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SPONTANEOUS OCCURRENCE OF PLACENTAL GLUTATHIONE S-TRANSFERASE-POSITIVE FOCI IN THE LIVERS OF LEC RATS

Masahito OYAMADA,[*1, *4]
Kimimaro DEMPO,[*1] Yoshinori FUJIMOTO,[*1]
Hidetoshi TAKAHASHI,[*1] Mutsumi I. SATOH,[*1]
Michio MORI,[*1] Ryuichi MASUDA,[*2]
Michihiro C. YOSHIDA,[*2] Kimihiko SATOH[*3]
and Kiyomi SATO[*3]
*[*1]Department of Pathology, Sapporo Medical College, S. 1, W. 17, Chuo-ku, Sapporo 060, *[*2]Chromosome Research Unit, Faculty of Science, Hokkaido University, N. 10, W. 8, Kita-ku, Sapporo 060 and *[*3]Second Department of Biochemistry, Hirosaki University School of Medicine, Zaifu-cho 5, Hirosaki 036*

Spontaneous occurrence of placental glutathione S-transferase (GST-P)-positive foci was observed in the livers of 5-month-old LEC rats. Quantitative studies revealed that GST-P foci appeared after the onset of hepatitis. The number and size of GST-P foci increased with age and more foci were induced in males than in females. No sex difference, however, was found in the incidence of hepatitis. Although hepatitis is necessary for the induction of GST-P foci, it is insufficient for their further growth. Since hereditary hepatitis first appears at around 4 months of age, leading to a high incidence of hepatocellular carcinomas in later life, the spontaneous occurrence of the foci may be related to the development of hepatocellular carcinoma.

Key words: Hepatitis — Hepatocellular carcinoma — Placental glutathione S-transferase — Animal model

Naturally occurring liver tumors in most strains of rats are unusual occurrences, though a high incidence of these tumors in a selected rat colony has been reported.[1] We recently reported that a new mutant (LEC) suffers from hereditary hepatitis associated with severe jaundice, 4 months after birth. Liver cancer, accompanied with chronic hepatitis, developed in the long-surviving rats.[2, 3] In this strain, hepatocellular carcinomas were observed in all the rats examined at over 18 months of age (unpublished results).

There have been numerous studies on multi-stage hepatocarcinogenesis in rats induced by chemical carcinogens, and on 'marker enzymes' of putative preneoplastic lesions.[4, 5] There is a consensus that at least some enzyme-altered foci are histogenetically related to the eventual malignancies in chemical hepatocarcinogenesis. In an attempt to learn whether there are preneoplastic enzyme-altered foci in the livers of LEC rats, we have carried out immunohistochemical staining of placental glutathione S-transferase (GST-P), which is a sensitive marker of preneoplastic foci in chemical hepatocarcinogenesis.[6, 7]

The LEC (Long-Evans with a cinnamon-like coat color) strain was established from non-inbred Long-Evans rats maintained under normal conditions at the Center for Experimental Plants and Animals of Hokkaido University, as described by Sasaki *et al.*[2] and Yoshida *et al.*[3] Twenty-seven (13 male and 14 female) rats were examined, between 2 and 16 months of age, in whose livers no tumors were macroscopically observed. LEA (Long-Evans with an agouti coat color) is one of the two inbred strains, LEA and LEC, which were isolated from a closed colony of randomly bred Long-Evans rats. Because LEA rats do not suffer from hepatitis, this strain was used as a negative control.

The liver was excised and cut into 2–3 mm thick sections with a razor blade. Some sections were fixed in Carnoy's solution for staining with hematoxylin and eosin (HE) and others were fixed in ice-cold acetone for immunohistochemical examination of GST-P. The number and size of GST-P-positive foci greater than 0.01 mm^2 in area were measured in enlarged photographs using a Digiplan image analyzer (Kontron, West Germany).

*[4] To whom all correspondence should be addressed.

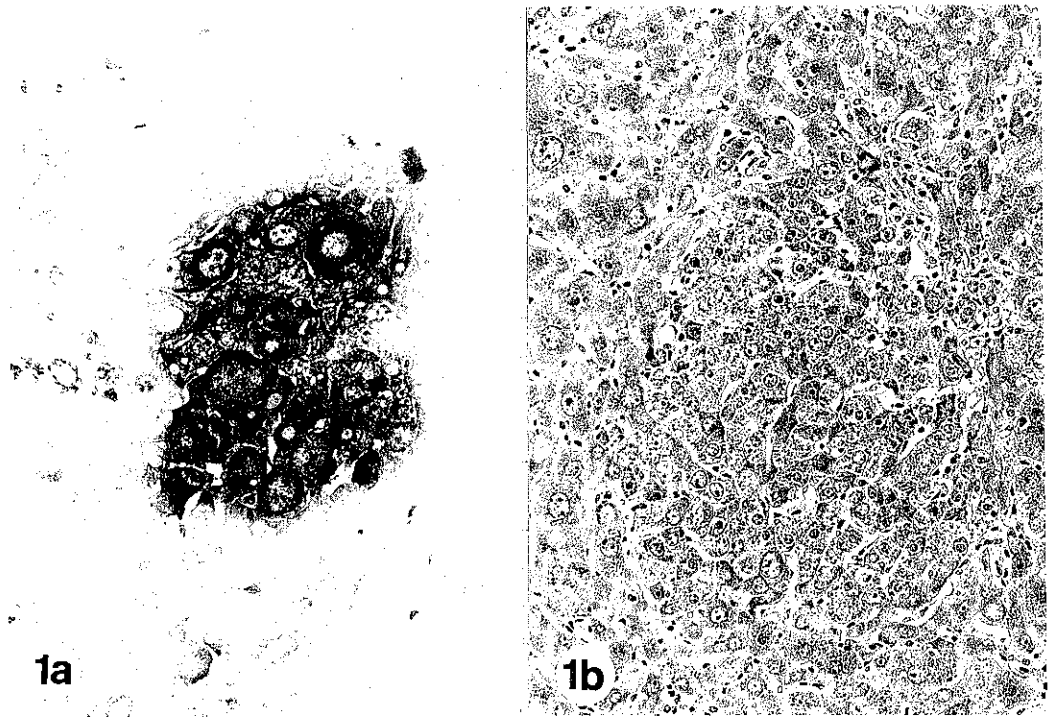

Fig. 1. GST-P-positive focus in the liver of a 5-month-old LEC rat. Serial sections showing immunostaining of GST-P (a) and HE staining (b).

Immunohistochemical staining of GST-P was performed by the avidin-biotin-peroxidase complex (ABC) method. Sections 5 μm thick were deparaffinized with benzene and an alcohol series. After inactivation of endogenous peroxidase with a methanol solution containing 0.6% H_2O_2, the sections were incubated in the following sequence: rabbit anti-GST-P (1:500), biotin-labeled goat anti-rabbit IgG (1:50, Vector Laboratories Inc., Burlingame, CA) and ABC (Vector Laboratories Inc.). The diaminobenzidine reaction was used to localize the bound peroxidase. The sections were counterstained with hematoxylin.

Enzyme-altered foci were observed in the livers of 5-month-old LEC rats after the development of hepatitis. There was a slight compression of the surrounding liver tissue, in which there were damaged hepatocytes with eosinophilic degeneration. Large nuclei were noticed in sections stained by HE (Fig. 1b). The lesions were clearly demarcated by the immunostaining of GST-P (Fig. 1a). Foci were found to be smaller than the liver lobules. The foci were composed of clear pale hepatocytes with an empty zone around the nucleus (Fig. 1b). Mitotic figures were occasionally detected in the lesion. Numerous and large foci were seen in the sections of the liver in 10-month-old LEC rats. The cytoplasm of altered hepatocytes was clear or basophilic (data not shown).

Figure 2 shows the average number of enzyme-altered foci per square centimeter of each liver section. There was a tendency for this number to increase with age. Enzyme-altered foci were observed in the livers of most of the rats older than ten months. There was a statistically significant correlation of this number with age ($P<0.05$); the correlation coefficient was 0.48. More foci were seen

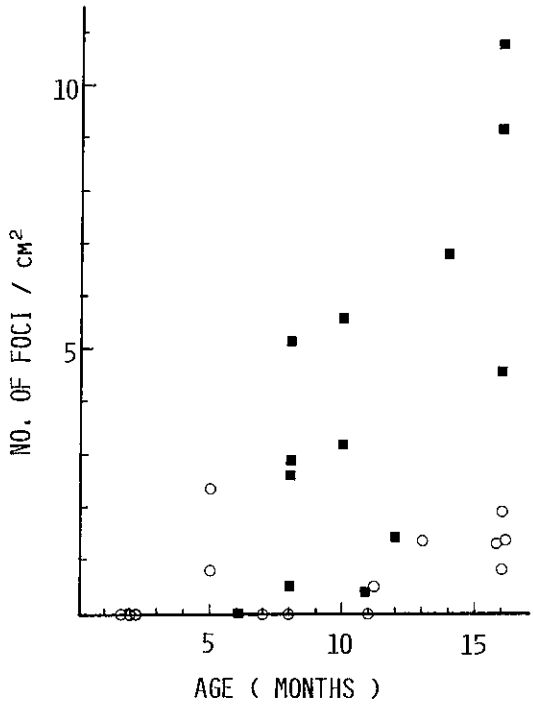

Fig. 2. The number of GST-P-positive hepatocytic foci per square centimeter of liver section. Male LEC rats (■) and female LEC rats (○).

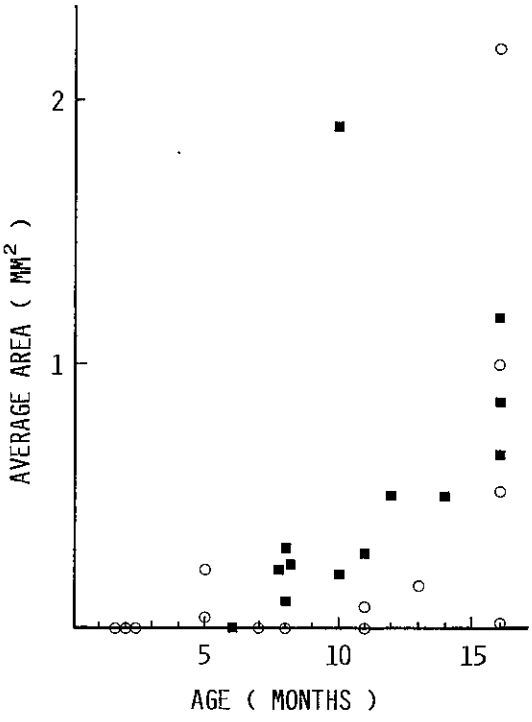

Fig. 3. The average size of GST-P-positive hepatocytic foci. Male LEC rats (■) and female LEC rats (○).

in the livers of male LEC rats than in female LEC rats. This appears to be related to the fact that a higher incidence of hepatocellular carcinomas was observed in males (97%; 28/29) than in females (58%; 18/31) examined at over 12 months of age (unpublished data). No foci were found in the livers of five LEA rats aged between 2 and 9 months.

Figure 3 shows the average size of enzyme-altered foci. The size increased with age, as did the number of foci. There was a significant correlation ($P<0.01$) between the size and age, the correlation coefficient being 0.56.

We also carried out histochemical staining of γ-glutamyl transpeptidase (GGT), widely used as a 'marker enzyme' of preneoplastic lesions in chemical hepatocarcinogenesis.[8] Because many hepatocytes in the portal areas outside the foci were stained positively by this method, we concluded that GGT is unsuitable for the detection of enzyme-altered preneoplastic foci in LEC rats, although the specific reaction to GGT was observed in most of the GST-P-positive foci (data not shown).

The present study has shown that enzyme-altered preneoplastic foci appeared in young (5-month-old) LEC rats, and the increase in number and size is an age-associated phenomenon. In many cases, hepatocellular carcinomas were positive for GST-P and coexisted with GST-P foci and nodules, although there was some heterogeneity (unpublished data). The development of hepatocellular carcinoma in LEC rats is probably related to the preneoplastic lesions in liver and at least some enzyme-altered foci may be histogenetically related to eventual liver cancer, as is generally accepted in chemical carcinogenesis.[4,5] Spontaneous occurrence of foci in altered hepatocytes of other strains has been reported by some authors.[9,10] However, we know of no reports indicating a high incidence of the foci in such young rats, except for one strain of sand rats (*Psammomys obesus*).

The necrosis of hepatocytes due to hepatitis and subsequent regeneration seem to be necessary, but insufficient, to cause the early appearance of enzyme-altered foci. No foci were seen in any of three LEC rats 2 months before the onset of hepatitis or in some LEC rats older than 5 months of age.

It is well-known that multiple stages, such as chronic hepatitis, cirrhosis and hepatocellular carcinoma, exist in the development of human hepatocellular carcinoma, especially that due to HB virus infection.[11] The LEC rat is a very useful animal model for investigating the mechanism of the transition from normal hepatocytes to precancerous lesions and to hepatocellular carcinomas.

Although a single autosomal recessive gene is considered to be responsible for hepatitis,[12] the pathogenesis of hepatitis remains to be resolved. We are now undertaking further experiments to clarify the relationship between hereditary hepatitis and hepatocellular carcinoma.

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