Different preparations of zymosan induce glycogenolysis independently in the perfused rat liver

Involvement of mannose receptors, peptide-leukotrienes and prostaglandins

Kazuhiro KIMURA,* Masakazu SHIOTA, Kiyotaka MOCHIZUKI, Mitsuaki OHTA and Tsukasa SUGANO
Laboratory of Veterinary Physiology, Department of Veterinary Science, College of Agriculture, University of Osaka Prefecture, Mozuumemachi, Sakai 591, Osaka, Japan

Zymosan (non-boiled) induced glycogenolysis biphasically, with no lag time, in the perfused rat liver. After the zymosan was boiled, it could be separated into two fractions, both of which stimulated glycogenolysis independently. The soluble fraction of boiled zymosan (zymosan sup) showed homologous desensitization, indicating that zymosan sup-induced glycogenolysis is a receptor-mediated event. Mannan (polymannose), which is known to be a biologically active component of zymosan, induced a glycogenolytic response similar to that produced by zymosan sup, and desensitized the response to the latter. Preinfusion of platelet-activating factor (PAF, 20 nM) or isoprenaline (10 μ M) did not extinguish the glycogenolytic response to zymosan sup, while the response to a secondary infusion of PAF was blocked. The glycogenolytic response to zymosan sup was completely inhibited by nordihydroguaiaretic acid (NDGA, 10 μ M), a lipoxygenase inhibitor, and by ONO-1078 (100 ng/ml), a leukotriene (LT) D₄ receptor antagonist. On the other hand, the glycogenolytic effect of zymosan pellet (the particulate fraction of boiled zymosan) was not affected by preinfusion of zymosan sup, and was inhibited by ibuprofen (20 μ M), a cyclo-oxygenase inhibitor. Prostaglandins (PGs) detected in the perfusate were augmented with infusion of zymosan pellet. Opsonization of the zymosan pellet by serum (complement) enhanced the glycogenolytic response without a lag period, and with a concomitant enhancement of PG output. Correlations between glucose production and PGs were $r = 0.832$ (PGD₂), $r = 0.872$ (PGF_{2 α}), $r = 0.752$ (PGE₂) and $r = 0.349$ (6-oxo-PGF_{1 α}). The glycogenolytic response to non-boiled zymosan was delayed and the biphasic glycogenolytic response was not observed when mannan was infused first. NDGA mimicked the effects of the preinfusion of mannan, while ibuprofen had no effect on the non-boiled-zymosan-induced glycogenolysis. These results suggest: (1) that non-boiled zymosan stimulates glycogenolysis through a mannose receptor-dependent, but unidentified, pathway, (2) that zymosan sup induces glycogenolysis via mannose receptor activation through the production of peptide-LTs but not PAF, and (3) that zymosan pellet causes glycogenolysis through the production of prostanoids, which is enhanced in the presence of complement.

INTRODUCTION

The synthesis and breakdown of glycogen in parenchymal liver cells is under strict hormonal regulation. In addition to the effects of the classical hormones, evidence has accumulated that non-parenchymal cell-derived factors, including prostanoids, platelet-activating factor (PAF) and interleukins, are also involved in glucose production [1–8].

Non-parenchymal cells, especially Kupffer cells and endothelial cells in the liver, have been shown to possess on their surfaces endocytic receptors for IgG, complement, mannose residues [9–12] and PAF [13]. Activation of Fc receptors by heat-aggregated IgG induces PAF production and then glycogenolysis in the perfused rat liver [14]. Exogenously infused PAF is capable of inducing a glycogenolytic response in a dose-dependent manner [15]. PAF-mediated events are characterized by homologous desensitization of the PAF-induced response [15] and by β -adrenergic agonist-induced heterologous desensitization of the PAF-induced response [16]. The glycogenolytic response to Fc receptor stimulation is also blocked by pretreatment with β -adrenergic agonists [14].

Fisher *et al.* [17] reported that phagocytosis of particle (zymosan)-induced glycogenolysis was mediated by PAF, as prior infusions of zymosan resulted in homologous desensitization of the glycogenolytic response to zymosan and hetero-

logous desensitization of the glycogenolytic response to PAF. Dieter *et al.* [18] reported that zymosan-induced glycogenolysis was sensitive to inhibitors of eicosanoid synthesis. On the other hand, zymosan has been shown to be ingested by peripheral macrophages through mannose/GlcNAc receptors [19], as zymosan has mannan (polymannose) as a biologically active component [20]. Thus it is likely that a mannose receptor is involved in the mechanism of zymosan-induced glycogenolysis. However, previous reports pay no attention to mannose receptors.

Here, we have re-investigated the effects of zymosan on glycogenolysis in perfused rat livers in terms of the involvement of mannose receptors. We found (1) that different preparations of zymosan induced glycogenolysis by different mechanisms, one of which is thought to act via a mannose receptor through the production of peptide-LTs, and (2) that the phagocytosis-induced glycogenolysis is mediated by prostanoid synthesis, which is potentiated in the presence of complement.

EXPERIMENTAL

Materials and animals

Zymosan, mannan and nordihydroguaiaretic acid (NDGA) were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Isoprenaline and ibuprofen were bought from Wako

Abbreviations used: LT, leukotriene; PAF, platelet-activating factor; PG, prostaglandin; NDGA, nordihydroguaiaretic acid; zymosan sup, soluble fraction of boiled zymosan.

* To whom all correspondence and reprint requests should be addressed.

Fig. 1. Effects of zymosan (non-boiled) on oxygen consumption and glucose production in perfused rat livers, and effects of NDGA on the zymosan-induced changes

Liver was perfused as described in the Experimental section. After 30 min pre-perfusion, zymosan (final concentration 150 μg/ml) was infused for 5 min (○). The oxygen consumption (a) and the glucose measured in the perfusate (b) are expressed as mean values of eight independent experiments (μmol/h per g of liver). When included, NDGA (●, 10 μM, $n = 4$), was infused 15 min before zymosan infusion. Statistical significance of differences between control (zymosan) and NDGA treatment are indicated by * $P < 0.05$.

Fig. 2. Effects of zymosan pellet (serum-treated or not) on oxygen consumption and glucose production in perfused rat livers

Zymosan pellet (○, $n = 8$), serum-treated zymosan pellet (●, $n = 6$) or heat-inactivated serum-treated zymosan pellet (▲, $n = 3$) were prepared as described in the Experimental section, and infused for 5 min. Other conditions were the same as in Fig. 1. Statistical significances between control (zymosan pellet) and serum or heat-inactivated serum treatment are shown by * $P < 0.05$; ** $P < 0.01$.

Pure Chemical Co. (Osaka, Japan). PAF and ONO-1078 [a leukotriene (LT) D_4 receptor antagonist] were generously provided by Ono Pharmaceutical Co. (Osaka, Japan). Other reagents were of analytical grade.

Male Sprague-Dawley rats weighing 180–210 g were used in all experiments. Animals were fed *ad libitum*.

Preparation of zymosan

Zymosan powder was suspended in perfusion medium at 135 mg/ml, and this suspension was used as non-boiled zymosan. To make the other preparations, the zymosan suspension was kept at 95 °C for 30 min as described by Dieter *et al.* [18]. The boiled suspension was centrifuged at 10000 g for 5 min, and the resulting supernatant designated 'zymosan sup'. The pellet, resuspended in the original volume of perfusion buffer, was used as zymosan pellet in experiments. In some experiments zymosan pellet was incubated with rat serum or heat-inactivated rat serum (56 °C, 30 min) at 37 °C for 30 min, washed three times with a large volume of perfusion medium, and finally resuspended in the original volume of medium. These suspensions were used as serum-treated and heat-inactivated serum-treated zymosan respectively.

Liver perfusion

All experiments were started between 09:00 and 12:00 h. Liver perfusion was performed essentially as reported previously [21], except that the liver was not excised. In brief, after anaesthesia

with pentobarbital (5 mg/kg), the portal vein and inferior vena cava were cannulated, and the liver was perfused at 32 °C at a constant flow rate (3.5 ml/min per g of liver) with oxygenated Krebs bicarbonate buffer in a flow-through mode. The buffer was continuously gassed with a humidified mixture of O_2/CO_2 (19:1) at 32 °C. The perfusion medium consisted of 115 mM-NaCl, 5.9 mM-KCl, 1.2 mM- $MgCl_2$, 1.2 mM- NaH_2PO_4 , 1.2 mM- Na_2SO_4 , 25 mM- $NaHCO_3$ and 2.5 mM- $CaCl_2$.

Other methods

The concentrations of oxygen in the effluent was continuously monitored with Clark-type electrodes, and oxygen utilized by the liver was estimated. Glucose in the perfusate was determined by the method of Bergmeyer & Bernt [22]. Prostaglandin (PG) D_2 in the perfusate was extracted with ethyl acetate [23] and then assayed with an EIA kit (Cayman Chemical Co., Ann Arbor, MI, U.S.A.). PGE_2 , $PGF_{2\alpha}$ and 6-oxo- $PGF_{1\alpha}$ in the perfusate were assayed by EIA with extraction.

Statistics

Values are expressed as means $\pm$ S.E.M., and the differences between means were analysed by Student's t test.

RESULTS

Infusion of non-boiled zymosan induced an immediate increase in glucose production and oxygen consumption (Fig. 1). At

Fig. 3. Effects of zymosan sup on oxygen consumption and glucose production in perfused rat livers, and inhibition of the zymosan sup-induced changes by NDGA or ONO-1078

Zymosan sup was infused for 5 min (○, $n = 8$) after 30 min pre-perfusion. When used, NDGA (●, $10 \mu\text{M}$, $n = 4$) or ONO-1078 (▲, 100 ng/ml , $n = 4$) were infused from 15 min or 5 min before stimulation respectively. Other conditions were the same as in Fig. 1. Statistical significances existed both between control (zymosan sup) and NDGA treatment, and between control and ONO-1078 treatment. (** $P < 0.01$.)

2 min after the start of infusion, a steep decrease in oxygen consumption, followed by suppression of glucose production, began. After cessation of infusion, oxygen consumption returned to the control level and a second peak of glucose release was observed. However, our results differed from those of Dieter *et al.* [18], who showed that zymosan stimulated glycogenolysis as a single peak after a lag period of about 1–1.5 min. To determine whether this difference was caused as a result of different preparations of zymosan, we prepared zymosan after boiling as described by Dieter *et al.* [18]. The boiled suspension was separated into two fractions by centrifugation, and both fractions stimulated glucose output. Zymosan pellet increased glucose production and oxygen consumption in parallel, with a lag time of 1.5 min (Fig. 2). On the other hand, zymosan sup caused biphasic glucose production, with a marked decrease in oxygen consumption after a short lag period (Fig. 3).

To determine which of the responses observed above were mediated by mannose receptors, mannan (polymannose) was infused (Fig. 4). Mannan caused changes in glucose production and oxygen consumption very similar to those induced by zymosan sup (Fig. 3). In repeated infusions of zymosan sup or mannan with intervals of 20 min between them, the glycogenolytic response to the second infusion of zymosan sup or mannan disappeared (Table 1). The administration of zymosan

Fig. 4. Effects of preinfusion of mannan on sequentially infused zymosan sup-induced or non-boiled-zymosan-induced glycogenolysis and changes in oxygen consumption in perfused rat livers

Mannan ($150 \mu\text{g/ml}$) was infused for 5 min and, after a 20 min interval, zymosan sup (●, $n = 4$) or zymosan (○, $n = 4$) was infused for 5 min (from 55 to 60 min). Other conditions were the same as in Figs. 1 and 3. Statistical significance of differences in glucose production and oxygen consumption are summarized in Table 1.

sup after infusion of mannan was also without effect on glycogenolysis (Fig. 4 and Table 1). These results indicate that zymosan sup may be rich in mannose. Zymosan pellet, added after infusion of zymosan sup or mannan, stimulated glycogenolysis in a manner similar to that observed in Fig. 2 (Table 1), indicating that zymosan was separated into different characteristic substances by boiling. On the other hand, the rise in glucose production with non-boiled zymosan was retarded by 1 min, as compared with the results shown in Fig. 1, after mannan was infused first (Fig. 4), and the marked suppression of oxygen consumption and biphasic glucose production seen in Fig. 1 were blocked.

Mannose receptors have been shown to be distributed in Kupffer cells and endothelial cells [10, 24–26]. Ovalbumin, which contains mannose residues, and mannose are thought to be cleared from the circulation via the mannose receptors on endothelial cells. When ovalbumin or mannose was infused, even at a high concentration (1.5 mg/ml), neither of them stimulated glucose production to any great extent: changes in glucose production induced by ovalbumin and mannose were $3.67 \pm 1.82 \mu\text{mol/h per g of liver}$ ($n = 4$) and $9.17 \pm 1.38 \mu\text{mol/h per g of liver}$ ($n = 3$) respectively.

We next determined whether the glycogenolytic response to mannose receptor activation was mediated by PAF. PAF (20 nM) also induced glycogenolysis as reported previously [15], and repeated infusion of PAF did not result in any glycogenolytic response (Table 1). Although the response to zymosan sup after PAF infusion was somewhat decreased compared with the response to zymosan sup alone, a significant increment of glucose production and suppression of oxygen consumption induced by zymosan sup were observed (Table 1), while the effects of PAF was desensitized. Infusion of PAF also stimulated glycogenolysis after zymosan sup was infused (Table 1). Furthermore, while pretreatment with a β -adrenergic agonist, isoprenaline ($10 \mu\text{M}$),

Table 1. Effects of zymosan sup, mannan, PAF and isoprenaline, and effects of sequentially infused zymosan, zymosan sup, mannan, zymosan pellet and PAF, on glucose production (a) and oxygen consumption (b) in the perfused rat liver

Experiments were performed as described in the legend to Fig. 4. After a 30 min pre-perfusion, zymosan sup, mannan (150 µg/ml) or PAF (20 nM) was infused for 5 min, and after a 20 min interval a second stimulus was infused for 5 min. In the case of isoprenaline (10 µM), the second infusion was performed after a 10 min interval, as described previously [16]. Data are means ± S.E.M. Statistical significances between the basal and the maximally stimulated values are indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. The first and second maximal values of glucose production in the case of zymosan sup, mannan and PAF are the maximal values of peak 1 and 2 respectively of glucose production, as seen in Figs. 3 and 4. The basal value for the second infusion was obtained at the time of the start of the second infusion. Figures in parentheses indicate numbers of experiments.

First infusion	Basal	Maximal		Second infusion	Maximal	
		First	Second		First	Second
(a) Glucose production (μmol/h per g of liver)						
Zymosan sup (8)	17.1 ± 1.1	38.4 ± 5.2**	47.6 ± 3.3***	Zymosan sup (4)	14.6 ± 1.4	18.3 ± 3.1
				Zymosa pellet (4)	13.5 ± 2.1	43.5 ± 2.7**
				PAF (3)	12.4 ± 2.4	25.1 ± 3.3* 39.2 ± 5.2**
Mannan (9)	16.5 ± 1.0	44.3 ± 4.1***	52.5 ± 2.9***	Zymosan sup (4)	13.6 ± 0.5	13.0 ± 0.56
				Zymosan pellet (4)	14.1 ± 1.9	45.1 ± 4.6**
				Zymosan (4)	13.9 ± 1.2	62.4 ± 3.6***
PAF (7)	17.9 ± 2.3	47.5 ± 6.5***	58.2 ± 4.0***	Mannan (3)	13.9 ± 1.3	17.9 ± 2.4
				Zymosan sup (4)	14.5 ± 1.9	2.7 ± 4.49* 38.7 ± 4.5**
				PAF (3)	11.4 ± 4.4	15.2 ± 1.4
Isoprenaline (7)	18.1 ± 1.1	36.6 ± 4.4**		Zymosan sup (4)	18.7 ± 0.8	30.8 ± 4.5* 38.9 ± 4.4**
				PAF (3)	19.8 ± 3.5	25.2 ± 4.5
(b) Oxygen consumption (μmol/h per g of liver)						
Zymosan sup (8)	86.4 ± 3.0	60.8 ± 2.2***		Zymosan sup (4)	83.9 ± 6.0	82.1 ± 4.8
				Zymosan pellet (4)	92.7 ± 5.3	107.9 ± 4.6
				PAF (3)	86.1 ± 4.5	69.4 ± 6.3
Mannan (9)	89.8 ± 3.9	64.3 ± 5.1**		Zymosan sup (4)	84.0 ± 2.2	85.2 ± 1.5
				Zymosan pellet (4)	87.7 ± 4.6	103.5 ± 2.1*
				Zymosan (4)	88.8 ± 6.6	113.0 ± 5.7*
PAF (7)	90.6 ± 5.6	64.4 ± 6.1**		Mannan (3)	86.3 ± 4.0	82.5 ± 5.1
				Zymosan sup (4)	86.6 ± 6.3	65.0 ± 5.9*
				PAF (3)	90.5 ± 4.5	90.7 ± 4.3
Isoprenaline (7)	91.0 ± 3.1	95.8 ± 3.3		Zymosan sup (4)	92.9 ± 4.6	72.9 ± 5.0*
				PAF (3)	85.4 ± 4.4	82.8 ± 4.8

diminished the glycogenolytic response to PAF, it was without effect on the response to zymosan sup (Table 1).

The glycogenolytic response and changes in oxygen consumption induced by zymosan sup were completely inhibited by NDGA, a lipoxygenase inhibitor (Fig. 3). Moreover, the responses were also sensitive to ONO-1078, an LTD₄ receptor antagonist (Fig. 3), indicating LTD₄-mediated events. NDGA also inhibited the decrease in oxygen consumption induced by non-boiled zymosan, and modulated the pattern of glucose release (Fig. 1). The response to zymosan pellet was not affected by NDGA; basal and maximal glucose production were 19.1 ± 2.52 µmol/h per g of liver and 52.3 ± 4.43 µmol/h per g of liver ($n = 3$) respectively.

When a cyclo-oxygenase inhibitor, ibuprofen at 20 µM, was used, the glycogenolytic response induced by the zymosan pellet was significantly suppressed, while the glycogenolytic response to non-boiled zymosan or zymosan sup was not (Table 2). The amount of PGD₂ in the perfusate was enhanced by infusion of zymosan pellet or non-boiled zymosan, but not by zymosan sup or mannan (Table 3).

Next, in order to determine whether a lack of mannan, as an opsonin, retards the glycogenolytic response to the zymosan pellet, we opsonized the zymosan pellet with rat serum. Serum treatment of the zymosan pellet caused a rapid increase in glucose production and oxygen consumption (Fig. 2). When rat serum was preheated at 56 °C for 30 min, the changes induced by the serum were almost the same as those with zymosan pellet

Table 2. Effects of ibuprofen on the glycogenolytic response to zymosan, zymosan sup, zymosan pellet and serum-treated zymosan pellet

Infusion of stimulus was performed as described in the legends to Figs. 1–3. When ibuprofen (20 µM) was used, it was infused from 15 min before stimulation. Basal (30 min after start of perfusion with or without ibuprofen) and maximal glucose production induced by the stimulus are demonstrated as means ± S.E.M. In the case of zymosan sup, both peaks of glucose release are indicated (see Fig. 3). Significant differences between before and after infusion are expressed by: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, and those between control and ibuprofen treatment are indicated by † $P < 0.05$; †† $P < 0.01$; ††† $P < 0.001$.

	Glucose production (µmol/h per g of liver)	
	Basal	Maximal
Zymosan pellet ($n = 8$)	15.3 ± 0.9	48.5 ± 3.8***
+ ibuprofen ($n = 5$)	12.2 ± 0.6	23.7 ± 2.1***†††
Serum-treated zymosan pellet ($n = 6$)	15.6 ± 1.6	65.8 ± 8.1***
+ ibuprofen ($n = 5$)	12.3 ± 1.4	30.5 ± 4.8***††
Zymosan ($n = 8$)	15.9 ± 2.1	71.6 ± 6.9***
+ ibuprofen ($n = 4$)	12.3 ± 0.7	86.1 ± 19.1**
Zymosan sup ($n = 8$)	17.7 ± 1.10	38.4 ± 5.2**/47.6 ± 3.3***
+ ibuprofen ($n = 4$)	15.9 ± 0.59	26.8 ± 3.0*/40.7 ± 5.4**

Table 3. Efflux of PGD₂, PGE₂, PGF_{2α} and 6-oxo-PGF_{1α} into the perfusate after various types of stimulation

The perfusates were collected before and during stimulation, as shown in Figs. 1–4 and Table 2. PGD₂ in the perfusate (a), and PGD₂, PGE₂, PGF_{2α} and 6-oxo-PGF_{1α} (b) were assayed as described in the Experimental section. Significant differences between before and after infusion are expressed by **P* < 0.05; ***P* < 0.01; ****P* < 0.001, and those between control and ibuprofen treatment are indicated by †*P* < 0.05; ††*P* < 0.01; †††*P* < 0.001.

(a)		PGD ₂ production (pmol/h per g of liver)			
Treatment	Time after infusion (min) ... Before	0.5–1.5	1.5–2.5	3.5–4.5	
Zymosan (<i>n</i> = 4)	12.6 ± 7.8	10.4 ± 5.8	97.1 ± 37.7***	364.7 ± 95.7***	
Zymosan sup (<i>n</i> = 4)	5.68 ± 0.28	–	6.9 ± 1.94	6.6 ± 0.26	
Mannan (<i>n</i> = 3)	26.9 ± 9.0	–	14.6 ± 5.7	12.7 ± 4.2	
(b)		PG production (pmol/h per g of liver)			
Treatment	Time after infusion (min) ...	Before	2–3	3.5–4.5	
Zymosan pellet (<i>n</i> = 4)	PGD ₂	22.6 ± 6.9	141.9 ± 25.2***	242.2 ± 37.2***	
	PGE ₂	12.5 ± 4.6	36.1 ± 7.2	59.5 ± 11.0*	
	PGF _{2α}	9.72 ± 1.3	75.8 ± 1.0***	128.5 ± 9.2***	
	6-oxo-PGF _{1α}	4.31 ± 1.44	6.08 ± 2.4	19.2 ± 9.8	
Zymosan pellet + ibuprofen (20 μM) (<i>n</i> = 4)	PGD ₂	7.90 ± 0.6	33.5 ± 11.2*†	24.9 ± 1.7***††	
	PGE ₂	20.7 ± 6.8	20.9 ± 5.2	25.3 ± 2.7†	
	PGF _{2α}	17.1 ± 1.5	32.6 ± 4.4*†††	48.2 ± 6.7*†††	
	6-oxo-PGF _{1α}	4.60 ± 1.3	4.82 ± 1.49	4.99 ± 1.17	
Serum-treated zymosan pellet (<i>n</i> = 4)	PGD ₂	25.7 ± 5.9	772.0 ± 11.6***	801.0 ± 33.7***	
	PGE ₂	17.5 ± 1.8	102.1 ± 7.9***	127.3 ± 7.0***	
	PGF _{2α}	12.9 ± 3.1	248.0 ± 31.3**	326.1 ± 14.0***	
	6-oxo-PGF _{1α}	9.25 ± 1.26	31.6 ± 2.7**	37.1 ± 0.28***	
Serum-treated zymosan pellet + ibuprofen (20 μM) (<i>n</i> = 4)	PGD ₂	15.8 ± 3.7	122.6 ± 59.0†††	124.4 ± 40.1†††	
	PGE ₂	14.7 ± 2.8	27.5 ± 5.1††	28.5 ± 4.4†††	
	PGF _{2α}	19.0 ± 3.3	63.2 ± 12.5*††	76.4 ± 18.9*†††	
	6-oxo-PGF _{1α}	4.70 ± 0.95	6.60 ± 1.48††	5.81 ± 1.32†††	

(Fig. 2). Serum alone had no effects on glucose production: the change in glucose production was 4.32 ± 2.12 μmol/h per g of liver (*n* = 3). These results indicate that complement facilitates the recognition of particles and enhances the production of glycogenolytic mediators as compared with the effects of zymosan pellet alone.

To determine which types of PGs are involved in zymosan pellet-induced or serum-treated zymosan pellet-induced glycogenolysis, PGF_{2α}, PGE₂, 6-oxo-PGF_{1α} and PGD₂ were measured. PGs detected in the perfusate increased significantly with infusion of zymosan pellet or serum-treated zymosan pellet, and opsonization by serum enhanced release of PGs as compared with zymosan pellet (Table 3). Stimulation of PG production was inhibited by ibuprofen (Table 3). When the relationships between glucose production and PGs in the perfusate were investigated, it was found that PGD₂ (*r* = 0.832), PGF_{2α} (*r* = 0.872) and PGE₂ (*r* = 0.752) were highly correlated with glucose production, while 6-oxo-PGF_{1α} (*r* = 0.349) showed no correlation. These results suggested that PGD₂, PGF_{2α} and PGE₂ are glycogenolytic mediators produced by the particles.

DISCUSSION

In the present study, we have clearly shown that different preparations of zymosan stimulate glycogenolysis in perfused rat liver (Figs. 1–3). After the zymosan was boiled, the resulting soluble fraction (zymosan sup) stimulated glycogenolysis in the same way as did mannan (Figs. 3 and 4; Table 1). Zymosan sup induced profound suppression of oxygen consumption and a biphasic increment of glucose production. This response was

thought to be independent of PAF and its receptor, as neither the glycogenolytic response nor the changes in oxygen consumption were blocked by PAF or isoprenaline (Table 1), while the PAF receptor was down-regulated by them [13–16]. However, the decreased glycogenolytic response observed in these experiments may imply the presence of similar post-receptor signal transduction pathways. PAF is capable of stimulating the release of various injurious products, including LTs, from peripheral macrophages [9,27]. As shown in Fig. 3, NDGA, an inhibitor of LT synthesis and an LTD₄ receptor antagonist, abolished the glycogenolytic response and the changes in oxygen consumption induced by zymosan sup. Moreover, (non-boiled) zymosan induced peptide-LT synthesis in resident mouse peritoneal macrophage [28]. These results suggest that mannose receptor activation, without phagocytosis, causes peptide-LT production.

The mechanism of induction of glycogenolysis by peptide-LT is still unclear, as direct actions of peptide-LTs on glycogenolysis in hepatocytes have not been reported. Infusion of exogenous peptide-LTs causes haemodynamic alternation and glycogenolysis in perfused rat livers [29–32]. Häussinger and co-workers [31,32] reported that peptide-LT began to stimulate glycogenolysis during infusion of LT, while Iwai & Jungermann [29,30] did not observe an increase in glucose output during infusion [29,30]. However, both groups reported that glucose output was potentiated after cessation of the infusion. Vasoconstriction induced by peptide-LT causes the restriction of perfusion of some of the parenchymal area [30], which indicates a restriction in oxygen supply. Anoxia is a trigger for glycogenolysis [33]. Thus it is likely that enhanced glycogenolysis after the end of peptide-LT infusion may be due to suppression of oxygen supply

in a certain region of the liver. Endogenously peptide-LT production stimulated by mannan or zymosan sup, as discussed above, induced inhibition of oxygen consumption (Figs. 3 and 4; Table 1) which was similar to that induced by exogenous peptide-LT [31]. Biphasic glucose production in the perfused liver was also observed after administration of high doses of PAF [15] or thromboxane analogue [31], along with haemodynamic changes. Therefore biphasic glucose production induced by mannan or zymosan sup might be due to vasocontraction caused by peptide-LT.

Carbohydrate receptors are present on various liver cells [9,10,24–26,34]. Small peptides which possess mannose residues are removed from the circulation mainly via uptake by endothelial cells in the liver [10,24]. On the other hand, Kupffer cells are unable to internalize small carbohydrate-terminal molecules such as asialofetuin [35,36]. In the present study, infusion of ovalbumin or mannose hardly stimulated glycogenolysis. Rat Kupffer cells have been demonstrated *in vitro* to be hepatic sites of endogenous LT production [37–39]; thus Kupffer cells might be responsible for mannan-induced glycogenolysis.

Homologous desensitization of mannan and zymosan sup (Fig. 4 and Table 1) indicates that mannose receptors on resident macrophages are down-regulated by their ligand. Mannose receptors on peripheral macrophages are specifically down-regulated by macrophage-activating agents [40], such as endotoxin, phorbol ester [41] and bacillus Calmette–Guerin (BCG) [42]. Activation of macrophage mannose receptors enhances the secretion of protease, and one possible mechanism of down-regulation is proteolysis of mannose receptors [43].

The particle (zymosan pellet) fraction, which lacked mannan (Table 1), stimulated glycogenolysis, and opsonization of the particles enhanced the glycogenolytic response (Fig. 2). The glycogenolytic responses to particles were sensitive to a cyclo-oxygenase inhibitor (Table 2), and were highly correlated with prostanoid release. These results suggest the involvement of prostanoid synthesis in particle-induced glycogenolysis.

Of these prostanoids, $\text{PGF}_{2\alpha}$ showed the best correlation, while PGD_2 was the major PG produced in perfused rat liver (Table 3). PGD_2 and PGE_2 stimulate glycogenolysis [3,4] and $\text{PGF}_{2\alpha}$ activates glycogen phosphorylase in rat hepatocytes [44]. However, $\text{PGF}_{2\alpha}$ is a more powerful activator of glycogenolysis than PGD_2 and PGE_2 at similar concentrations in perfused liver [45,46]. Thus the correlation between $\text{PGF}_{2\alpha}$ in the perfusate and glucose production was the strongest. In contrast, the concentration of PGD_2 in the perfusate was 2–3-fold higher than that of $\text{PGF}_{2\alpha}$. The concentration of PGE_2 was about half of that of $\text{PGF}_{2\alpha}$ (Table 3). In this regard, both PGD_2 and $\text{PGF}_{2\alpha}$ may play a major role in the process of glycogenolysis and PGE_2 may play a minor role, although the actual contribution of each PG is obscure.

Amounts of PGD_2 , PGE_2 and $\text{PGF}_{2\alpha}$ release induced by serum-treated zymosan pellet were three to five times greater than those induced by zymosan pellet (Table 3). One possible explanation is that phagocytosis of particles is accelerated by the presence of complement, and that this stimulates prostanoid synthesis in proportion to the uptake of particles. Another possibility is that activation of complement receptor also induces arachidonic acid cascade reactions. Although we could not identify which components of complement were involved in opsonization, complement C3b is known to act as a major opsonin [47]. C3b receptors are present only on Kupffer cells in the liver [10–12]. Activation of various receptors of the Kupffer cell surface activates the cyclo-oxygenase pathway [9]. Thus both of the possibilities discussed are likely to be involved in the enhancement of prostanoid synthesis.

Non-boiled zymosan induced glycogenolysis without any lag

period (Fig. 1), while a lag period appeared when the glycogenolytic response to mannan was blocked (Fig. 4 and Table 1), which implied that the mannose receptor might be down-regulated. It is possible that mannose acts as an opsonin, like complement (Fig. 2), and that the interaction of mannose-bearing particles (non-boiled zymosan) and liver cells through mannose receptors is accelerated and increased compared with that with the zymosan pellet (Figs. 1 and 2; Table 2). Moreover, suppression of LT synthesis by NDGA inhibited the decrease in oxygen consumption induced by non-boiled zymosan (Fig. 1). Therefore mannose-bearing-particle-induced glycogenolysis is thought to occur by a combination of mannose receptor activation and particle phagocytosis. Release of PGD_2 into the perfusate was enhanced after infusion of non-boiled zymosan, as with zymosan pellet (Table 3). However, the rise in PGD_2 release was slightly delayed compared with the rise in glucose production (Fig. 1 and Table 3), and the glycogenolytic response was quite insensitive to a cyclo-oxygenase inhibitor (Table 2). Other unidentified factors may be involved in mannose-bearing-particle-mediated glycogenolysis, and further work is necessary to clarify this mechanism.

We have clearly demonstrated that zymosan particles, irrespective of the presence of complement, induced glycogenolysis through the production of prostanoids in the perfused rat liver. The presence of complement enhanced the glycogenolytic response to the particles by potentiating prostanoid synthesis, and stimulation of mannose receptors caused glycogenolysis via peptide-LT production. These results support the notion that non-parenchymal cells modulate liver metabolism under various conditions [1,2].

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