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Hypersensitivity to endotoxin hepatotoxicity in rats with inflammatory lesions

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Summary. The ratio of sinusoidal nonparenchymal cells to hepatocytes in rat liver was significantly increased following induction of inflammation, and decreased after subsequent exposure to endotoxin, particularly in the region around the terminal portal venules. Rats with inflammatory lesions were more sensitive to endotoxin hepatocytotoxicity than normal controls, as judged from the dose-dependent increase in activity of serum transaminases and from the extent of liver tissue injury. In addition, these animals, which were already in a state of depletion of hepatic glycogen, demonstrated marked hyperglycemia 24 h after endotoxin administration in small doses of less than 2 mg/kg.
Key words. Inflammation; nonparenchymal cells; endotoxin; liver injury.

Inflammation causes some functional alterations in rat hepatocytes, such as enhanced glycoprotein synthesis[1], impaired drug metabolism[2], and delayed dye-uptake[3], although histologic lesions are virtually absent. On the other hand, toxic liver injury can be greatly enhanced by ordinarily harmless doses of bacterial endotoxin in animals[4].

The present communication describes that induction of inflammation causes an increase in sinusoid lining cells in rat liver, and that rats with local inflammation are susceptible to liver damage induced by endotoxin.

Materials and methods. Male inbred rats of the Buffalo (BUF) and F344 strains, raised in this laboratory, were used. These animals, aged 8 weeks, received 0.05 ml of a 1% suspension of heat-killed *Mycobacterium tuberculosis* in liquid paraffin (Freund's complete adjuvant) in the subplantar tissue of the right hind foot, and 11 days later were given i.v. endotoxin (lipopolysaccharide from *E. coli* 0127:B8, Sigma Chem. Co., USA), which was dissolved in saline at 2 mg/ml. Groups of pair-fed normal rats were injected with endotoxin alone and served as a control. Blood and liver samples were obtained from the animals 24 h after endotoxin injection. Transaminase activities and glucose levels in serum were determined using the kit-reagents of Wako Chem. Co., Tokyo. For histologic examinations, the left lobe of the liver was cut into slices vertically at the central position along the cross axis, and paraffin sections 3.5 µm thick were prepared and stained with hematoxylin and eosin. In order to determine the ratio of sinusoidal nonparenchymal (NP) cells to parenchymal (P) cells (hepatocytes), the periportal and centrilobular areas seen at a magnification of × 200 were selected at random and five photographs of each area where the portal or hepatic venule was positioned in the center of the field were taken for cell counting. Any portions containing inflammatory cells, which were identified by size and shape, were not photographed. The number of hepatocytes to be counted was 50 or more per one photograph. Liver tissue injury including hepatocyte necrosis and/or degeneration, inflammatory cell infiltration and granulation tissue formation was scanned throughout the entire slide of each tissue preparation and the degree of severity was arbitrarily graded from zero to three. All histological measurements were performed blindly by a single operator. In statistical comparisons, Student's t-test and the Wilcoxon signed rank test were used.

Results and discussion. F344 rats developed fully so called adjuvant polyarthritis 14 days after adjuvant injection, while BUF rats, which are known to be resistant to the disease[5], showed a large swelling only in the adjuvant-injected hind foot. There were no morphological signs of hepatopathy in these rats with inflammation. Nevertheless, the hepatic NP to P cell ratio was significantly increased with time after adjuvant injection in both the centrilobular and periportal regions in F344 rats, and in the centrilobular region in BUF rats, indicating elevation of the relative number of sinusoidal lining cells in the corresponding areas after induction of inflammation (fig. 1). Incidentally, BUF rats had an essentially higher basal NP to P cell ratio, particularly in the periportal region, than F344 rats ($p < 0.05$). Hepatic NP cells represent a heterogenous population consisting mainly of endothelial cells[6] and Kupffer cells[7]. The former cell population may be maintained largely by local proliferation[6]. Kupffer cells, on the other hand, originate predominantly from blood monocytes[8]. Inflammation is characterized by leukocytosis, so that an increase in the number of recruited monocytes from the circulation in the liver after development of inflammation could be expected.

Following endotoxin administration to BUF rats, the NP to P cell ratio in the periportal region, but not in the centrilobular region, was significantly decreased from the basal level, whether the animals had shown inflammation or not (fig. 2). A probable explanation for this seems to be the decrease in the relative number of Kupffer cells: these cells are preferentially distributed in the hepatic sinusoids around the portal tract[7], have the capacity to take up almost all administered endotoxin

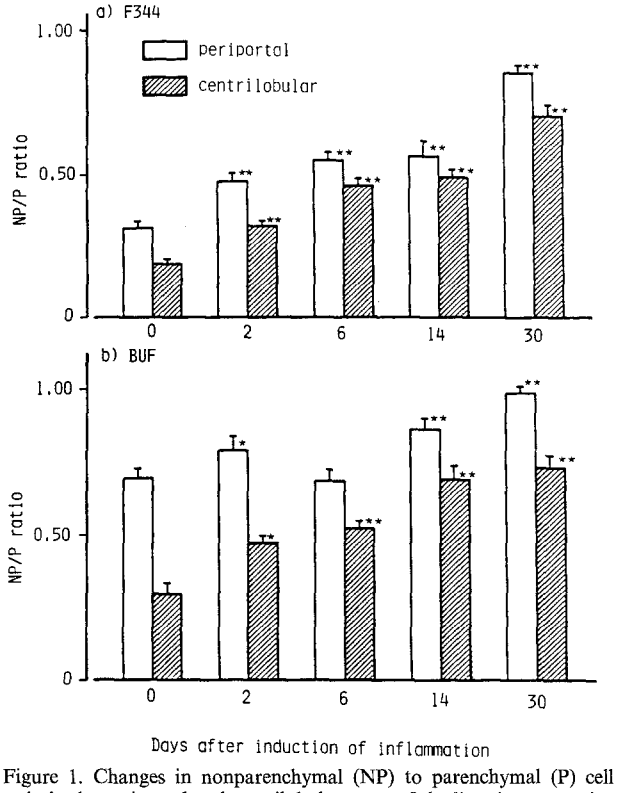

Figure 1. Changes in nonparenchymal (NP) to parenchymal (P) cell ratio in the periportal and centrilobular areas of the liver in two strains of rats following inoculation of Freund's complete adjuvant in the hind foot-pad. Bars show the standard errors of the means which were obtained from five animals. Significant differences against the respective 0-day values are expressed at 5% (*) and 1% (**), respectively.

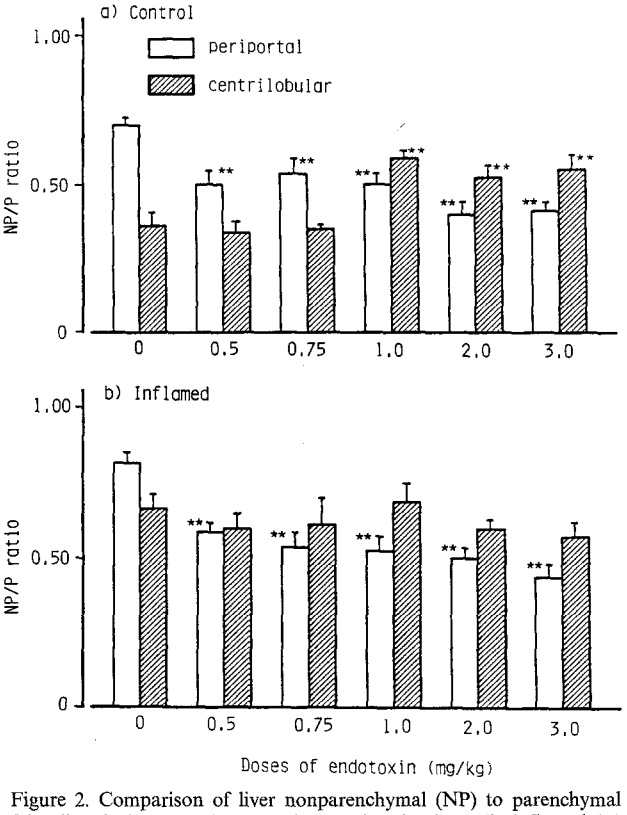

Figure 2. Comparison of liver nonparenchymal (NP) to parenchymal (P) cell ratio between the normal control and subacutely inflamed (11 days) groups of BUF rats 24 h after endotoxin administration in varying doses. Each dosage group consisted of five rats. Bars mean SE. Asterisks represent significant differences against the respective values from endotoxin-untreated rats at 1%.

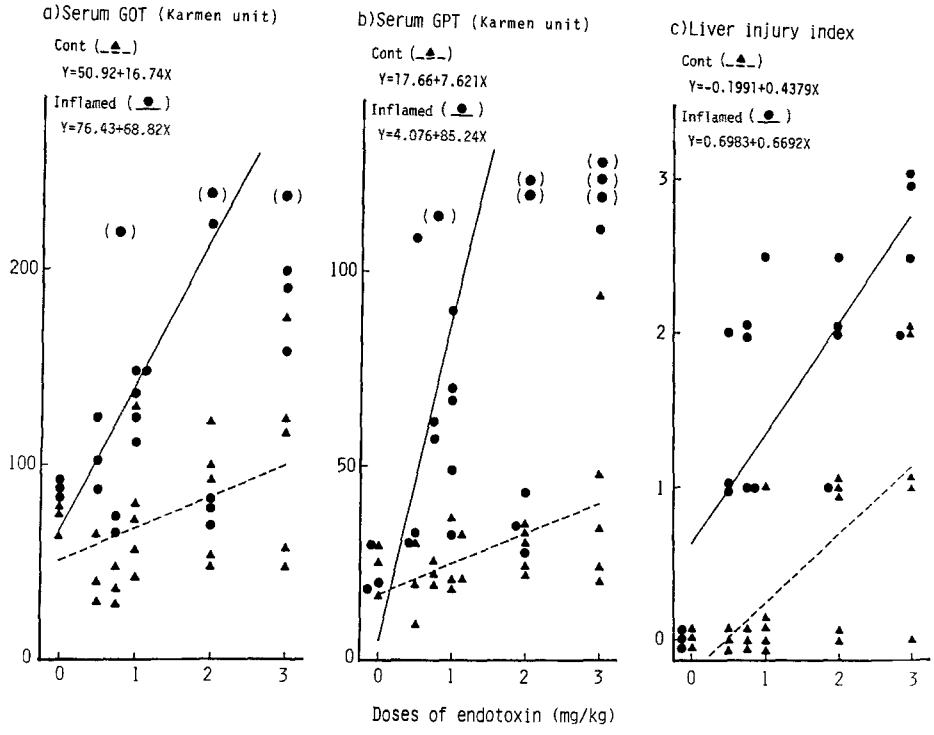

Figure 3. Serum transaminase activities and liver injury indices in endotoxin-treated BUF rats of the control and inflamed groups. Samples were obtained from the same animals used in figure 2. Points in parentheses represent the values exceeding 300 KU for GOT and 150 KU for GPT, respectively.

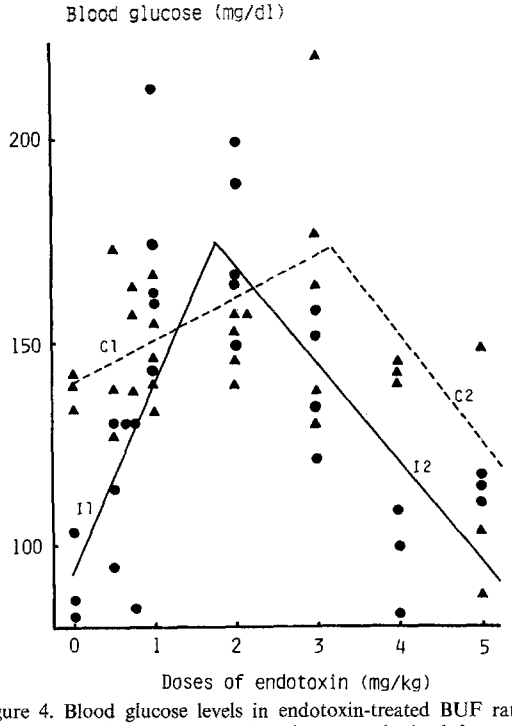

Figure 4. Blood glucose levels in endotoxin-treated BUF rats of the control and inflamed groups. Samples were obtained from the same animals as were used in figure 2. Equations for dose-response lines are C1: $Y = 139.5 + 11.38X$ and C2: $Y = 187.6 - 12.39X$ for the control group (_▲_), and I1: $Y = 95.22 + 46.17X$ and I2: $Y = 214.9 - 23.66X$ for the inflamed group (_●_), respectively.

from the portal blood[9], and sustain cellular damage by endotoxin earlier than morphological changes in parenchymal cell occur[10].

With increasing doses of endotoxin, hepatocyte necrosis associated with inflammatory cell infiltration became manifest in the livers of the treated rats of both groups; and in this connection, the NP to P cell ratio in the centrilobular area of the control rat liver, although the portion chosen for cell counting was histopathologically intact, was significantly increased, and reached highly elevated level of the corresponding value for rats with inflammation, which remained unchanged by endotoxin administration (fig. 2). Figure 3 illustrates that both serum transaminase activity and the liver tissue injury score were increased proportionally to the doses of endotoxin administered, and that rats suffering severe inflammation respond more to endotoxin hepatocytotoxicity than controls ($p < 0.01$). There was a close correlation between the serum glutamic pyruvic transaminase activity and the injury score (regression line: $Y = 0.578+0.005X$, correlation coefficient: $r = 0.6495$, $n = 45$, $p < 0.01$).

Endotoxin shock is characterized by profound hypoglycemia after a transitory hyperglycemia[11]. The initial hyperglycemia is considered to occur as a result of a rapidly enhanced glycogenolysis in the liver[12]. A marked depletion of glycogen in the hepatocytes from rats with severe inflammation has been reported[7,13]. As shown in figure 4, rats with hind foot swelling demonstrated a dose-dependent rise in blood glucose 24 h after the administration of endotoxin ranging from zero to 2 mg/kg, while controls failed to show any significant hyperglycemia under the experimental conditions employed here. The underlying mechanism of endotoxin-induced hyperglycemia in rats with inflammation remains to be studied.

Similar results concerning endotoxin hepatocytotoxicity as described above were also obtained from the experiments using F344 rats (data not shown).

Kupffer cells appear to be most important for endotoxin detoxication. However, Kupffer cells when stimulated by endotoxin release several effector substances inducing hepatocyte damage. These include lysosomal hydrolases, neutral proteinases, prostaglandins, endogenous pyrogen, lysozyme and others[4]. The increase in the number of NP cells in rat liver follow-

ing development of inflammation seems likely to contribute to an increase in the released amounts of such biologically active substances on subsequent exposure to endotoxin, leading to more extensive hepatocyte damage. The notion that hepatocellular injury may be mediated by recruited and activated macrophages has been presented by several authors[14,15]. However, another possibility, that the hepatocytes from rats with inflammation, which are already in a metabolically subnormal state[13], may be readily affected by the direct action of endotoxin, cannot be excluded.

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Effect of propane sultone pretreatment on Friend virus leukemogenesis in mice

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Summary. Propane sultone (PS) injected i.p. 24 or more hours before Friend leukemia virus increased the incidence of lymphoma in SJL/J mice and at a higher dose increased the incidence of erythroleukemia in B10SJF$_1$ mice. PS at the same time also decreased hematopoietic stem cell clonogenicity.
Key words. Mouse erythroleukemia; propane sultane; Friend leukemia virus; carcinogenesis; clonogenicity.

Various chemical carcinogens can affect cultured cells[1,2] or whole organisms[3-7] to make them more susceptible to viral oncogenesis. This report describes effects of propane sultone (PS) on Friend leukemia virus (FLV)-dependent oncogenesis in virus sensitive SJL/J mice and in relatively virus resistant B10SJF$_1$ mice.

PS, a sulfonated-hydrocarbon, monofunctional alkylating agent[8,9] is known to be carcinogenic in rats when ingested[10,11] or introduced parenterally[12,13]. In vitro PS transformed human epithelial cells with greater efficiency than human fibroblasts[14,15], suggesting similarity with natural situations where the frequency of carcinomas is greater than that of sarcomas. PS is used in certain industrial chemical processes[16,17] and is related to ethyl and methyl sulfonate, which are used in genetics to produce point mutations[18]. Like methyl methane sulfonate (MMS), PS attaches to DNA[17-19]. MMS is known to produce single strand breaks in DNA that resemble X-ray caused lesions and are quickly repairable[1], and the PS-caused damage to DNA, although not as well characterized as that of MMS, may be similar[2,8,9,20].

In vitro treatment of human fibroblasts with various carcinogens, which caused DNA damage that led to DNA-repair synthesis, increased the frequency of transformation produced by subsequent SV40 infection[2]. Our results show that PS and FLV, given in vivo at doses that cause little or no malignancy when either is given alone, can produce the malignancy characteristic of the virus in 80% of mice injected with both the chemical and viral carcinogen.

Materials and methods. Female, six-week-old SJL/J mice were purchased from Jackson Laboratories and held for 2 weeks before being entered into experiments. B10SJF$_1$ mice were bred by us from Jackson stock (C57BL/10J males × SJL/J females). Both male and female B10SJF$_1$ mice were used when they were 8–12 weeks old. There was no significant effect of sex or age on the response measured. Animal housing and maintenance were standard as described previously.

PS (Sigma) was diluted with physiological saline and injected i.p. at 100 or 175 mg per kg of b.wt. Groups that received only FLV were injected with saline instead of PS.

Our FLV stock and its preparation was described previously[3,21]. Approximately one tenth SED (spleen enlargement dose) was injected into SJL/J mice and 100 SED was injected into B10SJF$_1$ mice. The day of virus injection was taken to be day 0 in each experiment. Groups that received PS only were injected with saline on day 0.

The measurement of the induction of antibody against antigens on sheep red blood cells by the plaque forming (PFC) technique was previously described[22]. The enumeration of

Numbers of PFC and CFU-S in SJL/J mouse spleen cells after exposure of the animals to PS (100 mg/kg). Mean ± 1 SE of 3 or more experiments

Time after PS	Plaques per 10^6 spleen cells	% of control	CFU-S per 10^5 spleen cells	% of control
5 h	1318 ± 221	130	6.12 ± 0.34	66
1 day	1158 ± 148	115	5.20 ± 0.29*	51*
2 days	993 ± 76	98	3.91 ± 0.26	38*
5 days	1592 ± 264	158	9.56 ± 0.48	93
7 days	732 ± 121	72	10.60 ± 0.60	104
10 days	937 ± 73	93	–	–
20 days	1335 ± 198	132	–	–
Control	1010 ± 63	–	10.19 ± 0.28	–

*Indicates means that significantly differed from the control.