Effect of Anti-basic Liver Protein Antibody-Induced Liver Injury on Hepatic Drug-Metabolizing Enzymes in C57 BL/6J Mice

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We attempted to produce a model mouse with a liver injury resulting from an immunological mechanism in C57BL/6J mice, and the effect of hepatitis on the hepatic microsomal mixed-function oxidase system was studied. An experimental immunological liver injury model was caused by the intravenous injection of an anti-basic liver protein (BLP) antibody in mice which had been previously immunized with normal rabbit IgG (RGG) and complete Freund's adjuvant. C57BL/6J strain mice showed the highest susceptibility to the immunological liver injury. Typical histopathological changes in the liver included submassive hepatocellular necrosis and infiltration of lymphocytes into the portal tract and sinusoid area in a necrotic lesion. The liver injury in this model was markedly inhibited by the administration of prednisolone (20 mg/kg, p.o.), cyclophosphamide (15 mg/kg, i.p.), levamisole (10 mg/kg, p.o.), glycyrrhizin (50 mg/kg, i.p.) and cephaparhine (10 mg/kg, i.p.), which act on the immune system. Twenty-four hours after the injection of anti-BLP antibody, the activities of aminopyrine N-demethylase, aniline hydroxylase and NADPH-cytochrome c reductase and the content of cytochrome P-450 were mostly reduced, whereas cytochrome b_{5}, and NADH-ferricyanide reductase were not. These results suggest that the experimental liver injury model in C57BL/6J mice is useful as a model of liver injury mechanism, and its hepatitis was shown to inhibit the cytochrome P-450-dependent biotransformation of drugs in the mouse.

Keywords immunological liver injury; drug-metabolizing enzyme activity; C57BL/6J mice

The pharmacological effects and toxicity of drugs administered to humans are known to be altered according to various nonphysiologic or pathologic states.1) A number of reports have suggested changes in the pharmacokinetics, pharmacological effects, or toxicity of drugs, especially in animals and humans with liver injury produced by the administration of toxic chemicals.2)–9) Since pathologic changes resembling those in human hepatitis are not considered to be reproducible in experimental liver injury models using small animals, there have been no reports evaluating the pharmacological effects or the toxicity of drugs in immunological liver injury corresponding to human hepatitis. However, immunological mechanisms are known to be involved in the pathogenesis and progression of hepatitis.7)–9)

It is a generally accepted concept that the drug metabolizing activity of hepatic microsomes decreases when the liver is injured by virus, malnutrition, poison and so on. Also, Willson and Hart10) and Noguchi et al.11) reported that the hepatic drug-metabolizing enzyme activity changed following liver injury. The main cause of the decrease of this activity is from the damage of the mixed-function oxidase system in which P-450 plays the most important part.

Rational drug therapy following hepatitis requires an understanding of post-hepatitis drug metabolism. One approach to gaining this knowledge is to examine the effect of immunological liver injury on drug-metabolizing enzyme systems. Thus, the first objective of this study was to define a suitable model for experimental liver injury by an immunological mechanism resembling that in human hepatitis, and then studying the effect of this hepatitis on hepatic drug-metabolizing enzyme systems.

Materials and Methods

Animals

Inbred male C57BL/6J mice, DBA/2 male mice and ddY male mice, 5 weeks of age, were obtained from the Japan SLC (Hamamatsu) for use in liver injury experiments. The animals had free access to a commercial diet (CE-2, Japan Clea, Tokyo) and tap water, and were kept on a 12-hour light/dark cycle in a temperature controlled room. Male JW rabbits (Japan SLC, Hamamatsu) weighing 1.5 to 2.0 kg were used for the preparation of an antisera.

Drugs

Prednisolone acetate (Nihon Merck Ranyu Pharmaceutical Co., Ltd., Tokyo), cyclophosphamide (Shionogi Pharmaceutical Co., Ltd., Osaka), levamisole (Alldrich Chemical Co., Inc., Milwaukee, U.S.A.), glycyrrhizin (Minophagen Pharmaceutical Co., Ltd., Tokyo), cephaparhine (Kaken Shoyaku Pharmaceutical Co., Ltd., Tokyo) and complete Freund's adjuvant (CFA, Nacalai Tesque, Inc., Kyoto) were purchased. All other chemical used were of analytical grade.

Separation of Antigen

Basic liver protein (BLP), purified by the method of Mafune12) and Nagai et al.13) and reported in our previous article,14) was used as the antigen. The liver of C57BL/6J mice was perfused with physiological saline to remove blood. Physiological saline was added, and 50% homogenates were prepared in a Potter type homogenizer. After centrifugation (4°C, 8000 rpm, 30 min), the supernatant was adjusted to pH 4.8 with acetic acid, the resulting insoluble materials were eliminated by centrifugation, and the fraction that precipitated by the addition of saturated ammonium sulfate (35–60%) was recovered. This precipitate was dissolved in physiological saline and dialyzed against running water for 24 h and against 0.1 M Tris - HCl buffer (pH 8.0) and 0.05 M Tris - HCl buffer (pH 8.0). It was then chromatographed using a DEAE cellulose column equilibrated with 0.05 M Tris - HCl buffer (pH 8.0), and the eluted fraction was collected as BLP.

Preparation of Antiserum

Rabbits were immunized by injecting BLP (protein content 300 μg/ml) into C57BL/6J mice, and the same volume (2.0 ml) of emulsion of CFA, in the gluteral muscle and subcutaneously in the back and the plantar 4 times at 1-week intervals. Elevation in the antibody titer was confirmed 7 days after the last injection, and antisera were obtained from the blood drawn from the auricular vein. Antiserum was absorbed with homologous erythrocytes and kidney homogenate after inactivation of the complement at 56°C for 30 min, and were then precipitated with 30% saturated ammonium sulfate. The precipitated γ-globulin fraction containing IgG and IgM was dissolved in saline and dialyzed against 0.005 M Tris - HCl buffer (pH 8.0). The solution was applied on a diethylaminoethyl (DEAE) cellulose column equilibrated with 0.005 M Tris - HCl buffer (pH 8.0). The IgG fraction was obtained by collecting the passed effluent. It was dialyzed against phosphate buffer saline (PBS) and stored at −80°C until use.

Preparation of Rabbit γ-Globulin (RGG)

RGG was purified by a routine procedure using ammonium sulfate fractionation and DEAE cellulose
column chromatography. Anti-BLP Antibody-Induced Liver Injury Lesions were produced according to the method of Nagai et al., which was used to produce RGG accelerated nephrotoxic serum nephritis. CFA emulsion (0.5 ml) containing RGG at 4 mg/ml was injected intraperitoneally to the mice, and an anti-BLP antibody (0.6 ml, protein content 4.2 mg/ml) was injected into the tail vein 5 d later.

Biochemical Analysis and Histopathological Examination In order to evaluate the severity of the symptoms, blood was collected and the liver was removed and processed for biochemical and histological analysis at 24 h after injection of anti-BLP antibody. They were measured mainly for serum glutamic-oxaloacetic transaminase (GOT), and glutamic-pyruvic transaminase (GPT) using a diagnostic kit from Wako Pure Chemical Co., Ltd. (Tokyo). In some experiments, the activity of alkaline phosphatase (ALP), γ-glutamyltranspeptidase (γ-GTP), leucine aminopeptidase (LAP), choline esterase (CHE) and lactate dehydrogenase (LDH) and the amount of total bilirubin (TBil), total protein (TP), total cholesterol (TC), triglyceride (TG), phospholipids (PL), blood urea nitrogen (BUN), creatinine (CRE) and uric acid (UA) were also measured by an automatic serum analyzer. For the histopathological examination of liver, a portion of the median lobe of the liver was fixed in 10% normal formalin solution, embedded in paraffin, sectioned, and stained with hemotoxylin-eosin.

Effect of Hepatitis on Hepatic Drug Metabolism in Vivo Hexobarbital sleeping time and zoxazolamine paralysis time were determined in the hepatitis and control mice. The mice were given a single i.p. injection of hexobarbital (100 mg/kg) or zoxazolamine (100 mg/kg), and sleeping or paralysis times were defined as the time interval from the loss of reflexes to the reappearance of the righting reflex. The plasma or brain levels of hexobarbital and zoxazolamine at the time of awakening were determined as described elsewhere. Effect of Hepatitis on in Vivo Drug Metabolism The effect of hepatic on liver cytochrome P-450, cytochrome b5, microsomal NADPH-cytochrome c reductase, aminopyrine N-demethylase and aniline hydroxylase were determined as described elsewhere.

To obtain the microsomal fraction of liver preparations, the mice were killed by cervical dislocation. The liver was rapidly excised and then perfused with 1.15% KCl solution. All subsequent steps were carried out at 4°C. The minced liver was homogenized in 3 volumes of cold 0.1 M phosphate buffer (pH 7.4) using a Potter Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged at 10,000 rpm (9000 × g). The supernatant was centrifuged at 105,000 rpm (100,000 × g) for 60 min in a Hitachi model 65P-7 ultracentrifuge (model Rf-50 rotor). The pellet was kept at −80°C until use.

The microsomal cytochrome P-450 and cytochrome b5 concentrations were determined by the method of Omura and Sato. Determination of enzymatic activity of aminopyrine N-demethylase and aniline hydroxylase were carried out with the following cofactors: NADPH (0.5 mm), glucose-6-phosphate dehydrogenase (1 units), glucose-6-phosphate (5.0 mm) and MgCl2 (0.25 mm) in a total volume of 0.8 ml phosphat buffer (pH 7.4, 0.1 M). Four mg of microsomal protein were used in the enzyme assays. The reaction was started by adding 0.1 ml of the above buffer-containing substrate, and incubation was performed with shaking for 20 min at 37°C. The reaction was terminated by the addition of 1.0 ml of an equal of 10% trichloroacetic acid solution, the mixture was shaken well, and then centrifuged at 3000 rpm for 10 min. Form-aldehyde released from the aminopyrine (2.0 mm) was quantified according to the method of Nash. Aniline (1.0 mm) was used to determine aniline-4-hydroxylase activity. The rate of p-aminophenol formation was measured photometrically.

The activities of NADPH-cytochrome c reductase and NADH-ferri- cicyanide reductase were measured by the method of Phillips and Langdon and Mihara and Sato, respectively. The protein content was determined according to the binding of Coomassie brilliant blue G 250 to proteins following the method of Bradford, with bovine serum albumin as the standard.

Statistical Analyses The Student’s t-test was used to statistically evaluate the data. Significant differences were assumed when p < 0.05.

Results Liver Injuries in C57BL/6J, DBA/2, and ddY Mice The RGG-accelerated anti-BLP antibody-induced liver injury was compared among the three mouse strains, namely C57BL/6J, DBA/2, and ddY. BLP was prepared from C57BL/6J, anti-BLP antibody obtained from rabbits was injected into the mice of these three strains, and the blood GOT and GPT activities were determined after 12 h and at 1, 2, 3, 4, 5 and 6 d (Fig. 1). The blood levels of GOT and GPT showed peak levels 1 d after the antibody administration in mice of all three strains. Among the three strains, the increases in the blood GOT and GPT levels were most remarkable in the C57BL/6J mice. Histological findings in the liver were consistent with changes in the blood GOT and GPT levels.

Liver Injury in C57BL/6J Mice 1) Effects of RGG immunization. The blood levels of GOT and GPT were studied in an untreated group, a group administered normal rabbit IgG i.v., a group administered anti-BLP antibody i.v., a group immunized with RGG, a group immunized with RGG and then administered normal rabbit IgG i.v., and a group immunized with RGG and then administered anti-BLP antibody i.v.

In the normal rabbit IgG group, RGG group and RGG + normal rabbit IgG group, the blood GOT and GPT levels were similar to those of the untreated group. An increase in GPT was observed in the anti-BLP antibody group, and increases in GOT and GPT were noted in the RGG + anti-BLP antibody group. The results of histological examination of the liver were consistent with the increases in GOT and GPT. 2) Specificity of anti-BLP antibody. The magnitude of the liver injuries was studied by increasing the dose of anti-BLP antibody injected after RGG immunization. When anti-BLP antibody was administered at 0.1, 0.3, 0.6, and 1.0 ml to mice immunized with RGG, the degree of increase in blood GOT and GPT levels 24 h after antibody injection were dependent on the dose of the antibody (refer to the previous paper). 3) Serum biochemical and histological examinations. Various sero logical parameters and histological changes were examined 24 h after injection of anti-BLP antibody (0.6 ml) to mice 5 d after immunization with RGG and CFA. After

Fig. 1. Strain Difference in the Magnitude of Liver Injury Induced by RGG-Accelerated Anti-basic Liver Protein Antibody in Mice A, GOT; B, GPT. BLP from C57BL/6J mice was prepared according to the method of N. Mitani. BLP antibody was obtained from rabbits which had been immunized by injection of 2.0 ml of an emulsion containing BLP (300 μg protein/ml) and CFA intramuscularly or subcutaneously 4 times weekly C57BL/6J (A), DBA/2 (A), and ddY (C) mice were immunized by an intraperitoneal injection of 4.0 mg rabbit IgG (RGG) emulsified with 0.5 ml of CFA. Five days later, BLP antibody (0.6 ml/animal) was injected intravenously. In order to evaluate the severity of the liver injury, blood samples were collected at 12 h, 1, 2, 3, 4, 5, and 6 d after the injection of BLP antibody and were measured GOT and GPT activities. Values are the means of 6–8 mice. S.E.s were less than 20% of the respective means.
intravenous injection of anti-BLP antibody, GOT, GPT, and LDH showed clear increases, while ALP and T-bil decreased. Biochemical examinations were also performed in serum obtained 24 h after the administration of acetaminophen (800 mg/kg, i.p.). Increases in T-bil, GOT, GPT, LAP, and LDH and decreases in ALP, T-cho, TG and PL were observed (Table I). These results suggest that anti-BLP antibody-induced liver injury is limited to a detailed portion of the liver. No changes in the results of biochemical examinations of kidney injury were observed in either type of mice.

Figure 2A shows the histological profile of the liver of an untreated mouse. However, in the liver of mice administered anti-BLP antibody grossly, punctated necrotic areas observed in all lobes, with the occasional adhesion of hepatocytes to the peritoneum. In necrotic areas, inflammatory cells, which were predominantly neutrophils, were found to infiltrate into portal spaces around the necrotic areas (Fig. 2B). However, no concomitant histological changes were observed in the kidney, lung, or spleen, where histologic profiles were similar to those in untreated animals (data not shown).

**Effects of Drugs.** Effects of prednisolone, cyclophosphamide, levamisole, glycyrrhizin and cephranthine, which are used clinically for the treatment of hepatitis, were examined in this liver injury model. All of the drugs inhibited the increase in GOT and GPT activities, particularly GOT, in this liver injury model (Fig. 3). The prevention of liver injury by the drugs was evident also by histological examinations (data not shown).

**Effect on Hepatic Drug Metabolism.** Hexobarbital sleeping time and oxazolamine paralysis time were found to increase rapidly in post-hepatitis mice, as shown in Fig. 4. A significant increase was noted as early as 12 h after
admission of an anti-BLP antibody, with a maximum increase occuring at 24 h. Recovery of both hexobarbital sleeping time and zoxazolamine paralysis time was found at 144 h. Measurement of the concentration of both drugs in the brain and plasma immediately upon recovery from hypnosis showed that there were no differences in any of the groups examined. The brain-to-plasma ratios of drugs did not differ between the control and hepatitis mice. Furthermore, the time course of the decreased content of cytochrome P-450 was generally reflected in the altered hexobarbital sleeping time and zoxazolamine paralysis time following the administration of an anti-BLP antibody. Also, marked differences were observed in the difference spectra of CO-bound reduced microsomes from the control and hepatitis mice livers 24 h after anti-BLP antibody injection. The intensity of the peak at 450 nm declined with the concomitant appearance of a peak at 420 nm, which is ascribed to the denatured form of cytochrome P-450 (Fig. 5). In subsequent studies, therefore, hepatic drug metabolizing enzyme activities were assayed 24 h after the injection of anti-BLP antibody. The effect of hepatitis on hepatic microsomal cytochrome P-450 and cytochrome b₅ content and on the activities of NADPH-cytochrome c reductase, NADH-cytochrome c reductase, aminopyrine N-demethylase and anilino hydroxylase in hepatitis is

![Diagram](image-url)

**Fig. 3. Effect of Various Compounds on Serum GOT and GPT Activities in C57BL/6J Mice with Liver Injury Induced by RGG-Accelerated Anti-basic Liver Protein Antibody**

Each experiment consists of 8—10 mice. Mice were immunized by an intraperitoneal injection of rabbit IgG emulsified with CFA, and 3d later, BLP antibody was injected intravenously. Blood samples were collected at 24 h after injection of anti-BLP antibody and GOT and GPT activities were measured. Control mice were administered saline (0.1 ml/10 g mouse, i.p.). Prednisolone (20 mg/kg, p.o.), cyclophosphamide (15 mg/kg, i.p.), levamisole (10 mg/kg, p.o.), glycyrrhizin (50 mg/kg, i.p.) or cepharanthine (10 mg/kg, i.p.) was administered daily for 10 d before the injection of anti-BLP antibody using the schedule of Nagai et al.14 and as reported previously in our article.14a) Significantly different from control ($p<0.05$).

**TABLE I. Changes in Serum Biochemical Parameters in C57BL/6J Mice with Liver Injury Induced by RGG-Accelerated Anti-basic Liver Protein Antibody**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Hepatitis</th>
<th>Acetaminophen (800 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-bil (mg/dl)</td>
<td>0.75</td>
<td>0.60</td>
<td>1.40*</td>
</tr>
<tr>
<td>GOT (IU/dl)</td>
<td>181.5</td>
<td>3487.0*</td>
<td>15156.0*</td>
</tr>
<tr>
<td>GPT (IU/dl)</td>
<td>46.0</td>
<td>3146.0*</td>
<td>18665.5*</td>
</tr>
<tr>
<td>ALP (IU/dl)</td>
<td>110.5</td>
<td>45.5*</td>
<td>50.0*</td>
</tr>
<tr>
<td>r-GTP (IU/l)</td>
<td>5.5</td>
<td>5.0</td>
<td>5.5</td>
</tr>
<tr>
<td>LAP (IU/l)</td>
<td>33.0</td>
<td>79.0*</td>
<td>162.0*</td>
</tr>
<tr>
<td>CHE (mg/dl)</td>
<td>1425.5</td>
<td>1368.5</td>
<td>1306.5</td>
</tr>
<tr>
<td>TP (mg/dl)</td>
<td>5.40</td>
<td>4.60</td>
<td>4.35</td>
</tr>
<tr>
<td>T-cho (mg/dl)</td>
<td>87.0</td>
<td>80.5</td>
<td>51.5</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>126.0</td>
<td>92.0</td>
<td>69.0*</td>
</tr>
<tr>
<td>LDH (IU/l)</td>
<td>1111.0</td>
<td>11539.0*</td>
<td>40755.0*</td>
</tr>
<tr>
<td>PL (mg/dl)</td>
<td>186.5</td>
<td>141.5</td>
<td>114.0*</td>
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<tr>
<td>BUN (mg/dl)</td>
<td>29.9</td>
<td>23.3</td>
<td>27.0</td>
</tr>
<tr>
<td>CRE (mg/dl)</td>
<td>0.50</td>
<td>0.45</td>
<td>0.60</td>
</tr>
<tr>
<td>UA (mg/dl)</td>
<td>2.75</td>
<td>1.70</td>
<td>2.0</td>
</tr>
</tbody>
</table>

The experimental conditions were the same as in Fig. 1. Mice were sacrificed 24 h after intravenous injection of anti-BLP antibody or after intraperitoneal administration of acetaminophen (800 mg/kg), and serum parameters were determined. Values are the means of 6—8 mice; S.E.s were less than 20% of the respective means.

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**Fig. 4. Time-Course of Prolongation of Hexobarbital Hypnosis and Zoxazolamine Paralysis in Hepatitis Mice**

Each experiment consists of 6—8 mice. The experimental conditions were the same as in Fig. 1. Mice received BLP-antibody and at specified time intervals received hexobarbital (100 mg/kg, i.p., circle) or zoxazolamine (100 mg/kg, i.p., triangle). The duration of hypnosis or paralysis was measured. Controls received saline (0.1 ml/10 g mouse). Values plotted are means (±S.E.M.) of percentage compared with control. Closed symbols are significantly different from the control ($p<0.05$).
Fig. 5. Time-Course of Cytochrome P-450 Content in Hepatitis Mice

Each experiment consists of 6–8 mice. The experimental conditions were the same as in Fig. 1. (A) Hepatic microsome was collected at indicated times after the injection of anti-BLP antibody and cytochrome P-450 content was measured. Values are the means of S.E.s and were less than 20% of the respective means. △, control; ○, hepatitis. Closed symbols are significantly different from the control (p<0.05). (B) Hepatic microsomes were collected 24 h after the injection of anti-BLP antibody, and adjusted to 2 mg of protein per ml. CO bound (a) or dithionite reduced (b) difference spectra of hepatic microsomes isolated from the control (right panel) and hepatitis (left panel) were recorded.

| Table II. Status of Hepatic Drug-Metabolizing Enzyme System in Hepatitis Mice |
|---------------------------------|----------------|----------------|
| **Parameters**                  | **Control**   | **Hepatitis**  |
| Cytochrome P-450<sup>a</sup>    | 0.71 ± 0.029  | 0.16 ± 0.008<sup>b</sup> |
| Cytochrome b<sub>5</sub>         | 0.23 ± 0.016  | 0.20 ± 0.014  |
| AM N-demethylase<sup>c</sup>    | 13.7 ± 12.37  | 34.5 ± 2.48<sup>d</sup> |
| AN hydroxylase<sup>d</sup>      | 62.8 ± 4.25   | 41.4 ± 3.53<sup>d</sup> |
| NADPH Cyt. c reductase<sup>e</sup> | 0.064 ± 0.0042 | 0.057 ± 0.0039 |
| NADPH Cyt. c reductase<sup>f</sup> | 1.32 ± 0.108  | 0.79 ± 0.061<sup>f</sup> |

The experimental conditions were the same as in Fig. 1. Animals were sacrificed at 24 h after the injection of anti-BLP antibody, and drug-metabolizing enzyme activities were measured. Each value represents the mean value ± S.E. obtained from 6–8 animals. a) nmol/mg protein, b) nmol/20 min/mg protein, c) units/mg protein. Abbreviations: AM: aminopyrine, AN: aniline, Cyt.: cytochrome. Significant difference from the control values are indicated as (p<0.05).

shown in Table II. Whereas the cytochrome b<sub>5</sub> content and NADPH-cytochrome c reductase activity were not affected, hepatitis significantly decreased the activities of aminopyrine N-demethylase, aniline hydroxylase and NADPH-cytochrome c reductase, and the cytochrome P-450 content.

Discussion

Development of Model Hepatitis

Antigens localized in specific tissues are called tissue-specific antigens and have long been studied in connection with problems of tissue differentiation, tumor-specific antigens, and gene expression. In addition, as these antigens appear in the blood in particular diseases, they have been regarded as clinical markers. Recently, there have been a number of reports that antibodies to tissue-specific antigens are involved in the injury of particular tissues in autoimmune diseases. 25–29

BLP discovered by Mafune<sup>12</sup> is a basic protein specific to and rich in the liver compared with other organs. This BLP is reported to be different from known liver-specific antigens in its physical properties and immunological responsiveness. However, it is considered to be most suitable as a liver-specific antigen material, because it is abundant in the liver and has previously reported characteristics of liver-specific antigens, such as commonness within a species and the appearance of autoantibodies.

The fact that rat anti-BLP antiserum, obtained by immunizing a rabbit with rat BLP, reacts with the liver of the rabbit, suggests the self production of a antibody to BLP. Proliferation of rat fetal hepatocytes in culture is inhibited by the addition of rat anti-BLP antibody.12 In this process, a large number of dead hepatocytes appeared in the culture medium, presumably because of the cytotoxic effect of rat anti-BLP antibody on rat fetal hepatocytes. Therefore, the anti-BLP antibody appears likely to be involved as an autoantibody in immunological liver injury.

In the present study, we attempted to produce a model mouse with a liver injury resulting from an immunological mechanism using BLP as the antigen.

The liver injury was produced in mice by intravenous injection of an anti-BLP antibody derived from C57/BL/6J mice 5 d after intraperitoneal administration of RGG obtained from normal rabbits with CFA emulsion. When the anti-BLP antibody was administered to C57BL/6J, DBA/2, and ddY mice, liver injury was severest in C57BL/6J mice. In this strain, the severity of liver injury was dependent on the dose of anti-BLP antibody. Serum biochemical studies showed that abnormalities of the parameters of liver functions and histological lesions were demonstrated only in the liver, but not in the kidney, spleen, or lung. The liver injury in this model was markedly inhibited by the administration of prednisolone, cyclophosphamide, and levamisole, which act on the immune system.30–32 From these results, liver injury in this model was considered to be caused by an immunological mechanism, in contrast to liver injuries induced by the administration of chemicals such as carbon tetrachloride and acetaminophen.

The total bilirubin level, including direct-reacting and

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indirect-reacting bilirubin, is known to increase in humans with hepatitis, but the serum total bilirubin level tended to decrease in this mouse liver injury model after the injection of anti-BLP antibody. Furthermore, in the early stage of human hepatitis, the structure of hepatic cords appears normal, a few hepatocytes are swollen, degenerated, or necrosed, and a mild infiltration of migrating cells such as macrophages, lymphocytes, and neutrophils are observed. In advanced stages, marked hepatocellular necrosis is observed in the entire liver, and a very small number of hepatocytes are left, exhibiting features of acute hepatic atrophy, and the limiting lamina that demarcates a hepatic cord from Glisson's capsule is destroyed due to the disintegration of hepatic cords and the infiltration of inflammatory cells. In the present liver injury model, however, the histopathologic profile of the liver differed slightly from that in human hepatitis. Pathological changes in human hepatitis are generally considered not to be reproducible in small animals, so this point needs improvement.

No drug specifically effective for hepatitis has been developed, and the disease is usually treated with anti-inflammatory agents and immunomodulators. In the present experimental model, the anti-inflammatory steroid, prednisolone, and cyclophosphamide with an immunosuppressive effect, markedly reduced liver injury. The development of liver injury was also inhibited by levamisole, glycyr rhizin and cepharanthine, with immunomodulating effects.

These findings suggest that this mouse liver injury model is useful for clarification of the immunological mechanism involved in liver injury, or as a model of a particular pathologic state. Moreover, considering the fact that drugs acting on the immune system have little effect on hepatitis induced by chemicals such as acetaminophen and carbon tetrachloride, the present model developed by an immunological process is considered to be useful also for screening of drugs for the treatment of immunoreactive liver injuries.

**Mixed-Function Oxidase Activity** Furthermore, we were able to determine the effect of immunological liver injury on both in vivo and in vitro oxidative drug metabolism in mice. The results suggest that immunological liver injury decreased both in vivo and in vitro drug metabolism. As mentioned above, sleeping and paralysis times are often utilized as the first evaluation of a given treatment's influence on cytochrome P-450-mediated hepatic drug metabolism. Hexobarbital and zoxazolamine were selected for these studies because of their widespread use as indicators of cytochrome P-450 activity and their characteristic in vitro metabolism and in vivo pharmacokinetic use. Hexobarbital metabolism is more related to cytochrome P-450 than to cytochrome P-448 dependent enzyme activity, and conversely, zoxazolamine metabolism is more related to cytochrome P-448 than to P-450. Since both hexobarbital sleeping time and zoxazolamine paralysis time were primarily increased 24 h after the administration of an anti-BLP antibody, and because there was no significant difference in plasma or brain drug levels at awakening between the control and hepatitis animals, it is suggested that both cytochrome P-450 and P-448 dependent drug metabolizing enzyme systems are inhibited by hepatitis. Hepatitis thus produced has a blood decreasing effect on drug metabolism similar to cimetidine, tilorone and ozone, which have been observed to increase both hexobarbital sleeping time and zoxazolamine paralysis in rodents. Also, the time course of the decreased content of cytochrome P-450 was generally reflected in the altered hexobarbital sleeping time and zoxazolamine paralysis time after the administration of an anti-BLP antibody.

Furthermore, immunological liver injury produced significant decreases in the metabolism of the hexobarbital, aminopyrine and aniline, NADPH-cytochrome c reductase activity and cytochrome P-450 content, but not in the NADPH-ferricyanide reductase activity, or cytochrome b5 or microsomal protein content.

The effects of hepatic diseases on the microsomal metabolism of xenobiotics have been studied in several laboratories, and some conflicting observations and interpretations of these effects have been published. Thus, the effects of hepatitis on the hepatic mixed-function oxidase (MFO) system may be temporal. The decrease of hepatic MFO system seems to be a specific depression for cytochrome P-450 since other associated components, viz. NADPH-ferricyanide reductase and cytochrome b5, were not altered significantly during the study. The decrease in the hepatic MFO system during hepatitis may be due to a specific alteration in the synthesis, function or catalysis of cytochrome P-450. This is further supported by the appearance of a peak at 420 nm. Hence, the loss in cytochrome P-450, and associated activities, may be attributed to an accelerated degradation of cytochrome P-450, rather than to a decreased synthesis of this hemoprotein. The mechanism of the decreased effect of hepatitis on the hepatic MFO system can not be explained at present. Studies of the mechanisms of impairment effects of hepatitis on the hepatic MFO system are in progress in our laboratory.

A decrease in the hepatic MFO system by hepatitis as well as by other hepatic injury may affect the efficacy and pharmacological proper of drugs, as well as the metabolism of endogenous substances, with possible physiological, pharmacological and toxicological consequences.

In conclusion, the data presented in this paper establish the impairment of the hepatic MFO system during hepatitis. The denaturation/degradation of cytochrome P-450 appears to be a possible cause of this impairment of the hepatic MFO system.

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