The roles of mast cells and Kupffer cells in rat systemic anaphylaxis

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Shibamoto T, Shimo T, Cui S, Zhang W, Takano H, Kurata Y, Tsuchida H. The roles of mast cells and Kupffer cells in rat systemic anaphylaxis. Am J Physiol Regul Integr Comp Physiol 293: R2202–R2209, 2007. First published October 10, 2007; doi:10.1152/ajpregu.00613.2007.—Mast cells and other cells such as macrophages have been shown to mediate systemic anaphylaxis. We determined the roles of mast cells and Kupffer cells in hepatic and systemic anaphylaxis of rats. Roles of mast cells were examined by using the mast cell-deficient white spotting (Ws/Ws) rat; the Ws/Ws and wild type (+/+) rats were sensitized with ovalbumin (1 mg). Roles of Kupffer cells were examined by depleting Kupffer cells using gadolinium chloride or liposome-encapsulated dichloromethylene diphosphonate in the Ws/Ws and Sprague-Dawley rats. An intravenous injection of 0.6 mg ovalbumin caused substantial anaphylactic hypotension in both the Ws/Ws and +/+ rats; however, the occurrence was delayed in the Ws/Ws rats. After antigen, portal venous pressure increased by 13.1 cmH2O in the +/+ rats, while it increased only by 5.7 cmH2O in the Ws/Ws rats. In response to antigen, the isolated perfused liver of the Ws/Ws rats also showed weak venoconstriction, the magnitude of which was one tenth as large as that of the +/+ rats, indicating that hepatic anaphylaxis was primarily due to mast cells. In contrast, Kupffer cell depletion did not attenuate anaphylactic hepatic venoconstriction in isolated perfused livers. In conclusion, mast cells are involved mainly in anaphylactic hepatic presinusoidal portal vеноconstriction but only in the early stage of anaphylactic systemic hypotension in rats. Macrophages, including Kupffer cells, do not participate in rat hepatic anaphylactic venoconstriction.

ANAPHYLAXIS IS AN IMMEDIATE, type-1 hypersensitivity reaction that occurs after exposure of sensitized organisms and tissues to sensitizing antigen. The most common life-threatening feature of acute anaphylaxis is cardiovascular collapse and shock (29). Although anaphylaxis is classically mediated by histamine released in response to antigen cross-linking of IgE bound to high-affinity Ig-E receptors, FceRI, on mast cells, both human and rodent studies indicate that this classical pathway does not account for all anaphylactic responses (3, 10, 21, 26). Indeed, systemic anaphylaxis can be induced in genetically mast cell-deficient mice (13, 15). Moreover, lethal anaphylactic shock was as likely to develop in mast cell-deficient W/Wv as in normal mice (1, 13, 15, 31). In contrast to mice, the role of mast cells in anaphylactic hypotension is not clear for rats: Nishida et al. (22) reported the lack of anaphylactic hypotension in the genetically mast cell-deficient rats “white spotting”; Ws/Ws (23) sensitized with the nematode Nippostrongylus brasiliensis, whereas Guo et al. (12) found similar hypotensive responses in both mast cell-deficient Ws/Ws and their wild-type (+/+) control rats. Thus, the necessary participation of mast cells in anaphylactic hypotension in rats is now being seriously questioned.

On the other hand, two distinctive pathways of systemic anaphylaxis have been demonstrated in mice (11, 30: one mediated by mast cells, IgE, FceRI, and histamine (classical pathway-associated human allergy), and an alternative pathway involving IgG, FcγRIII, macrophages, and platelets, and platelet-activating factor (PAF). However, to our knowledge, the role of macrophages in systemic anaphylaxis in rats has not ever been evaluated.

It is well known that the vasoconstriction of the liver contributes to the control of systemic circulation: the passive blood mobilization to and from the liver, which is caused by hepatic vasoconstriction, influences the venous return to the heart and hence systemic circulation (20). Indeed, we have recently demonstrated that the liver is involved in rat anaphylactic hypotension, by showing that elimination of liver and splanchnic circulation attenuated the antigen-induced fall in systemic arterial pressure in anesthetized Sprague-Dawley (SD) rats (27). On the other hand, it has been recently demonstrated that the effector cells responsible for regional anaphylactic responses are not necessarily mast cells but could be varied depending on the organs and tissues exposed to antigens (4). However, determination of the effector cells in hepatic anaphylaxis is not performed; it is not known whether mast cells or macrophages are responsible for the anaphylactic reaction in the liver. Actually, both mast cells and macrophages are abundantly distributed in the livers. In particular, macrophages distribute quite densely in the liver, as intravascular resident macrophages, that is, Kupffer cells. They constitute 80 to 90% of the fixed-tissue macrophages ( reticuloendothelial system) and account for ~15% of the liver cells (33).

Thus, we determined here the roles of mast cells and Kupffer cells in rat hepatic anaphylaxis and anaphylactic hypotension. To clarify the participation of mast cell in rat systemic and hepatic anaphylaxis, we used the genetically mast cell-deficient Ws/Ws rats (23), together with their wild-type (+/+) rats as controls. To determine the role of macrophages in rat hepatic anaphylaxis, we used the isolated perfused liver from sensitized rats whose macrophages were chemically depleted by intravenous injections of either gadolinium chloride (14) or liposome encapsulated dichloromethylene diphosphonate (32). In this study, to clarify more details on the roles of effector cells in hepatic vasoconstriction, we

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determined the changes in hepatic vascular resistance distribution by measuring the sinusoidal pressure with the double occlusion pressure (28, 34).

MATERIALS AND METHODS

Animals

Twenty male Ws/Ws (234 ± 15 g) and twenty-one male +/+ (230 ± 14 g) rats, as well as twenty-four male SD rats (285 ± 17 g) were used in this study. Ws/Ws rats have a 12-base deletion in the tyrosine kinase domain of the c-kit cDNA and are deficient in mast cells, whereas +/+ rats have a normal number of these cells (23). All animals used were purchased from Japan SLC (Shizuoka, Japan) and were maintained at 23°C and under pathogen-free conditions on a 12:12-h dark-light cycle and allowed food and water ad libitum. The experiments conducted in the present study were approved by the Animal Research Committee of Kanazawa Medical University.

Sensitization

Rats were actively sensitized by the subcutaneous injection of an emulsion made by mixing equal volumes of complete Freund’s adjuvant (0.5 ml) with 1 mg ovalbumin (grade V; Sigma, St. Louis, MO) dissolved in physiological saline (0.5 ml), as described previously (27).

Macrophage Depletion

Gadolinium chloride. Gadolinium chloride (GdCl₃) (Sigma-Aldrich), which not only blocks phagocytosis but also eliminates Kupffer cells (14), was dissolved in saline and was intravenously injected via tail vein at 2 mg/100 g into six Ws/Ws rats, seven +/+ rats, and eight SD rats at 24 h before the experiment.

Dichloromethylene diphosphonate. Suspensions of multilamellar liposomes encapsulating dichloromethylene diphosphonate (Cl₂MDP) were prepared according to the method of van Rooijen (32). In brief, 86 mg phosphatidylcholine (Sigma) and 8 mg of cholesterol (Sigma) were dissolved in 20 ml methanol/chloroform (1:1) in a round-bottomed flask connected to a rotary evaporator. After evaporation, 10 nL PBS (10 mM, pH 7.4) containing 1.89 g Cl₂MDP (Sigma) was added, and the suspension was sonicated 2 h later. They were dissolved in 20 ml methanol/chloroform (1:1) for 30 min. The liposomes were then centrifuged (100,000 g) for 30 min. The liposomes were then resuspended in 4 ml PBS and were extruded through polycarbonate membranes with a pore size of 0.45 μm; 2 ml was intravenously injected into six SD rats at 24 h before the experiment.

In Vivo Experiment

Two weeks after sensitization, +/+ rats (n = 8) and Ws/Ws rats (n = 8) were anesthetized with pentobarbital sodium (70 mg/kg ip) and placed on a thermostatically controlled heating pad (ATC-101B; Unique Medical, Tokyo, Japan), which maintained body temperature at 36–37°C throughout the experiment. The adequacy of anesthesia was monitored by the stability of blood pressure and respiration under control conditions and during a pinch of the hindpaw. Supplemental doses of anesthetic (10% of initial dose) were given as necessary. The basic methods for anesthetized rat experiments were described previously (27). The left carotid artery was catheterized to measure systemic arterial pressure (Psa). The right external jugular vein was catheterized, and the catheter tip was positioned at the confluence of the superior vena cava and the right atrium. This catheter was used for an intravenous injection of antigen and measurement of the central venous pressure (Pcv). Heart rate (HR) was measured by triggering the R wave of electrocardiogram. Following a midline incision, a catheter (ID 0.47mm, OD 0.67mm) was inserted into the main portal vein for continuous measurement of the portal venous pressure (Ppv). After closure of the abdomen, the baseline measurements were started.

The Psa, Pcv, and Ppv were continuously measured via pressure transducers (TP-400T, Nihon-Kohden, Tokyo, Japan) with the reference point at the levels of right atrium. These pressures and HR were continuously displayed on a thermal physiograph (RMP-6008). Outputs were also digitally recorded at 20 samples/s (PowerLab, ADInstruments, Castle Hill, NJ). Hemodynamic parameters were observed for at least 20 min after surgery until a stable state was obtained. After the baseline measurements, 0.6 mg of the ovalbumin antigen was administered via the jugular vein catheter.

Isolated Liver Preparation

Two weeks after sensitization, the Ws/Ws, +/+ , and SD rats were anesthetized with pentobarbital sodium (70 mg/kg ip) and mechanically ventilated with room air. The basic methods for isolated perfused rat livers were described previously (27). In brief, a polyethylene (PE) tube was placed in the right carotid artery. After laparotomy, the hepatic artery was ligated, and the bile duct was cannulated with the PE tube (0.5 mm ID, 0.8 mm OD). After infra-arterial heparinization (500 U/kg), 8–9 ml of blood was withdrawn through the carotid arterial catheter. The intra-abdominal inferior vena cava (IVC) above the renal veins was ligated, and the portal vein was cannulated with a stainless-steel cannula (2.1 mm ID, 2.5 mm OD) for portal perfusion. After thoracotomy, the supradiaphragmatic IVC was cannulated through a right atrium incision with the same size stainless cannula,
and then portal perfusion was begun. To eliminate an influence of circulating blood cells, the perfusate was the blood-free Krebs-Henseleit solution (118 mM NaCl, 5.9 mM KCl, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 1.2 mM NaH₂PO₄, 25.5 mM NaHCO₃, and 5.6 mM glucose) added with 5% bovine albumin (Sigma). The liver was rapidly excised, suspended from an isometric transducer (TB-652T, Nihon-Kohden), and weighed continuously throughout the experimental period.

The sensitized livers were perfused at a constant flow rate in a recirculating manner via the portal vein. The perfusate (40 ml) was oxygenated in the venous reservoir by continuous bubbling with 95% O₂ and 5% CO₂ and was pumped using a Masterflex pump from the venous reservoir through a heat exchanger (37°C). The height of the reservoir and the portal flow rate were adjusted to obtain the initial isogravimetric state as described below. The Ppv and Phv were measured using pressure transducers (TP-400T, Nihon-Kohden) attached by sidearm to the appropriate cannulas with the reference points at the hepatic hilus. To occlude inflow and outflow perfusion lines simultaneously for measurement of the double occlusion pressure (Pdo), two solenoid valves were placed in a position so that each sidearm cannula was between the corresponding solenoid valve and the liver (34). Portal flow rate (Qpv) was measured with an electromagnetic flow meter (MFV 1200, Nihon-Kohden, Japan), and the flow probe was positioned in the inflow line. Bile was collected drop by drop in a small tube suspended from the force transducer (SB-1T, Nihon-Kohden). One bile drop yielded 0.018 g, and the time between drops was measured for determination of the bile flow rate (27). The hepatic vascular pressures, Qpv, liver weight, and bile weight were monitored continuously and displayed through a thermal physiograph (H20849). One bile drop yielded 0.018 g, and the time between drops was measured for determination of the bile flow rate (27).

Hepatic hemodynamic parameters were observed for at least 20 min after the start of perfusion until an isogravimetric state (no weight gain or loss) was obtained by adjusting Qpv and the height of the reservoir to a Phv of 0–1 cmH₂O. After the baseline measurements, the perfused livers were challenged with ovalbumin injected into the reservoir. The doses of ovalbumin were 1 mg for the Ws/Ws and +/+ rats, 0.01 mg for the SD rats.

The hepatic sinusoidal pressure was measured by the double occlusion method (34). Both the inflow and outflow lines were simultaneously and instantaneously occluded for 13 s with the solenoid valves, using the custom-made program (Liver software, Bio-medical Science, Kanazawa, Japan), after which Ppv and Phv rapidly equilibrated to a similar or identical pressure, which was Pdo. In each experimental group, Pdo was measured at baseline and at the Ppv peak following by a gradual recovery to 67 ± 24 mmHg at 60 min. In contrast, the Ws/Ws rats showed a gradual decrease in Psa: Psa did not change from the baseline of 127 ± 8 mmHg for 1.5 min after antigen and then began to decrease gradually. Actually, Psa during the initial 2 min after antigen was significantly higher in the Ws/Ws rats than in the +/+ rats. At 4 min, Psa in the Ws/Ws rats decreased to 59 ± 12 mmHg, the levels comparable to that in the +/+ rats (60 ± 20 mmHg). The bottom levels of Psa were similar between two groups. In the +/+ rats, Ppv increased from the baseline of 7.8 ± 1.0 cmH₂O.

RESULTS

Response of the Anesthetized Rats to Antigen

Figure 1, A and B, shows a representative example of the response to an intravenous injection of the ovalbumin antigen in an anesthetized +/+ and Ws/Ws rat, respectively. Figure 2 shows the summary data of time course changes in Psa, Ppv, and Pcv. After an injection of antigen in the +/+ group, Psa rapidly decreased from the baseline of 116 ± 8 mmHg to 66 ± 13 mmHg at 1 min after the antigen and then continued to decrease progressively to the nadir of 51 ± 10 mmHg at 9 min, followed by a gradual recovery to 67 ± 24 mmHg at 60 min. In contrast, the Ws/Ws rats showed a gradual decrease in Psa; Psa did not change from the baseline of 127 ± 8 mmHg for 1.5 min after antigen and then began to decrease gradually. Actually, Psa during the initial 2 min after antigen was significantly higher in the Ws/Ws rats than in the +/+ rats. At 4 min, Psa in the Ws/Ws rats decreased to 59 ± 12 mmHg, the levels comparable to that in the +/+ rats (60 ± 20 mmHg). The bottom levels of Psa were similar between two groups. In the +/+ rats, Ppv increased from the baseline of 7.8 ± 1.0 cmH₂O.

Data Analysis

All results are expressed as the means ± SD. Statistical analyses were performed with repeated-measures ANOVA, and a P value less than 0.05 was considered significant. When a significant difference was obtained, post hoc analysis was performed with the Bonferroni post-test method.
to the peak of 20.9 ± 4.2 cmH₂O at 1 min after antigen, and then gradually decreased to 7.8 ± 1.0 cmH₂O at 20 min. The Ws/Ws rats showed a more gradual and increased in Ppv after antigen: Ppv reached the peak of 16.5 ± 2.7 cmH₂O at 2 min after antigen. The increment of Ppv of the Ws/Ws rats was 56% of that of the +/+ rats (7.4 ± 2.6 vs. 13.1 ± 3.8 cmH₂O). After antigen, Pcv significantly decreased by 1.4 and 1.1 cmH₂O in both the +/+ and Ws/Ws groups, respectively. The decrease in Pcv of the +/+ group tended to be greater, but not significantly, than that of the Ws/Ws group. Heart rate did not significantly change after antigen in either group.

Response of Perfused Livers to Antigen

When antigen was injected into the perfusate of either +/+ or Ws/Ws livers, hepatic venoconstriction occurred, as evidenced by an increase in Ppv, as shown in Fig. 3. The +/+ rat livers showed marked venoconstriction similar to that in the in vivo +/+ rats: Ppv increased by 13.4 cmH₂O from the baseline of 6.4 ± 0.3 to 19.4 ± 1.6 cmH₂O. This anaphylactic hepatic venoconstriction observed in the wild-type +/+ rat was characterized by selective presinusoidal constriction. This is based on a slight increase in Pdo (from 2.2 ± 0.2 to 2.7 ± 0.3 cmH₂O) compared with a marked increase in Ppv, which resulted in a large increase in the Ppv-to-Pdo gradient (12.5 ± 1.4 cmH₂O), an indicator of Rpre, and a marginal increase in the Pdo-to-Phv gradient (0.5 ± 0.2 cmH₂O), an indicator of Rpost. Actually, at 1.5 min after antigen, Rpre increased significantly at 4 times the baseline from 0.091 ± 0.011 to 0.362 ± 0.049 cmH₂O·ml⁻¹·min⁻¹·10⁻⁴ g liver weight⁻¹, while Rpost tended to increase, but not significantly, from the baseline of 0.042 ± 0.005 to 0.051 ± 0.008 cmH₂O·ml⁻¹·min⁻¹·10⁻⁴ g liver weight⁻¹, as shown in Fig. 4. In contrast to the control +/+ rats, the mast cell-deficient Ws/Ws rat liver showed only slight venoconstriction, which occurred after a longer latent period following an injection of antigen: Ppv increased only by 1.4 cmH₂O from the baseline of 6.8 ± 0.3 to 8.2 ± 1.1 cmH₂O at 2.5 min after antigen (Figs. 4 and 5), which was ~10% of the increase in the +/+ rats.

This marginal increase in Ppv contrasted with a substantial antigen-induced increase in Ppv in the anesthetized Ws/Ws rats, as described above.

The presence of antigen-induced hepatic venoconstriction, albeit to a small degree, in the mast cell-deficient rats indicates that the effector cells other than the mast cell should be involved in this hepatic anaphylactic reaction. Thus, we determined the role of Kupffer cell, the resident intravascular macrophage in liver, by depleting them with GdCl₃. However, pretreatment with GdCl₃ did not affect the responses to antigen of the perfused livers derived from either the sensitized Ws/Ws or +/+ rats, as shown in Fig. 4. These findings suggest the absence of significant roles of Kupffer cells in hepatic anaphylaxis of +/+ and Ws/Ws rats.

To further confirm the absence of Kupffer cell involvement in rat hepatic anaphylaxis, we examined the rat hepatic anaphylaxis in the different strain of SD rat whose Kupffer cells were eliminated with different drugs of GdCl₃ or Cl₂MDP. Sensitized SD rats were pretreated with GdCl₃ or Cl₂MDP, and then their livers were examined for the isolated perfused liver experiment. Figure 5 shows a representative example of the response of the isolated perfused control SD rat livers without pretreatment with GdCl₃ or Cl₂MDP. The response of the sensitized SD rat livers was characterized by selective presinusoidal constriction similar to that of the +/+ rat, although the increase in Ppv after antigen lasted longer than in the +/+ rats. As shown in Fig. 6, pretreatment with either GdCl₃ or Cl₂MDP did not affect the response to antigen of the sensitized SD rat livers. These findings further indicated the absence of participation of Kupffer cells in rat hepatic anaphylactic venoconstriction.

DISCUSSION

There are three major findings in the present study. The first finding (derived from the anesthetized rat experiments) is that the mast cell-deficient Ws/Ws rats showed antigen-induced hypotension, the degree of which was similar to the control +/+ rats, although the hypotension developed much more...
slowly. The second was related to the antigen-induced increase in PPv in vivo and isolated perfused livers. In the anesthetized Ws/Ws rats, antigen induced a substantial increase in PPv, the magnitude of which was 57% of the increase in the control +/+ rats. In contrast, the antigen-induced increase in PPv of the isolated perfused Ws/Ws rat livers was only 10% of the increase in the +/+ rat livers. This result may suggest that the intrahepatic mast cells are not responsible for the anaphylactic reaction that occurs in the livers and that portal hypertension seen in the anesthetized Ws/Ws rats may be mainly caused by mediators released from extrahepatic tissues. Finally, Kupffer cell depletion by the use of GdCl3 and Cl3MDP did not influence the hepatic anaphylactic venoconstriction of perfused livers from either SD or +/+ rats. This finding suggests that Kupffer cells are not involved in hepatic anaphylactic venoconstriction.

We confirmed the finding of Guo et al. (12), who reported that similar anaphylactic hypotension was observed in ovalbumin-sensitized Ws/Ws and +/+ rats. However, they did not refer to the differences in the early changes in Psa after antigen between these two groups. We clearly showed that there is a delay in the occurrence of hypotension in Ws/Ws rats compared with the +/+ rats. Furthermore, the increase in PPv after antigen also delayed in the Ws/Ws rats rather than the +/+ rats. Similar delay in occurrence of systemic anaphylaxis was also reported in the sensitized mast cell-deficient mice, when they were challenged with antigen (5). The presence of this time lag for the occurrence of anaphylactic responses in the mast cell-deficient rats indicates that the early stage of anaphylactic hypotension depends on mast cells. Then, a question arises about how the mast cells are involved in the early phase of anaphylactic hypotension. With respect to the mechanism for the early stage of anaphylactic hypotension in anesthetized rats, Bellou et al. (2) reported that histamine and serotonin, both of which are stored in the secretory granules of mast cells and released in response to antigen, are involved in the initial decrease in Psa after an injection of the antigen in the sensitized Brown Norway rats. Actually either histamine or serotonin, when administered intravenously into the anesthetized rats, causes a short-lasting decrease in Psa, presumably because of dilatation of systemic arterioles (2). Thus, the initial anaphylactic reaction related to mast cell degranulation could not occur in the mast cell-deficient Ws/Ws rats, resulting in delay of anaphylactic hypotension. The other anaphylaxis-associated chemical mediators such as PAF and leukotrienes might be biosynthesized from the cells other than mast cells, resulting in delayed hypotension in the Ws/Ws rats.

We have proposed that hepatic venoconstriction plays an important role in anaphylactic hypotension in SD rats (27), based on the finding that the elimination of hepatic and splanchnic circulation by ligation of the celiac and mesenteric arteries combined with total hepatectomy attenuated the antigen-induced reduction of Psa. Although the exact mechanism for the beneficial effect of these surgical procedures on the anaphylactic hypotension is not known, we assume that anaphylaxis-induced portal hypertension may account for the profound decrease in Psa. The pathophysiological process may be as follows: anaphylaxis causes hepatic venoconstriction, as observed in the isolated perfused sensitized liver, resulting in portal hypertension, which then causes congestion of the upstream splanchnic organs, with resultant decrease in venous return and effective circulating blood volume, and finally augmentation of anaphylactic hypotension. From this standpoint, the results of the present in vivo experiments are consistent with this assumption in that an antigen-induced increase in PPv of the Ws/Ws rats after antigen was delayed compared with that of the +/+ rats, resulting in a delay in development of anaphylactic hypotension.

We have, for the first time, demonstrated that hepatic anaphylaxis was caused by almost only mast cells. This conclusion was derived from the findings that, in response to antigen, PPv of the isolated Ws/Ws rat livers increased only slightly by 10% of that of the control normal +/+ rat livers. The perfusate of the isolated perfused livers was cell-free albumin Krebs solution, and it did not contain blood cells such as basophils, platelets, or neutrophils. Thus, this indicates that intrahepatic mast cells and their chemical mediators account for 90% of anaphylactic hepatic venoconstriction. In addition, we demonstrated that Kupffer cells do not participate in this hepatic anaphylactic venoconstriction. The absence of involvement of...
Kupffer cells in rat hepatic anaphylaxis was also confirmed in SD rats, in which Kupffer cells were depleted by two different drugs of GdCl₃ and Cl₂MDP.

The occurrence of anaphylactic hypotension in the Ws/Ws rats suggested that mediators were not released from mast cells but from other cells, including neutrophils, basophils, and platelets in these mast cell-deficient rats. Kimura et al. (18) reported that antigranulocyte antibody suppressed active and passive anaphylactic shock in the genetically mast cell-deficient mice of WBB6F1-W/Wv mice. Basophils express high-affinity Ig-E receptors (FcεRI), which bind Ig-E-antigen complexes and cause anaphylactic reaction (9). Platelets have been shown to participate in systemic anaphylaxis in mice (4). Platelets may mediate both allergen-induced vascular permeability and leukocyte recruitment in skeletal muscles in the absence of mast cells. Actually, platelets have been described as having FcεRII (FcεRII/CD23) on their plasma membranes (6, 16, 17) that can bind IgE with low affinity. In addition, these blood cells have granules that contain mediators, such as serotonin, and generate platelet-activating factor and many other proinflammatory mediators (24). Finally, other potential cell types that can release potent biologically activated mediators through IgE-dependent mechanisms are the eosinophil and lymphocytes via FcεRII (CD23) (7). It should be noted, however, that sensitization of rats also results in the production of antigen-specific IgG antibody (25), which can induce passive systemic anaphylactic responses (30); because mouse platelets have FcγR (19), which may also participate in allergic immune response (21), we cannot discount a role of IgG in this allergic response.

The weak venoconstrictive response of the isolated livers of the Ws/Ws rats contrasts to the substantial increase in Ppv observed in anesthetized Ws/Ws rats, which comprised 57% of that of the control normal +/+ rats. These findings suggest that portal hypertension seen in the anesthetized Ws/Ws rats might...
be caused by anaphylactic mediators primarily released not from the liver itself, but from the extraparenchymal tissues such as splanchic organs.

We previously showed that almost selective presinusoidal contraction is the characteristic for the hepatic anaphylaxis of isolated SD rat livers perfused with blood (27) or blood-free perfusate (8). We confirmed this previous finding in the present experiment on isolated SD rat livers perfused with ovalbumin-Krebs solution. Furthermore, in the present study, we demonstrated that the isolated perfused +/- rat liver also showed similar selective presinusoidal contraction, although the venoconstrictive response was short-lasting compared with that in the SD rat livers (see Figs. 3 and 5).

**Perspectives and Significance**

It is well established that systemic anaphylaxis is mediated by IgE, FcεRI, mast cells, and histamine in humans. Recently, it has been suggested that an alternative pathway involving IgG, FcγRIII, macrophages, and PAF is more important in the anaphylactic response to antigen challenge in mice (11, 30). However, the role of mast cells or macrophages in the rat anaphylaxis has not been well evaluated. In this study, using the mast cell-deficient rat, we have demonstrated that mast cells are involved mainly in anaphylactic hepatic venoconstriction but only in the early stage of anaphylactic systemic hypotension in rats sensitized with ovalbumin. However, macrophages, including Kupffer cells, do not participate in rat hepatic anaphylactic vasoconstriction. The lack of involvement of the macrophage in rat anaphylaxis contrasts with the concept of the alternative pathway of mouse anaphylaxis, in which macrophages and IgG are definitely involved (11, 30). This may be due to the differences in the immune system between these two species. It is proposed that the immune system responsible for anaphylaxis of the mouse is similar, in part, to that of the human (11). However, human anaphylaxis depends on mast cells and IgE, and IgG-mediated anaphylaxis has not been described in human subjects (11). Further experimental studies are required to represent the systemic anaphylaxis in human subjects from the immunological and physiological standpoint.

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