the first verified ulcer episode. In another 33% their first ulcer had been verified within less than one year. Current symptoms were classified severe enough to partly or definitely inhibit normal work in 93% of patients. During follow up (n = 305) 31% had no relapse, 26% one, 23% 2-3 and 20% >3 relapses or constant symptoms.

OQL scores: PGWB showed a low degree of general well being at entry, the values returned to those found in a normal population after healing. Evaluation of the GSRS showed severe symptoms at entry with a reduction during follow up.

We conclude that our "uncomplicated" DU patients have symptoms and relapse pattern as described in hospital outpatients. General well being (QOL) is low in untreated patients.

24 Pain and Quality of Life in Acute Duodenal Ulcer (DU): Effect of Ranitidine
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The effect of antisecretory drugs on the sensory and affective components of DU pain, and on the quality of life of DU patients, have not been studied so far. Patients (pts) and methods: 101 pts with epigastric pain and DU at endoscopy (diameter at least 5 mm) were included in this multicentre prospective study. All were treated with effervescent ranitidine 300 mg per day for 4 weeks. The following parameters were assessed: a) disappearance of DU pain by self-