served one young infant girl (8 month) with the bleeding from the 1.5 month of life. During a diarrhea in the 6 month of life a excretion polypos of 1.5–2.5 cm diameter was observed. Polypos juveniles was recognized in the histopathological investigation. One month later the second polypos was excreted and bleeding the lower digestive tract and anaemia (Hb 7.6 g/dl) was observed. Colography did not give the definite reason for the bleeding. Colonoscopy was performed (video film) and a tumor in ascendens colonis was found what made another investigation impossible. The 8 x 10 cm tumor was removed surgically. Postoperatively, the general condition of the infant is satisfactory, free from complications.

**11 Use of PCR to Individually Tailor Interferon Dosage in Chronic Hepatitis C**

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Although interferon-α (IFN) is widely used in the treatment of chronic hepatitis, the optimal dosage remains to be defined. Given the high cost of IFN, it is desirable to use the lowest effective dose. This dose is probably variable from one patient to another. We therefore decided to study the feasibility of detecting HCV-RNA by PCR to individually tailor doses and to compare the response rate obtained with that of a standard dose schedule.

Patients with chronic hepatitis C were divided into two groups. All patients received 18 mioU IFN 2a/week in three doses for 6 weeks followed by reduction to 9 mioU/week. For patients in group 1, this dose was maintained for the following 18 weeks. However, those patients who normalised transaminases with the high dose but who relapsed later on had their dose increased to 18 mioU/week again. For patients in group 2, PCR was performed monthly and the dose reduced every 2 months provided that serum HCV-RNA was negative. Dose reductions were to 4.5 mioU/week, then 3 mioU/week and finally 1.5 mioU/week. The study is ongoing and we report here the results after 6 months treatment.

**Results:** There were 22 patients in gp 1 and 28 patients in gp 2. Four patients were withdrawn before the end of 6 month period in gp 1 because of non-response and in gp 2, three patients were withdrawn for non-response and 1 from other cause of side-effects. At the start of therapy, ALT were similar in both groups (115 ± 25 vs 116 ± 15.54). At 6 months ALT levels were significantly reduced but with no difference between the two groups (45.5 ± 15.54 vs 27.5 ± 13.38). On an intention to treat basis ALT was normalised in 11/22 (50%) in gp 1 and 15/28 (53.6%) in gp 2. HCV-RNA was negative in 12/28 (43%) patients in gp 2. In group 1, at month 6, 15 patients (83%) were receiving 9 mioU/week and 3 patients (17%) 18 mioU/week. In gp 2, 5 patients (21%) were receiving 3 mioU/week. Of these, 4 (80%) were HCV-RNA negative 8 patients (33%) received 4.5 mioU/week (6 (75%) HCV-RNA negative, 7 patients (29%) received 9 mioU/week (2 (22%) HCV-RNA negative) and 4 patients (17%) received 18 mioU/week, none of whom were HCV-RNA negative.

**Conclusions:** This study shows that after an induction dose, it is possible to use PCR to individually tailor interferon dosage allowing the use of lower doses than currently recommended, without compromising response rates, thus lowering side effects. This study also shows that in responding patients the dose can usually be lowered, whilst in non-responders, increasing the dose above 9 mioU/week is not beneficial. There is significant variation between patients in the dose of IFN required.

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**12 Human Lymphocyte Proliferative Response to the Hepatitis B Virus Pre-S1 Synthetic Antigen**

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The course of hepatitis B virus (HBV) infection depends on the host's immune response to the antigens of the virus. The Pre-S1 antigen is a very important component of mature virion and plays an essential role in virion attachment and assembly.

Pre-S1(20–49) antigen was analysed for its ability to stimulate peripheral blood mononuclear cells (PBMCs) from patients with chronic hepatitis B (CHB) infection, convalescents after HBV infection, carriers of hepatitis B surface antigen (HBsAg) and normal healthy individuals. PBMCs were also examined for their proliferative response after in vitro stimulation with phytohemagglutinin (PHA), monoclonal antibody to CD3 molecule (anti-CD3 MoAb) and allogeneic cells (mixed lymphocyte reaction, MLR).

The PBMCs of the majority of patients after recovery from hepatitis B infection responds with significant proliferation to stimulation with Pre-S1.

**Pre-S1-induced T-cell specific proliferation was not detectable in healthy individuals. Compared to the vigorous T-cell response to Pre-S1 antigen in convalescents, the T-cell responses of chronically infected and HBsAg-positive patients were significantly depressed. The proliferative responses to PHA and anti-CD3 MoAbs of PBMCs of CHB patients were significantly lower in comparison to the responses of PBMCs of convalescents and healthy individuals. Proliferative responses of T-cells from CHB and HBsAg-positive patients to alloantigens in MLR were significantly diminished as compared to the response of cells from convalescents and from healthy control patients.**

Our data confirm the suggestion that unresponsiveness to the Pre-S1 antigen facilitates chronic hepatitis B infection and/or viral persistence.

**13 Low Substitution Rate in Precore/Core Gene of Hepatitis B Virus in Asymptomatic Carriers**

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Hepadna viruses are DNA viruses, but they replicate via a RNA intermediate. High substitution rate in nucleotide sequences of the hepatitis B virus precore/core gene has been found in patients with chronic hepatitis B virus infection, but a relatively lower evolution rate has also been reported. To investigate the accurate substitution rate of the hepatitis B virus precore/core gene, sera of 12 asymptomatic carriers from 4 families with clustered hepatitis B virus infection were analyzed by polymerase chain reaction and direct sequencing. The diversities of precore/core nucleotide sequences in these asymptomatic carriers were 0.16% to 1.3% as compared with a prototype adr sequence. The diversities of nucleotide sequences of the precore/core gene among members from different families were from 0.02% to 0.44%, and the diversities between mothers and children of the same family were only 0.1% to 0.16%. The substitution rate in nucleotide sequence of hepatitis B virus precore/core gene was estimated to be between 0 and 9.8 x 10⁻⁵ per site per year with a median of 0.

We conclude that the evolution rate of hepatitis B virus precore/core gene in asymptomatic carriers could be as stable as those of DNA genomes.

**14 Detection, Incidence and Prognostic Significance of Allograft CMV Infection Following Orthotopic Liver Transplantation**

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CMV infection remains a serious complication after liver transplantation and its trigger function on acute and chronic rejection is discussed. The aim of the present study was to find the best method to detect CMV infection of the transplant, to determine its incidence and to examine its clinical relevance and prognostic significance. A total of 294 specimen (biopsies and explants) from 60 orthotopic liver transplantation (OLTX) patients were examined by routine light microscopy, in situ hybridisation (ISH), immunohistology (IHM) and CMV-DNA PCR. 27 of the 294 specimen were examined by PCR following DNA extraction from snap-frozen material. The probability for a transplant to acquire CMV-infection and its prognostic significance on patient survival was determined (Kaplan and Meier). In addition, the correlation between CMV-infection and acute as well as chronic rejection was examined. The combination of ISH and IH revealed the highest number of CMV-positive biopsies (15%), with IH alone detecting 80% of them. In the subgroup examined by CMV-DNA PCR the detection of positivities was not increased. The probability for the transplant to acquire CMV-infection within the first year after transplantation is 40% with a peak of infections within the first 6 weeks after OLTX and a probability of 35%. From 27 rejection episodes in transplants with an early CMV-infection, 20 occurred before and 7 after CMV-infection indicating a trigger function of acute rejection on CMV-infection but not vice versa. Out of 25 transplants with an observation period greater than 60 days, 31% of grafts with CMV infection developed chronic rejection whereas, none of 9 grafts without CMV infection did. Finally, patients with a CMV infection of the graft had a significantly reduced one-year survival rate (58%) compared to patients without graft CMV-infection (87%, p = 0.03), with cause of mortality mainly due to infectious complications. These data demonstrate the clinical importance and prognostic significance of the early detection of CMV infection.
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 in HCV.

In a homogeneous group of HCV-positive women studied 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, lymphocyte activation by measuring interleukin-2 receptor expression using two colour flow cyrometry. 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 3HSeCAT. sICAM-1 was elevated in all three disease groups compared to controls. PBC (median 602 nMl, range 226-3276; p < 0.0001), PSC (1077, 393-219, <0.0001) and ALD (393, 96-908; 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 3HSeCAT. These latter parameters are direct measurements of cholestasis and hepatocellular function respectively.

No correlation was detected with lymphocyte interleukin-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.

**Expression of ICAM-1 (CD54) in Human Hepatocytic 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; insight to 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 cDNA probe. Hep G2 and SK-Hep1 were stimulated with agonists; alpha-tocopherol, interferon-gamma, IL-1α, IL-1β, IL-6, TNF, PMA, CD3 stimulation, and the cytokines IL-2, IL-4, IFN-γ, and IL-10, respectively. 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 upon protein kinase C. 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 signalling via protein kinase C. Our observation indicate that hepatocytes share at least some of the immunoregulatory pathways described for other cell types.

**Protective Effect of Zinc Sulfate (ZS) on Mortality, Narcosis and Hepatocellular 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 hepatocellular necrosis due to acute ethanol intoxication.

**Methods:** A mortality: 70 rats were intoxicated with an LD50-IP dose of 30% ethanol (4.5 g/kg). Saline or ZS in doses of 5, 15, 50 and 100 mcg/kg were IP administered thirty minutes afterwards. Mortality was observed during 24 h. B: Narcosis: 30 rats were IP intoxicated with a narcotic dose of 30% ethanol (4.5 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 Majchrowicz by an investigator who was unaware of

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, 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 B8 was 33% vs 15.2% of controls, HLA DR3 occurred in 42.8% vs 12%, HLA DR4 28.5% vs 12.7%, and HLA DQw2 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, 3. more exactly; HCV-positive blood transusion 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 Perenatal Infection

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The results of several studies concerning the prognosis of the hepatitis C viral (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-immunoglobulin).

Methods: In 1978, in various centres of the former GDR, several outbreaks of hepatitis C had been occurred in women treated with anti-D-immunoglobulin 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 ALAT-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