American Journal of Pathology, Vol. 144, No. 1, January 1994

Animal Model

The LEC Rat: A Model for Human Hepatitis, Liver Cancer, and Much More

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The LEC rat is an inbred mutant strain with spontaneous hepatitis isolated from Long-Evans rats. Since approximately 40% of LEC rats die of fulminant hepatitis, the rat serves an animal model for studying the pathogenesis and treatment of human fulminant hepatitis. The remaining 60% of LEC rats survive and develop chronic (prolonged) hepatitis and subsequently develop liver cancer. Therefore, the LEC rat serves an important animal model for studying the significance of chronic hepatitis in the development of human liver cancer, which often develops in association with chronic hepatitis. The LEC rat can also be used as an animal model of Wilson's disease, since recent studies have disclosed high copper accumulation in the liver and low ceruloplasmin concentration in the serum of this mutant rat. (Am J Pathol 1994, 144:200–204)

Hepatitis, mostly viral in nature, affects more than 200 million people world wide. Although it is well known that liver cancer frequently develops in association with chronic hepatitis, the role of hepatitis in the occurrence of liver cancer has not been fully understood. An animal model that mimics the hepatitis and liver cancer sequence would be of great assistance in the research of human liver carcinogenesis.

The LEC rat is a suitable animal model for studying the significance of hepatitis in liver cancer development.[1,2] This mutant strain spontaneously manifests chronic hepatitis and subsequently develops preneoplastic lesions and liver cancer with an extremely high frequency (Figure 1). Thus, the natural history of the LEC rat shows a remarkable similarity to the clinical course of human liver cancer development.

Spontaneous hepatitis in LEC rats has recently been shown to be closely related to high copper accumulation in the liver, perhaps due to impaired excretion of hepatic copper into the blood and bile. Therefore, it is theoretically possible to examine whether or not chronic hepatitis is related to the occurrence of liver cancer by manipulating the occurrence of hepatitis by reducing copper concentration in the laboratory chow. In addition to suitability for liver cancer research, the LEC rat can serve as an animal model of human fulminant hepatitis and Wilson's disease because of its high mortality in hepatitis and abnormalities in the copper metabolism, respectively.

Biological Features

The LEC rat is a mutant strain of Long-Evans rats, designated from its peculiar cinnamonlike coat color, discovered in 1983 at the Center for Experimental Plants and Animals of Hokkaido University, Sapporo, Japan.[3]

The spontaneous hepatitis of LEC rats is characterized by a rather sudden onset at approximately 4 months after birth. The affected rats manifest jaundice and elevated serum levels of alanine and aspartate aminotransferases. Approximately 40% of

Supported in part by a Grant-in-Aid for cancer research from the Ministry of Health and Welfare and from the Ministry of Education, Science, and Culture of Japan.

Accepted for publication September 22, 1993.

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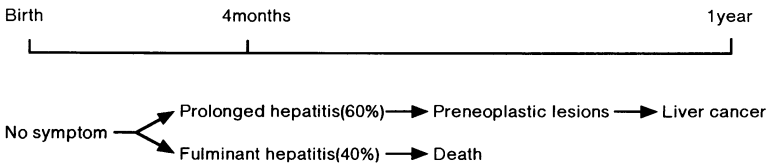

Figure 1. *Natural history of the LEC rat.*

the animals die of fulminant hepatitis within 1 week of manifestation of jaundice. Both sexes are affected, but females are more vulnerable to fulminant hepatitis. Serum levels of alanine and aspartate aminotransferases reach up to 500 IU/L, and affected rats manifest symptoms of hepatic failure such as anemia, dysuria, and hemorrhagic tendency. Histological examination of the liver at this period reveals massive to submassive coagulative necrosis of hepatocytes with minimal inflammatory cell infiltration, predominantly in the central areas of liver lobules.

The livers of LEC rats contain large amounts of copper,[4] and the genetic link between copper accumulation and the occurrence of hepatitis has been demonstrated.[5] Copper concentration in the liver is already high soon after birth and increases with age to reach 500 ng/100 g liver weight at 4 months old, which is 50 to 80 times that in the normal rat liver. Histochemical examination of LEC rat liver for copper by the magnesium-dithizonate silver-dithizonate method revealed that copper accumulated preferentially in hepatocytes and was distributed diffusely throughout the cytoplasm (Figure 2). Copper accumulates in virtually all hepatocytes throughout the entire liver lobule, but shows a tendency to localize in higher amounts in the cytoplasm of perilobular hepatocytes. The molecular mechanisms by which accumulated copper causes hepatotoxicity remain unknown. However, because our biochemical studies have indicated that a small

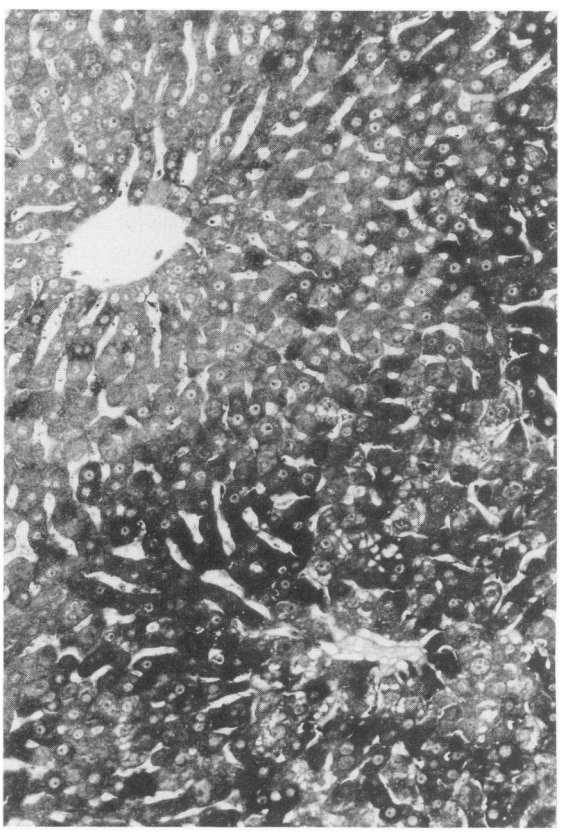

Figure 2. *Accumulation of copper in the liver tissue stained by the sulfide silver method. Copper accumulates more densely in the periportal hepatocytes (×170).*

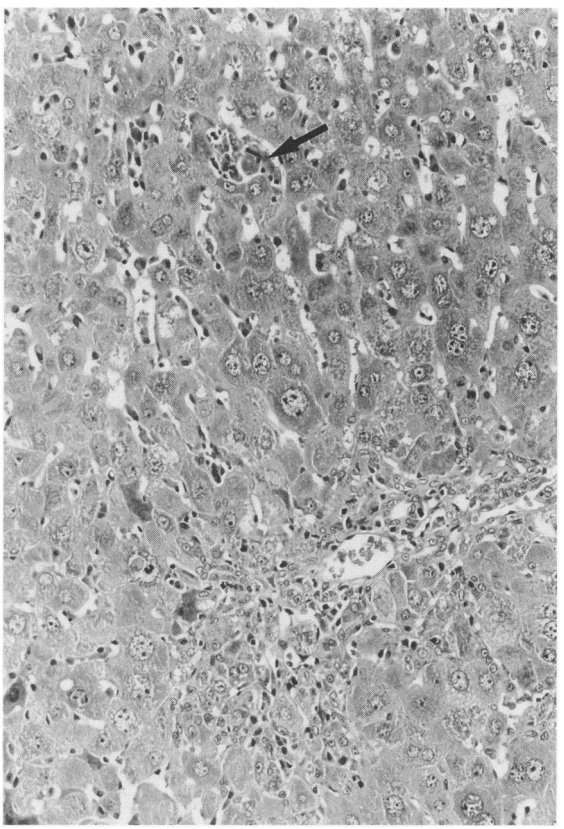

Figure 3. *Megalocytotic change of the hepatocytes with nuclear enlargement is a characteristic histopathological feature of chronic hepatitis in LEC rats. Oval cell proliferation at the periportal area and coagulative necrosis (arrow) of hepatocytes are prominent (H&E, ×170).*

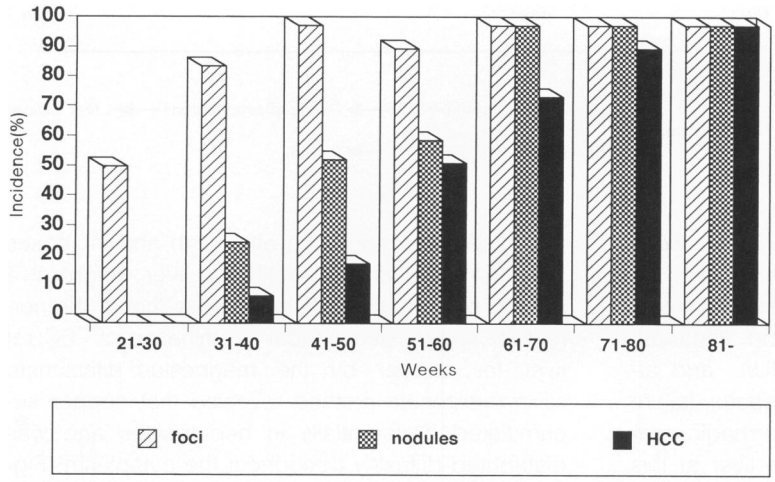

Figure 4. *Incidence of foci, hyperplastic nodules, and hepatocellular carcinomas in the livers of LEC rats. 168 rats (males and females) were examined at the periods from the 20th week to 122nd week of life.*

amount of cytoplasmic copper is in the nonmetallothionein fraction,[6] though most copper is rightly bound to metallothionein, we consider that this minor copper component might be responsible for occurrence of hepatitis (hepatocyte injury) through the generation of free radicals.

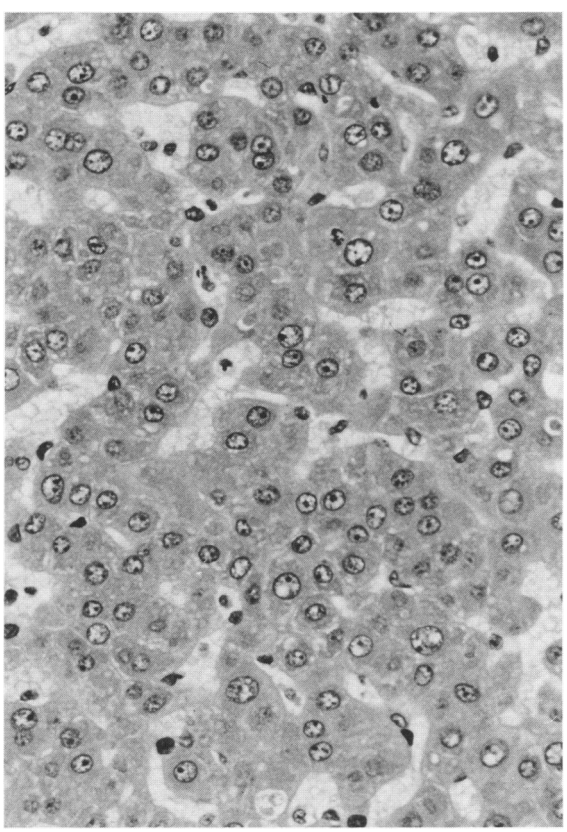

Figure 5. *Histological appearance of a well differentiated hepatocellular carcinoma developed spontaneously in a LEC rat survived more than 1 year (H&E, ×240).*

The remaining 60% of rats survive and develop chronic hepatitis. Spotty coagulative necrosis of hepatocytes and the appearance of giant hepatocytes with abundant eosinophilic cytoplasm and enlarged polyploid nuclei are the characteristic histopathological features of chronic hepatitis in LEC rats (Figure 3).[7] Few inflammatory cells are seen in Glisson's sheath but oval cell proliferation at the periportal areas is prominent. A slight elevation of serum α-fetoprotein level is observed, which corresponds to the appearance of oval cell proliferation.[9]

Preneoplastic lesions, including hyperplastic foci and nodules, develop in the liver of LEC rats with chronic hepatitis (Figure 4). These lesions display almost identical immunohistochemical and biochemical features to those seen during chemical hepatocarcinogenesis in rats, which suggests that multistep carcinogenesis takes place in the LEC rat liver. Preneoplastic lesions are positive for placental-type glutathione S-transferase.[10]

Ultimately, liver cancer develops in virtually all LEC rats surviving longer than 1 year. Most are histologically classified into well-differentiated hepatocellular carcinomas (Figure 5). They show increased expression of c-*myc*, whereas no mutations of *ras* genes are detected.[11] DNA is damaged by the oxygen radicals activated by excess copper, and elevated levels of an oxidative DNA damage product, 8-hydroxyguanine, are detected in the livers of the rats with chronic hepatitis.[12] Another distinctive biological feature of LEC rats that may account for the development of spontaneous liver cancer is the extremely high sensitivity to chemical carcinogens, especially to diethylnitrosamine.[13] Results of our preliminary genetic linkage analysis indicated that this sensitivity is independent of copper accumulation in the liver.

Additionally, LEC rats show 1) combined immunodeficiency, mainly maturation arrest of helper T cell and low level of serum IgG; 2) high sensitivity to a low dose of radiation; and 3) intolerance to alcohol. These and other features of LEC rats have been described in detail.[1]

Usefulness of the Model

As described earlier, the LEC rat provides a suitable animal model for studying the role of hepatitis in the development of liver cancer. Excessive copper accumulation in the liver has been proven to be responsible for hepatitis. Accumulated copper from laboratory chow is deposited in hepatocytes as the consequence of impaired excretion into the blood and bile. Therefore, it is possible to manipulate the occurrence of hepatitis in LEC rats by changing the dietary copper content or by treating rats with chelating agents such as D-penicillamine or trientine. Results of our preliminary experiment showed that feeding LEC rats with low copper diet before the onset of hepatitis significantly prevented the occurrence of hepatitis, whereas administration of excess copper before the onset of hepatitis induced fulminant hepatitis.[14] Thus, one may assess how the sequential development of liver cancer is modified by preventing or accelerating hepatitis.

At the same time, the LEC rat serves as an animal model of Wilson's disease. It mimics the human Wilson's disease in the excess copper accumulation in the liver, low serum ceruloplasmin,[15,16] and low excretion of copper into bile. Because the key defect that causes such abnormalities in the copper metabolism has not been elucidated in Wilson's disease, the search for the gene responsible for hepatitis in LEC rats might cover a clue to the pathogenesis of Wilson's disease. However, this condition is not exactly identical with human Wilson's disease, in that copper does not accumulate in the brain (hepatolenticular degeneration) or cornea (Kayser-Fleischer ring), which are pathognomonic signs of Wilson's disease.

Availability

LEC rats under a specific pathogen-free condition are available from Charles River Japan, Inc., 3-19-5 Shinyokohama, Kouhoku-ku, Yokohama 222, Japan. The vendor supplies LEC rats at approximately 1 month of age, approximately 3 months before the onset of hepatitis.

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