gression represents an important surrogate end point for evaluation of the vulnerability of a given patient and for assessment of treatment impact on natural history. Activity grade is not very useful to predict fibrosis progression since there is no study demonstrating that activity is predictive of fibrosis progression independent of fibrosis stage and, in fact, fibrosis alone is the best marker of ongoing fibrogenesis (4). Because of the informative value of fibrosis stage it is worthwhile for clinicians to assess the speed of the fibrosis progression. In a large population we observed that fibrosis progression rate was not normally distributed with an asymmetrical distribution suggesting the presence of at least three populations: one population of "rapid fibrosers", a population of "intermediate fibrosers" and one population of "slow fibrosers" (5). Using the- median fibrosis progression rate, and without treatment, the median expected time to cirrhosis was 30 years; 33% of patients had an expected median time to cirrhosis of less than 20 years and 31% will never progress to cirrhosis, or would do so in more than 50 years. There are no clear cut explanation to the individual susceptibility of developing liver fibrosis. However several factors have been clearly shown as associated with higher fibrosis progression rate: duration of infection, late age, male gender, consumption of alcohol and HIV coinfection with low CD4 count. For example, the estimated probability of progression per year for men aged 61-70 years was 300 times greater than that for men aged between 21 and 40 years and 10 times greater than that for women aged 61-70 years. Virus-associated factors such as genotype, viral load, quasi species are not associated with fibrosis.

Better knowledge of factors associated with individual susceptibility will allow a better definition of the patients' risk of developing fibrosis.

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Alcoholic and nonalcoholic steatohepatitis

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Chronic alcohol abuse may lead to two different types of liver injury. Most drinkers develop fatty liver, which by itself is a reversible alteration and has a low risk of progressing to liver cirrhosis. Approximately 20-40% of heavy drinkers, however, develop a special type of alcoholic liver disease, namely alcoholic steatohepatitis (ASH), which rapidly progresses to liver cirrhosis in most of the affected patients. ASH is characterized by the ballooning of hepatocytes, steatosis, hepatocellular necrosis and apoptosis, pericellular and perivenular fibrosis, inflammation with predominantly polymorphonuclear granulocytes, cholestasis and activation of Kupffer cells (1). Furthermore, a hallmark lesion seen in hepatocytes is the appearance of cytokeratin (CK)-containing cytoplasmic inclusions, termed Mallory bodies (MBs), which is accompanied by a disruption of the CK intermediate filament cytoskeleton (2-4). These alterations, however, are not specific for ethanol-induced toxic liver injury, but can also be found in patients without evidence of alcohol abuse. Occurrence of nonalcoholic steatohepatitis (NASH) is associated with obesity, noninsulin-dependent diabetes, intestinal bypass surgery, bacterial contamination of the small bowel, as well as with several drugs, such as amiodarone or perhexiline maleate, which are known to be inhibitors of mitochondrial ß-oxidation (5). Identical hepatocytic alterations to that seen in human liver biopsies with ASH or NASH can be experimentally induced in mice by chronic intoxication with the porphyrogenic drugs griseofulvin (6) or 3,5-diethoxcarbonyl-1 ,4-dihydrocollidine (DOG) (7). Analysis of the protein composition of MBs revealed that besides CKs also non-CK components, namely, the stress-inducible MM 120-1 antigen, a 62-65 kDa MB component recognized by the antibody SMI 31, and ubiquitin, which is a common constituent of a variety of cytoplasmic inclusions occurring in different chronic degenerative diseases, are present in MBs (8, 9). The role of these different components in MB formation as well as the relevance of MBs and the cytoskeletal alterations in the course of alcoholic hepatitis is still unclear.

To obtain further insight into the role of the different MB components and the alterations of the OK cytoskeleton in the pathogenesis of ASH and NASH, we investigated mice in which either of the two OK genes expressed in hepatocytes, namely CK8 and CK18, had been inactivated (10, 11). Since OK intermediate filaments are obligatory heteropolymers, no intermediate filaments can be formed in the absence of one of these two partner proteins resulting in hepatocytes devoid of a cytoplasmic OK network (12, 13). DDC intoxication of *CK8—/—* mice showed a higher toxicity than in wild-type mice. After 3 months of intoxication seven out of 12 CK8—/— animals had died, whereas all 13 K8+/+ mice survived. Analysis of metabolic alterations in these mice revealed that mice lacking CK8 develop a much severer porphyria than wild-type mice. Analysis of livers from DDC-fed CK8-/— mice with double-label immunofluorescence microscopy showed that in the absence of CK8 no MBs were formed. Moreover, none of the non-OK MB components accumulated in these livers, indicating that OK is the core protein in MBs and that all other MB components either bind to or coassemble with CK. These *in vivo* data are in line with previously obtained *in vitro* data where we found that overexpression of CK by transient transfection of cells is sufficient to lead to induction of the MM 120-1 protein and to association of the MM 120-1 protein with cytokeratin aggregates, mimicking the initial phase of MB formation. To our surprise, DDC intoxication of mice with only one inactivated CK8 allele did not lead to alterations of the cytokeratin cytoskeleton nor to the appearance of MBs, although all other signs of DDC intoxication such as the loss of lamin B2 from the nuclear lamina, development of porphyria, proliferation of bile ductules were present. This different behavior of wild-type and heterozygous CK8 mutant mice has, therefore, to be attributed to the loss of one CK8 allele. To obtain more information on the functional consequences of the disruption of one CK8 allele, the mRNA concentrations of CK8 and OKi 8 were analyzed using a quantitative RT-PCR assay. DDC intoxication led to an approximately 5-fold overexpression of both CK8 and CK18 mRNAs in wild-type mice. In CK8+/— mice, DDC caused a similar increase in the concentration of 0K18

mRNA. The concentration of 0K8 mRNA, however, raised only to approximately 50% of OKi 8 mRNA level. This indicates that in the absence of the second CK8 allele the DOG-induced overexpression is half maximal, and that in the situation of a relative excess of 0K18 over 0K8 no MBs are formed. Furthermore, these data showed that the effect of DOG intoxication on CK mRNA expression is independent from the GK cytoskeleton, suggesting that the overexpression reflects a direct or indirect response to the toxic injury.

Further important clues as to the role of GK in alcoholic liver disease have come from experiments in GK18 knockout mice. CK18—/— mice responded completely differently to DOG intoxication than did GK8 knockout mice. Although CK18-/— like GK8—/— mice are devoid of a CK IF cytoskeleton, CK18-/— animals showed no increased sensitivity to DOG. Therefore, the lethality seen in DOG-treated CK8-deficient mice cannot be attributed to the loss of the CK cytoskeleton but has to be related to the imbalance of nonassembled GK polypeptides or CK oligomers. This suggests a completely new mechanism for how CK may interfere with cellular processes. The specificity of these interactions is underlined by the fact that there are substantial differences in the consequences for the liver between mice which lack GK8 and mice which lack GK18. These differences are also pertinent with respect to MB pathogenesis. In contrast to GK8 defective mice, DOG intoxication of CK18-/— mice led to the formation of classical MBs consisting of CK8, the MM 120-1 and SMI 31 protein, and ubiquitin. These findings further show that CK8 can be stabilized in cells under certain pathological conditions even without the corresponding partner CK.

In conclusion, from these experiments it is obvious that CK8 is the key protein in MB formation. The increased toxicity in DOG-fed heterozygous and homozygous GK8 gene-deleted mice widens our view of the cellular function of GK and demonstrates that overexpression of GK8 protects hepatocytes from toxicity. Moreover, the fact that mice which were able to form MBs had fewer signs of toxicity than those which did not form MBs implies that the MB itself is not detrimental to the hepatocyte but rather is a product of a new cellular defense mechanism involving GKs.

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Hemochromatosis: Recent molecular findings

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Hemochromatosis is the clinicopathological syndrome caused by toxic accumulation of iron in the tissues: it may be demonstrably hereditary, sporadic or associated with another disorder, "secondary". Hemochromatosis causes liver disease, endocrine failure, joint disease and cardiomyopathy and is a true multisystem disease. Secondary hemochromatosis occurs when excess iron is administered or taken up by the intestine as a little understood effect of chronic dyserythropoietic anemia. Early treatment of iron overload prevents irreversible liver damage and is associated with normal life expectancy, provided cirrhosis has not supervened.

Hereditary hemochromatosis (HFE) is characterized by onset in the adult, juvenile (<35 years) or neonatal periods. Recently, the class I human lymphocyte antigen (HLA)-linked HFE gene, initially termed HLA-H, for the prevalent adult form of hemochromatosis that maps to chromosome 6p21. .3 has been identified by Feder *et al.*. This gene encodes a class I gene product that is almost ubiquitously expressed on cell membranes and specifically on the basolateral membrane of intestine epithelial cells. Two mutations in HFE, Cys282->Tyr and His83->Asp, have been associated with adult hemochromatosis, although the principal mutation Cys282->Tyr (C282Y) disrupts a ß 2-microglobulin binding domain essential for cell surface expression of class I molecules: this is the major determinant of iron overload. Between 85% and 10-0% of adult hemochromatosis patients are homozygous for C282Y and as predicted, the carrier frequency is —5% of the population. Studies in several pedigrees however have failed to find G282Y in some adult and most juvenile patients with hemochromatosis. Moreover, some adult C282Y homozygotes may have no signs of disease or abnormal parameters of iron metabolism. H63K appears to be a minor determinant of disease. Penetrance of the C282Y mutation in homozygous form, increases with age and about two-thirds of men over the age of 40- appear to show signs of iron-related tissue injury and/or organ failure. Pedigree analysis has also shown that some juvenile patients with severe iron overload have a disease gene with no linkage to chromosome 6p markers.

The identification of C282Y in HFE undoubtedly represents an important step in understanding iron metabolism, although beyond our knowledge that ß2-microglobulin knock-out mice develop iron storage disease and liver tumors and pathology that resembles the human disease, the role of HFE is quite unknown. Clearly the G282Y mutation is neither sufficient to explain all instances of human iron