In normal mice, plasma histamine levels were 29.4±10.1 pmol/ml. When 0.1 μg of lipopolysaccharide (LPS) was intravenously injected into Propionibacterium acnes (P. acnes)-primed ICR mice, histamine levels increased remarkably to 61.2±15.9 pmol/ml (p<0.001). An increase was also observed in liver tissues. Oral administration of histidine at 200 mg/kg once daily for 5 d before intravenous LPS injection increased the plasma alanine aminotransferase (ALT) activity to 2936.5±356.3 IU/l, a significant change compared with the controls (2244.8±425.5 IU/l, p<0.05). The 24 h survival rate after LPS injection was 72.7% in the mice treated with 50 mg/kg of ranitidine, in contrast with 50% in the control group although the treatment did not significantly decrease the plasma ALT activity. On the other hand, 50 mg/kg of pyrilamine significantly reduced plasma ALT activity (p<0.001). The results suggested that histamine levels are related to hepatic damage in the P. acnes plus LPS induction of liver injury.

Key words Propionibacterium acnes; lipopolysaccharide (LPS); histamine

Heat-killed Propionibacterium acnes (P. acnes) plus lipopolysaccharide (LPS)-induced liver injury has been used frequently as an animal model of autoimmune active hepatitis.1—3) When LPS is administered to P. acnes-primed ICR mice, death from massive hepatic cell necrosis usually occurs within 24 h.4,5) It was reported that this liver injury can be prevented by treatment with immunosuppressants such as FK506,6) and cyclosporin A.7,8) In addition, Toshima et al.9) demonstrated positive correlations between liver injury and the number of Kupffer cells, residual hepatic macrophages, which produce free radicals and cytokines.10) Therefore, these results suggested that in this model, activated Kupffer cells, hepatic macrophages and splenocytes play an important role during the development of P. acnes plus LPS-induced liver injury.11)

It is well known that histamine is one of the major chemical mediators in the regulation of inflammatory responses, immunity and allergic reactions.12—14) Histamine has been shown to be involved in various physiological functions via H1, H2, and H3 receptors.15-18) Pharmacological studies have also suggested that histamine affects T cell sensing from receptors,19,20) and interaction of histamine with its specific receptors exerts inflammatory reactions by mediation of inflammatory cytokines.21) However, these studies were limited to specific pathophysiolgies, and less attention has been paid to the influence of the histamine concentration in plasma, and the liver tissues in response to liver injury. Thus, in the present study, we investigated the relation between liver injury and histamine levels in plasma, and liver tissues. We also investigated the effect of histidine, a precursor amino acid of histamine, and a histamine receptor antagonist on LPS-induced liver injury following priming with P. acnes in mice.

MATERIALS AND METHODS

Animals Seven-week old male ICR mice were purchased from CLEA Japan Inc. (Shimizu, Japan). The animals were kept in a specific pathogen-free animal room at 23±1 °C with a 12 h light and dark cycle (lights on from 0600 to 1800 h), and were provided standard laboratory chow (CE-2; CLEA Japan, Inc.) and tap water. The animals were kept for 1 week before the experiment. The care and treatment of the animals conformed to the guidelines established by the Japanese Society of Nutrition and Food Science (Law No. 105 and Notification No. 6 of the Japanese government).

Preparation of P. acnes and Reagents P. acnes (ATCC 6919) was cultured with brain heart infusion medium (Difco Laboratories, Detroit, MI, U.S.A.) supplemented with L-cysteine (0.03%) and Tween 80 (0.03%) under anaerobic conditions using the Anaerobic System (Forma Scientific, Inc., OH, U.S.A.) for 48 h at 37 °C. Cultured cells were centrifuged at 10000 g for 15 min at 4 °C and washed with phosphate-buffered saline (PBS). The bacterial pellet was resuspended with PBS and the cells killed by heat treatment at 80 °C for 30 min, then lyophilized to prepare the heat-killed P. acnes powder. LPS from Escherichia coli 055:B5 was purchased from Difco Laboratories. These samples were dissolved in PBS solution immediately before use. Pyrilamine maleate (histamine H1-receptor antagonist), ranitidine (histamine H2-receptor antagonist), and thioperamide (histamine H3-receptor antagonist) were purchased from Sigma Chemical (St. Louis, MO, U.S.A.), and dissolved in PBS before use. L-Histidine (Sigma) was dissolved in water before use. The activity of plasma alanine aminotransferase (ALT) was measured using kits for the measurement of enzyme activity (Wako Pure Chemical Industries Ltd., Osaka, Japan).

P. acnes Plus LPS-Induced Liver Injury Heat-killed P. acnes was injected through the tail vein at 0.3 mg/mouse, and after 5 d, LPS (0.1 μg/mouse) was injected intravenously. These animals were sacrificed for analysis of liver injury 5 h after the LPS injection. Blood samples were taken from the heart of the mouse under anesthesia with diethyl ether into a tube containing 2% sodium heparin. The tubes were centrifuged at 5000 rpm for 5 min and the supernatant was used as a sample. All samples were stored at −20 °C until the assay. Plasma ALT activity, which is a marker of hepatocyte injury, was determined with an automatic serum analyzer.
Histamine Levels in Plasma, Liver and Spleen Tissues

Blood was taken from the hearts of mice under ether anesthesia, into a tube containing 2% sodium heparin. Then, the tubes were centrifuged at 3000 rpm for 15 min at 4°C to obtain plasma as a sample. The plasma was treated with 60% perchloric acid to adjust the concentration to 3%, and then centrifuged at 15000 rpm for 5 min at 4°C. The supernatant was stored at 4°C until the histamine analysis. Liver and spleen tissue samples, obtained after the mice were sacrificed by drawing blood, were weighed and mixed with a 5-fold volumes of 3% perchloric acid containing 5 mM Na₂-EDTA. Then the tissues were homogenized with a Polytron at maximum setting for 30 s under ice-cold conditions, and centrifuged at 15000 rpm for 20 min at 4°C. The supernatant was filtered through a 0.45 μm membrane filter and used as an assay sample.

The concentrations of histamine in plasma, liver, and spleen tissues, were determined by HPLC-fluorometry as described by Yamatodani et al. 20) The system was operated at room temperature. Samples were injected directly into a column packed with TSKgel SP2SW Cation Exchanger (6 mm I.D. x 150 mm, Tosoh, Tokyo, Japan). Histamine was eluted with 0.25 M potassium phosphate, at a flow rate of 0.6 mL/min. The histamine was post-labeled with α-phthalaldehyde in an alkaline condition, and detected fluorometrically in an F1080 Fluorometer (Hitachi, Tokyo, Japan), and monitored at 360 and 450 nm, respectively.

Statistical Analysis All values are expressed as the mean±S.D. Statistical analyses were performed with Student’s t-test. Differences were considered to be significant when the probability value was less than 0.05.

RESULTS

Effect of Splenectomy in P. acnes Plus LPS-Induced Liver Injury ALT measurements were performed using plasma obtained from mice 5 h after LPS administration. As shown in Fig. 1A, the ALT level in the splenectomy A group in which the spleen was removed from mice 24 h before the P. acne injection was significantly (p<0.01) lower than that in the P. acnes plus LPS-induced liver injury control. However, there were no significant differences between the splenectomy B group in which mice were splenectomized 24 h before the LPS injection and the liver injury control group. In addition, the ALT level on administration of P. acnes without the LPS injection (P. acnes alone group) increased significantly compared to that in normal mice (p<0.01). But the difference was not significant in the LPS alone group.

When mice were given intravenous injections of P. acnes, their spleen weight was 365.4±32.8 mg/tissue, significantly increased (p<0.001) compared with the control mice (134.2±9.9 mg/tissue). In mice injected with LPS 5 d after P. acnes, spleen weight was 369.8±44.6 mg/tissue and there was no significant difference with the P. acnes alone group. That in the LPS alone group was 164.8±16.9 mg/tissue, which was 122.8% the value for untreated normal mice (Fig. 1B).

Histamine Levels of Plasma, Liver and Spleen Tissues in P. acnes Plus LPS-Induced Liver Injury In normal mice, the plasma histamine level was 29.4±10.1 pmol/ml. While in mice treated with P. acnes plus LPS, it increased remarkably (61.2±15.9 pmol/ml, p<0.001) as shown in Fig. 2A. Figure 2A also shows that the histamine content of spleen tissue in mice treated with P. acnes plus LPS was 59.8±7.1 pmol/ml, a remarkable increase as compared with normal mice (40.4±7.0 pmol/ml, p<0.01). The same result was observed in liver tissue. The results suggested that the histamine level plays an important role in the induction of inflammatory liver injury.

We also evaluated the effect of histidine, which is a precursor of histamine, on the P. acnes plus LPS-induced liver injury model. As shown in Fig. 2B, 200 mg/kg of histidine was orally administered once a day for 5 d before the intravenous LPS injection. The activity of plasma ALT 5 h after the LPS administration was 2936.5±356.3 IU/l and was significantly high compared with the control (2244.8±425.5 IU/l, p<0.05).

Effect of Histamine Receptor Antagonists in P. acnes Plus LPS-Induced Liver Injury Next, 50 mg/kg of pyrilamine, 50 mg/kg of ranitidine, and 5 mg/kg of thioperaamide, doses determined in our preliminary experiments, were injected intraperitoneally, respectively, 24 and 1 h before the ad-
ministration of LPS. The same dose of saline was given in the other group as a control. The 24 h survival rate were 72.7% in the ranitidine-treated group, compared with 50% in the saline group (Fig. 3A). The histamine H2-receptor antagonist reduced the death rate slightly in *P. acnes* plus LPS-induced liver injury. As shown in Fig. 3B, the plasma ALT activity was 2103.8 ± 144.4 IU/l in the saline group, while it was 1392.3 ± 183.3 IU/l (p<0.001) in the pyrilamine-treated group at 5 h after LPS injection. The histamine H1-receptor antagonist significantly lowered plasma ALT activity relative to the control. The histamine H1 and H2-receptor antagonist suppressed the hepatic damage as shown by the 24 h survival rate and plasma ALT activity, however thioperamide was not significantly effective in the liver injury model.

**DISCUSSION**

The injection of LPS into normal mice does not induce liver injury, while the same treatment of *P. acnes*-primed mice induces severe liver injury, indicating that *P. acnes* renders mice susceptible to LPS. As previously reported at 1 week after priming with *P. acnes*, the large numbers of macrophages and lymphocytes that infiltrate the portal area in the priming phase are essential for the subsequent liver injury elicited by a low dose of LPS.21,22) Mizoguchi *et al.* reported that when liver adherent cells including Kupffer cells isolated from the liver tissue of mice after *P. acnes* injection in *vitro*, remarkable activity of the cytotoxic factor which is thought to cause liver injury was found in the culture supernatant.23) Several studies have focused on the analysis of cytokine production mechanisms. As a result, in liver injury induced with a low dose of LPS, it was found that there was a marked increase in the blood level of tumor necrosis factor (TNF), which is an important agent that induces inflammation.24) Moreover, higher blood levels of these cytokines may not persist for a prolonged period, since they may be instantly or rapidly consumed. This liver injury can be prevented by treatment with immunosuppressants. Therefore, macrophages, and splenocytes are considered to play an important role in the inflam-

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**Fig. 2.** Histamine Levels of Tissues, and Effects of the Histamine Metabolic Precursor in *P. acnes* Plus LPS-Induced Liver Injury

Seven-week-old male ICR mice were used in the *P. acnes* plus LPS-induced liver injury test. Heat-killed *P. acnes* was injected through the tail vein at 0.3 mg/mouse, and after 5 d, LPS dissolved in PBS was injected intravenously in a volume of 0.1 μg/mouse. Plasma histamine was assayed 5 h after the LPS injection (A). Histidine (200 mg/kg per d) diluted with water was given orally at a dose of 0.1 ml/10 g of body weight from 1 d before the administration of *P. acnes* for 5 d continuously before the intravenous LPS injection (B). These animals were sacrificed 5 h after the LPS injection, and the results represent the mean ± S.D. of the values obtained from 5 animals in each group. Significantly different from the control mice at *p<0.05, **p<0.001 as determined with Student’s *t*-test.

**Fig. 3.** Effect of Histamine Receptor Antagonists in *P. acnes* Plus LPS-Induced Liver Injury

Seven-week-old male ICR mice were used in the *P. acnes* plus LPS-induced liver injury test. Heat-killed *P. acnes* was injected through the tail vein at 0.3 mg/mouse, and after 5 d, LPS dissolved in PBS was injected intravenously in a volume of 0.1 μg/mouse. The 24 h survival rate after LPS injection were observed in (A). These animals were sacrificed 5 h after the LPS injection, and the results represent the mean ± S.D. of the values obtained from 5 animals in each group (B). Pyrilamine (50 mg/kg), ranitidine (50 mg/kg), and thioperamide (5 mg/kg) were injected intraperitoneally at a dose of 0.1 ml/10 g of body weight 24 h, and 1 h before the administration of LPS, respectively. Significantly different from the control mice at *p<0.001 as determined with Student’s *t*-test.
matory reaction in mice as immunomodulatory cells.

In the present experiment, splenocytes provided important information on the liver injury. When LPS is administered to *P. acnes*-primed mice, the plasma ALT activity markedly increases within 5 h of the injection. However, ALT activity was significantly lower than in the controls when the spleen was removed from mice before the *P. acnes* injection. On the other hand, ALT activity was not markedly influenced by splenectomy at 5 d after the injection. For the 5 d, the migration of lymphocytes from the peripheral lymphoid organs to liver tissues, and infiltration by inflammatory cells of the liver may have occurred to cause a massive hepatic cell necrosis. Even though splenectomy suppresses liver injury as splenocytes are not able to migrate to liver, the plasma ALT activity was higher than in normal mice, *P. acnes*-treated mice, and LPS-treated mice. These results suggest that many networks regulate each other in the induction of liver injury.

Little is known about the relation between *P. acnes* plus LPS-induced liver injury and histamine levels in both plasma and liver. When mice were treated with *P. acnes* plus LPS, the plasma histamine levels remarkably increased as compared with normal mice. An increase in the level of histamine was also observed in liver and spleen tissues. It is well known that histamine is mainly stored in mast cells, basophils and enterochromaffin-like cells. Although in the present study, we were unable to clarify the mechanism of this liver injury-induced histamine response, recent evidence indicates that macrophages are responsible for the increase in histidine decarboxylase (HDC), histamine-forming enzyme, relied on histamine production in the liver caused by the injection of hepatotoxins, and an increase in the number of liver macrophages may have dual causes such as the local replication of resident macrophages and influx of precursor cells. Umezu *et al.* reported that the marked increase in liver histamine content in injured liver was presumably derived from an increase of mast cells in the inflamed area of the liver. This has been associated with the production of acute-phase proteins in the liver, that is, the inflammatory cytokine-induced synthesis of protein such as complement component C3 in hepatocytes is greatly modulated by histamine added to the system. Ishii *et al.* indicated that the blood histamine concentration increases in patients with chronic hepatitis, and in animals treated with hepatotoxin. Suzuki *et al.* reported similar results.

In addition, repeated injection of histamine induces liver injury in animals, and administration of a HDC inhibitor to patients with hepatitis relieves the symptoms. Our result that the plasma, and liver histamine concentration were markedly increased in the course of *P. acnes* plus LPS-induced liver injury, suggest that histamine is implicated in the host stress response and regulates stress-induced physiological changes.

We also evaluated the effect of histidine on *P. acnes* plus LPS-induced liver injury in the present study. When mice were pretreated with 200 mg/kg of histidine once a day for 5 d before intravenous LPS injection, plasma ALT activity was significantly increased compared with controls. Although we do not know the mechanism by which histidine affects this response, in previous reports, LPS increased HDC activity *in vitro* and *in vivo*, leading to production of histamine. The finding that the *P. acnes* plus LPS-induced liver injury deteriorated following pretreatment with histidine suggests that such excessive production of histamine is controlled even when in the liver is injured. At least in part, it may make some contribution to the development of inflammation. The effects of histamine receptor antagonists were studied using this experimental model. Mortality after 24 h was improved, from 50% in the control to 27.3% in the rani-tidine-pretreated mice. Ranitidine protected the mice from *P. acnes* plus LPS-induced lethal shock, and improved the survival rate during the course of *P. acnes* plus LPS-induced liver injury. Pyrilamine was found not to affect the survival rate, but it markedly lowered the plasma ALT activity compared to the control. However, thiopental, a selective antagonist of the histamine type 3 receptor, had no effect. We found that ranitidine and pyrilamine protect against *P. acnes* plus LPS-induced liver injury. Ranitidine competitively inhibits the interaction of histamine via H2 receptors, and is widely used for the treatment of peptic disease, since ranitidine shows a protracted and intense inhibition of gastric acidity. Recently, Okajima *et al.* demonstrated that ranitidine could reduce liver injury directly by inhibiting neutrophil activation, or indirectly by inhibiting the production of TNF-alpha, which is a potent activator of neutrophils. However, famotidine, another H2 receptor antagonist, did not have this effect in their experiment.

It has also been reported that histamine inhibits LPS-induced synthesis of TNF-alpha, and interleukin-1 in peripheral blood mononuclear cells, and the effects are reversed by H2 receptor antagonists. However, these results were limited to *in vitro* effects, and the role of histamine in the regulation of inflammatory reactions is still poorly understood. On the other hand, Nakamura *et al.* reported that dimaprit, a selective histamine H2 receptor agonist, inhibited the production of TNF-alpha in galactosamine plus LPS-induced rapid hepatitis, a mouse model of acute liver injury caused by activated Kupffer cells *etc.* hepatic non-parenchymal cells. However, the inflammatory process was not implicated in the *P. acnes*-primed liver injury model, because our findings indicate that peripheral lymphoid organs are one of the sources of inflammatory reaction cells. Overall, our observations indicate that histamine plays a critical role in the *P. acnes* plus LPS-induced liver injury process. Additionally, although our results show that treatment with ranitidine and pyrilamine is useful for protecting mice from *P. acnes* plus LPS-induced liver injury, the therapeutic efficacy of ranitidine and pyrilamine might not be explained solely by the blockade of the histamine receptor in only this course of inflammatory responses, and further studies are necessary to examine other possibilities.

REFERENCES


