Kupffer Cell Activity Is Involved in the Hepatoprotective Effect of Dietary Oligofructose in Rats with Endotoxic Shock

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ABSTRACT In the present study, we tested the hypothesis that dietary oligofructose (FOS) can modulate both the response to an endotoxic shock induced by lipopolysaccharide (LPS) administration and the activity of resident hepatic macrophages, i.e., Kupffer cells. Male Wistar rats (n = 5–9 per group) were fed a standard diet or a diet supplemented with 10 g/100 g FOS for 3 wk. LPS (10 mg/kg) or saline were injected i.p. after dietary treatment. After LPS injection, serum levels of tumor necrosis factor (TNF)-α, a proinflammatory cytokine, and prostaglandin E_2 (PGE_2), an immunosuppressive mediator, were higher in FOS-treated rats than in control rats. Alanine aminotransferase (ALT) activity was ~50% lower than in controls 24 h after LPS administration in FOS-treated rats, suggesting less hepatic injury; this was confirmed through histological analysis. FOS treatment increased the number of large phagocytic Kupffer cells, as assessed by histological examination of the liver after colloidal carbon injection into the portal vein. Precision-cut liver slices (PCLS) from FOS-treated rats released more TNF-α and PGE_2 into the incubation medium than PCLS from control rats, independently of LPS challenge in vitro. This would suggest that the higher Kupffer cell phagocytic activity and secretion capacity due to FOS supplementation improve LPS clearance in liver tissue and reduce hepatocyte alterations. This study supports the hypothesis that oligofructose might decrease liver tissue injury after endotoxic shock and sepsis. J. Nutr. 134: 1124–1129, 2004.

KEY WORDS: • *Kupffer cells* • *sepsis* • *oligofructose* • *precision-cut liver slices* • *lipopolysaccharide*

Modulation of immune function by nutrients is an emerging research area in the field of nutrition. The bacterial population throughout the gastrointestinal tract (GIT)[2] could constitute an important determinant of disease resistance/susceptibility, i.e., through interactions with nutrients (1). Fermentable dietary fibers, which modulate intestinal flora composition, were reported to increase the number of cecal and colonic macrophages as well as the number of Peyer's patches, and modulate immune parameters in gut-associated lymphoid tissue (2). Interesting studies showed that dietary supplementation with fermentable fructooligosaccharides not only protected animals against enteric infection, but also promoted resistance toward systemic infection induced by i.p. injection with *Salmonella typhimurium* or *Listeria monocytogenes* (3). The link between events occurring in the colon and the protection against systemic infection remains hypothetical. The liver occupies a critical place because it is linked directly to the GIT through the portal venous system. The high local blood flow results in a high rate of interaction, not only with nutrients or nutrient metabolites, but also with foreign antigens. This latter property assigns a central immunologic function to the liver (4). Kupffer cells, localized mainly in the periportal area in hepatic sinusoids, represent 80–90% of fixed macrophages of the whole body. Kupffer cells thus constitute the first macrophage population outside the gut to come into contact with bacteria, endotoxin [lipopolysaccharide (LPS)] and microbial debris (5). Consequently, Kupffer cells play a major role in clearing circulating LPS from the blood (6,7). Several reports suggested that intestinal bacterial overgrowth can modify Kupffer cell response to septic stimuli. For example, Kupffer cells from germ-free rats are reduced in number and fail to respond to LPS in Kupffer cell:hepatocyte coculture (8). In addition, mice, which have a gut flora monoassociated with *Lactobacillus acidophilus*, exhibit twice the number of Kupffer cells compared with germ-free controls (9).

When gram-negative bacteria, normally restricted to the GIT, invade the bloodstream, circulatory collapse, multiple organ failure, and ultimately death can occur (10). Gram-negative sepsis remains an important cause of morbidity and mortality in intensive care units despite recent advances in critical care (11). The arrays of pathophysiologic features that accompany gram-negative bacterial sepsis appear to be qualitatively similar to those encountered after LPS insult (11–13). LPS, a component of the gram-negative bacterial cell wall, is responsible for initiating a series of a highly complex cascading events leading to damage in multiple organs, including the liver and the lung. Results obtained by Suzuki et al. (14) indicate that i.p. injection of LPS at the high dose of 10 mg/kg into rats induced a condition compatible with septic multiple

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[2] Abbreviations used: ALT, alanine aminotransferase; AST aspartate aminotransferase; FOS, oligofructose; +FOS, oligofructose-treated rats; −FOS, control rats; GIT, gastrointestinal tract; LPS, lipopolysaccharide; +LPS, LPS challenge; −LPS, saline injection; PCLS, precision-cut liver slice; PGE_2, prostaglandin E_2; TNF-α, tumor necrosis factor-α.

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organ dysfunction syndrome as defined by the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference (15). For example, in that rat model of septic syndrome, serum alanine aminotransferase (ALT) activity increased and lung injury was observed 12 h after LPS administration (14). Several mediators are secreted by macrophages upon LPS stimulation (16,17). Some of them are cytokines that contribute to multiple organ failure, notably, TNF-α. Intravenous injection of TNF-α induces a shock syndrome similar to that caused by LPS (10). Macrophages are also able to secrete immunosuppressive mediators, such as interleukin 10 or PGE_2. The latter was shown to protect against LPS-induced liver injury by down-regulating the production of proinflammatory cytokines (10,18).

Oligofructose (FOS), an inulin-derived oligosaccharide, is a carbohydrate that escapes digestion in the upper GIT but is fermented extensively in the ceco-colon mainly by bifidobacteria. Because they promote the growth of lactic acid bacteria, they led to the concept of prebiotics (19). The present study aimed to determine whether a prebiotic such as FOS might help rats face the endotoxic shock induced by an injection of high doses of LPS, which are known to produce liver injury. We analyzed general variables that are typically altered after LPS administration such as lung, spleen and liver histology, serum levels of transaminases and inflammatory mediators, body temperature, glycemia and triglyceridemia (14–17,20–22). We determined whether FOS is able to modulate Kupffer cell activities, such as their phagocytic function after colloidal carbon injection and their capacity to secrete inflammatory mediators in vitro. For the in vitro study, we used precision-cut liver slices (PCLS) in culture. This original model allows us to maintain an intact liver architecture as well as cellular heterogeneity and interactions. We showed recently that PCLS released immune mediators such as TNF-α and PGE_2 into the medium during at least 20 h of incubation in basal conditions or in response to inflammatory stimulus such as LPS. Moreover inhibition of Kupffer cells by injection of $GdCl_3$ in rats, 1 d before PCLS preparation, strongly reduced the production of those mediators, suggesting that Kupffer cell function was retained inside PCLS upon incubation (23,24).

MATERIALS AND METHODS

Chemicals. Chemicals of the purest grade available were purchased from Sigma or Roche Diagnostics Belgium. William's medium E, fetal bovine serum, L-glutamine, penicillin and streptomycin were purchased from Invitrogen.

Animals and diet. Male Wistar rats weighing 100–125 g (Harlan) were housed in individual cages in a room with temperature control and an automatic 12-h light:dark cycle. After an acclimatation period of 5 d before the experiment, control rats (−FOS) were fed a powdered AO4 standard diet (UAR, Villemoisson-sur-Orge, France), whereas oligofructose treated-rats (+FOS) received the same diet containing 10 g/100 g Raftilose P_{95} (Orafti). The AO4 standard diet contained the following (g/100g dry diet): 19 protein; 70 total carbohydrates; 3.2 lipids; 7.3 minerals and vitamins (25). Raftilose P_{95} is a mixture of glucosyl-$(fructosyl)_n$-fructose and $(fructosyl)_m$-fructose with an average degree of polymerization of 5. The energy value for the control and FOS diet was 13.86 and 13.08 kJ/g, respectively. Body weight and food intake were monitored twice each week. After 3 wk of treatment, LPS from *Escherichia coli* 0127:B8 (10 mg/kg) or NaCl (9 g/L) were injected i.p. Food was withdrawn after LPS or saline administration. Blood was collected from the tail vein 0, 2, 4, 6 and 8 h after LPS or saline injection and rats were killed under pentobarbital (Nembutal, 60 mg/kg body) anesthesia 24 h after LPS or saline administration. Blood was collected from the vena cava and stored at −80°C for further analysis. Liver, lung and spleen specimens were fixed in formalin, embedded in paraffin and sections were stained with hematoxylin-eosin for histological analysis. All rats received care in compliance with institution's guidelines from the National Academy of Sciences (26). Experiments were approved by the Ethics Committee of the University Catholic of Louvain, Brussels, Belgium.

Rectal temperature evaluation. Rectal temperature was recorded using thermometer (Homeothermic Blanket Control Unit, Harvard Apparatus). All measurements were made at an ambient temperature of 22 ± 2°C.

Colloidal carbon uptake by the liver. Phagocytic activity of Kupffer cells toward colloidal carbon was assessed on tissue slices as described previously (27). Briefly, colloidal carbon (Pelikan no. 17 dissolved in saline 1:10; 1.5 mL/kg) was injected into the portal vein 20 min before liver sampling for further histological study. Semiquantification of large Kupffer cells (≥10 μm length) was performed under microscopic analysis on 5 different liver sections (3 fields of 10 mm^2 per liver section) for each rat.

Biochemical variables. ALT and aspartate aminotransferase (AST) activities as well as glucose and triglycerides concentrations were measured in the serum by standardized enzymatic procedures using kits from Elitech (ALT/GPT SL, AST/GOT SL, glucose PAP SL and triglycerides ESPAS SL, respectively).

Culture of precision-cut liver slices (PCLS). Three weeks after −FOS and +FOS treatments, in situ perfusion of the liver of rats was performed with ice-cold Krebs-Ringer solution before liver removal. Rat surgical procedures were carried out under pentobarbital (Nembutal, 60 mg/kg body) anesthesia. Tissue cores (diameter 10 mm) were prepared from the freshly excised liver and PCLS (~220 μm thick) were rapidly prepared in oxygenated ice-cold Krebs-Ringer buffer using a Krumdiek slicer according to a previously described procedure (23,24,28). After 30 min of preincubation at 4°C followed by 1 h at 37°C in William's E medium [supplemented with penicillin (100 kIU/L), streptomycin (100 mg/L), glutamine (2 mmol/L) insulin (100 nmol/L) and fetal bovine serum (100 mL/L)], PCLS were

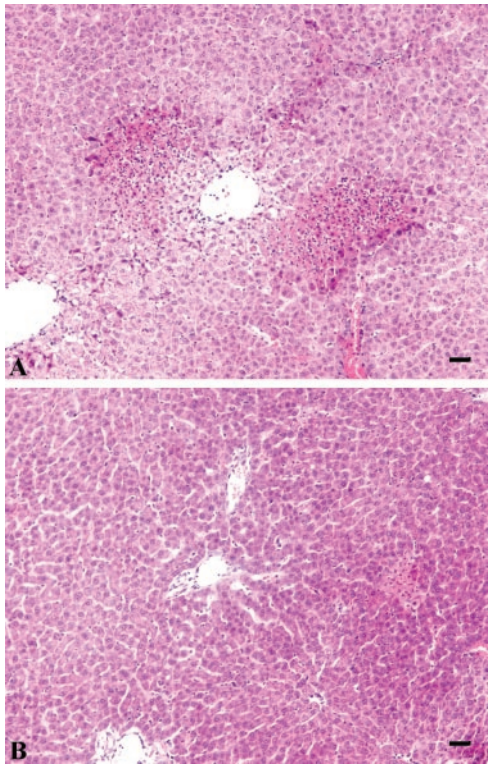

FIGURE 1 Histological analysis of liver section 24 h after LPS administration to rats fed a control diet (*A*) or a diet supplemented with FOS (*B*). Hematoxylin-eosin staining; bar = 50 μm.

transferred into fresh medium (without fetal bovine serum, with 1 g/L bovine serum albumin) and incubated for 20 h in the presence or absence of LPS (100 μg/L). The vials, containing a maximum of 4 slices (2 mL medium slice), were saturated with 95% O_2:5% CO_2 and placed in a shaking water bath at 37°C for 20 h.

PGE_2 and TNF-α assays. PGE_2 and TNF-α concentrations were measured in frozen aliquots of serum or incubation medium by immunoassay kits (PGE_2 Immunoassay, DE0100 and Quantikine rat TNF-α immunoassay, RTA00 from R&D Systems).

Statistical analysis. All measurements were made in duplicate. Values are presented as means ± SEM. All statistical analyses were performed with the SPSS statistical program for Windows system (SPSS). When variances were heterogeneous, logarithmic transformations of individual values were used before statistical analysis. Student's *t* test was applied to compare the effect of FOS supplementation vs. control diet, independently of inflammatory stimulation (by LPS). To compare the time course effect of FOS feeding under +LPS conditions on ALT, AST, body temperature, glycemia and TNF-α, two-way ANOVA was performed. Two-way ANOVA with the factors FOS (+FOS and −FOS) and the LPS (+LPS and −LPS) was performed to analyze serum triglycerides and PGE_2 at each time as well as mediator secretion in vitro. Differences were considered significant at $P < 0.05$.

RESULTS

Body weight measured just before LPS challenge was lower in groups treated with FOS (268 ± 4 g, $n = 13$) than in controls (291 ± 7 g, $n = 14$; $P < 0.05$). Weekly recorded food intake tended to be lower ($P = 0.23$) in +FOS rats (442 ± 17 g, $n = 13$) than in −FOS rats (416 ± 11 g, $n = 12$) throughout the experiment. The relative liver weight (g/100 g body) was not modified by FOS and/or LPS treatments (data not shown). The FOS diet increased the cecal wall weight 60% independently of LPS treatment (1.41 ± 0.13 g, $n = 5$ for +FOS/−LPS vs. 0.87 ± 0.05 g, $n = 5$ for −FOS/−LPS; *$P < 0.05$). Neither LPS nor FOS treatments affected spleen weight (data not shown).

Analysis of systemic response to LPS

Some rats died during the dark period in +LPS groups, independently of FOS treatment (2 of 9 in each group, +FOS and −FOS rats).

Organ integrity. We assessed organ injury by histological examination 24 h after LPS administration. Marked lung congestion, edema, alveolar septal thickening and an influx of inflammatory cells were observed 24 h after LPS administration. Moreover, splenic red pulp was strongly congested, whereas necrotic foci with karyorrhectic cells appeared in white pulp. FOS treatment did not modify these histological alterations in lung or spleen (data not shown). LPS caused necrotic foci with leukocyte infiltration in the liver of some −FOS rats (4 of 7 −FOS/+LPS rats) (**Fig. 1***A*). When these necrotic foci were present in the liver of +FOS rats (2 of 7 +FOS/+LPS rats), they were smaller and less numerous (Fig. 1*B*).

Serum enzymes, glycemia and temperature. AST and ALT activities were analyzed in the serum of +LPS rats; their levels increased with time after LPS challenge independently of FOS treatment. AST activity was not modified by dietary FOS treatment (data not shown). However, ALT activity was significantly lower 24 h after LPS injection in rats previously fed FOS (**Fig. 2***A*). Rats responded to LPS injection with a transient hypothermia 2 h after injection (Fig. 2*B*); this response was more pronounced in +FOS rats than in controls. Moreover, in +FOS/+LPS rats, body temperature had not returned to initial values 24 h after LPS challenge. Two hours after injection, LPS also induced a transient hyperglycemia followed by a period of hypoglycemia (Fig. 2*C*). After 24 h, only +FOS rats had not returned to glycemia values measured at time 0 h.

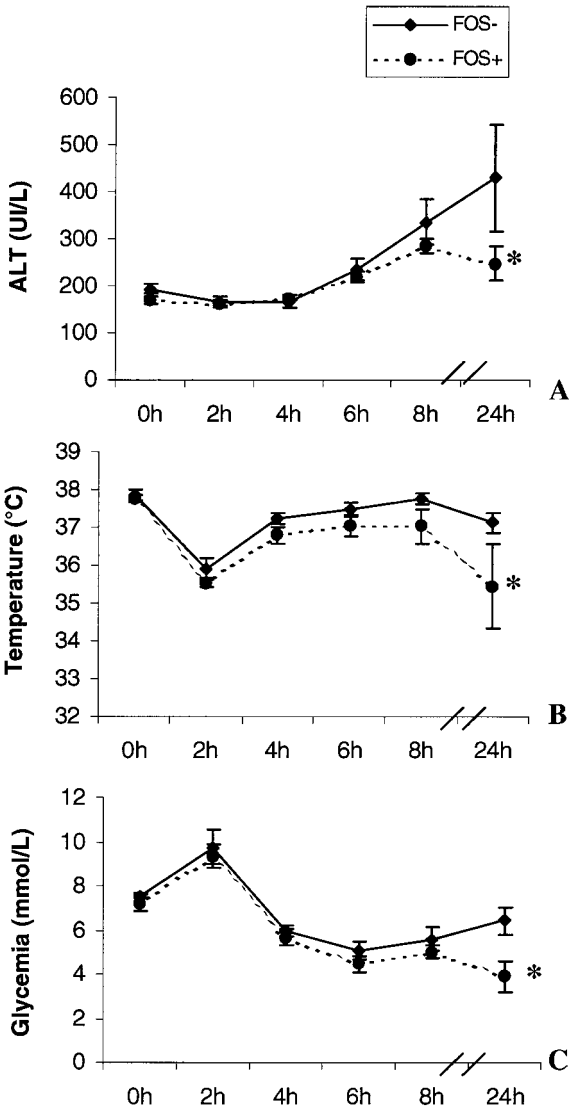

FIGURE 2 ALT activity in the serum (*A*), rectal temperature (*B*) and glycemia (*C*) after LPS challenge in rats fed a control diet (−FOS) or a diet supplemented with FOS (+FOS). Values are means ± SEM ($n \geq 5$). *Significant effect of FOS treatment ($P < 0.05$, two-way ANOVA).

Serum triglycerides. Triglyceride concentration increased during the first hours and remained elevated until 8 h after LPS injection (**Fig. 3**). The FOS diet decreased triglyceridemia compared with −FOS rats, independently of LPS challenge. However, serum triglycerides were higher in +FOS/+LPS (1.22 ± 0.23 mmol/L) rats than in −FOS/+LPS rats (0.90 ± 0.11 mmol/L) at 24 h.

Serum TNF-α and PGE_2. TNF-α was detectable in the serum only after LPS injection. The concentration of TNF-α increased strongly 2 h after LPS administration and then decreased progressively until 24 h (**Table 1**). TNF-α concentration in the serum of rats previously treated with FOS was slightly but significantly higher than in controls. LPS administration raised PGE_2 levels in the serum after 8 h (**Fig. 4***A*). Basal levels, as well as LPS-induced increases in serum PGE_2, were higher in +FOS rats than in controls. Contrasting effects

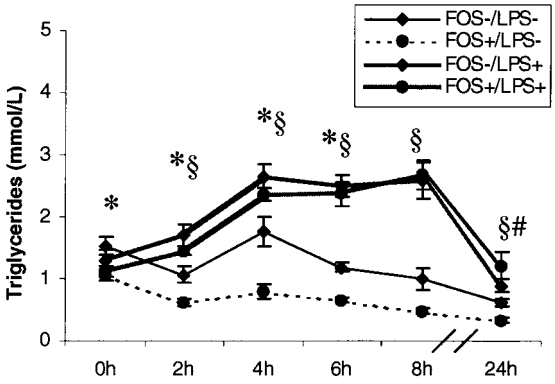

FIGURE 3 Serum triglycerides after LPS challenge (+LPS) vs. saline injection (−LPS) in rats fed a control diet (−FOS) or a diet supplemented with FOS (+FOS). Values are means ± SEM ($n \geq 5$). *Significant effect of FOS treatment; §significant effect of LPS treatment; #significant interaction between FOS and LPS treatments ($P < 0.05$, two-way ANOVA performed at each time).

of LPS on serum PGE_2 24 h after the LPS vs. saline challenge were observed, i.e., the PGE_2 level was lower in −FOS/+LPS rats than in −FOS/−LPS rats, whereas it was higher in +FOS/+LPS rats than in +FOS/−LPS rats (Fig. 4*B*).

Secretion of inflammatory mediators by PCLS in vitro

Unstimulated PCLS in culture released large amounts of TNF-α and PGE_2 into the medium (**Fig. 5**). The presence of LPS at 100 μg/L in the medium increased TNF-α concentration without affecting PGE_2 secretion. PCLS from rats fed FOS for 3 wk before PCLS preparation secreted more PGE_2 and TNF-α into the medium, independently of the presence of LPS in the culture medium.

Analysis of colloidal carbon uptake by the liver

Kupffer cells that had taken up colloidal carbon appeared in control rats as large and irregular cells localized mainly in the periportal zone (**Fig. 6***A*). Supplementation of FOS in the diet for 3 wk increased the number and the size of phagocytic cells in the liver tissue (Fig. 6*B*). Quantitative analysis revealed that the number of large Kupffer cells/yield (see Materials and Methods for yield definition) near the portal space was higher in rats fed a diet supplemented with FOS (18.0 ± 0.4, $n = 4$) compared with control rats (16.0 ± 0.3, $n = 4$; $P < 0.05$).

TABLE 1

TNF-α release in serum after LPS challenge in rats fed a control diet or a diet supplemented with FOS[1,2]

Time, *h*	Control	FOS
	ng/L	
2	11389 ± 1845	16412 ± 4004
4	922 ± 247	981 ± 226
6	373 ± 51	542 ± 62
8	264 ± 50	328 ± 34
24	18 ± 1	30 ± 5

[1] Values are means ± SEM, $n = 8$ (control) and 9 (FOS).
[2] Significant effect of FOS treatment ($P < 0.05$, two-way ANOVA performed on logarithmic values).

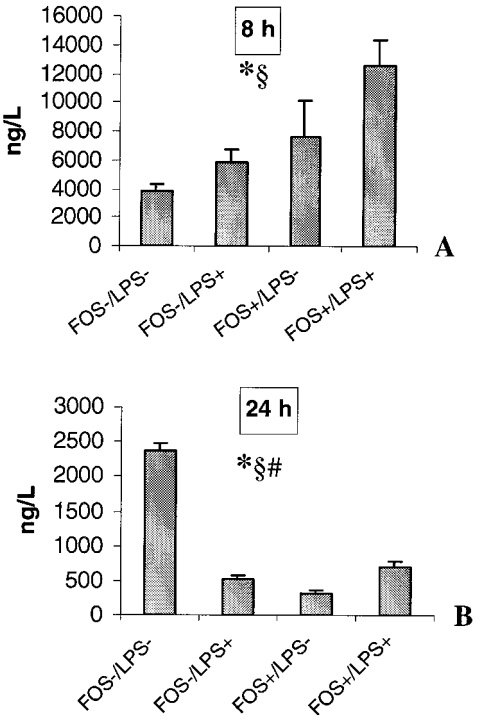

FIGURE 4 PGE_2 concentrations in the serum 8 and 24 h after LPS injection (+LPS) vs. saline injection (−LPS) in rats fed a control diet (−FOS) or a diet supplemented with FOS (+FOS). Values are means ± SEM ($n \geq 5$). *Significant effect of FOS treatment; §significant effect of LPS treatment; #significant interaction between FOS and LPS treatments ($P < 0.05$, two-way ANOVA performed on logarithmic values).

DISCUSSION

The first observation of an effect of dietary fructans on systemic infection was a decreased mortality due to systemic pathogen injection in mice fed a diet containing FOS (10 g/100 g) (3). In the present study, there was no effect on mortality rate due to dietary treatment because 2 of 9 rats in each experimental group died a few hours after LPS injection. However, rats that were followed up after LPS injection had confirmed biological metabolic disturbances, which may have been modulated by FOS treatment, and may have participated in the hepatoprotective effect of FOS, discussed below. A correlation between TNF-α concentration in serum and hypothermia after LPS administration was suggested (20). Results obtained in this study support the idea that peripheral TNF per se, or other factors that are induced by TNF, provide the cryogenic signals that are involved in the maintenance of the hypothermia phase that occurs in response to LPS. In both groups, the progressive decrease in TNF-α occurring within 2 to 8 h was accompanied by the restoration of body temperature. The glycemic profile after LPS challenge, observed in the present study, agrees with the one described by Bosh et al. (21). LPS caused a transient hyperglycemia, due in part to increased glycogenolysis in the liver (29), followed by a progressive decrease in glycemia, resulting from glucose utilization by peripheral tissue, and mainly by immune cells (21,30). Glycemia was lower in FOS-fed rats than controls 24 h after LPS injection.

Triglyceridemia due to FOS treatment was significantly lower in rats that did not receive LPS injection. This phenomenon has been well described and may be due to decreased hepatic lipogenesis and triglyceride-VLDL secretion by the

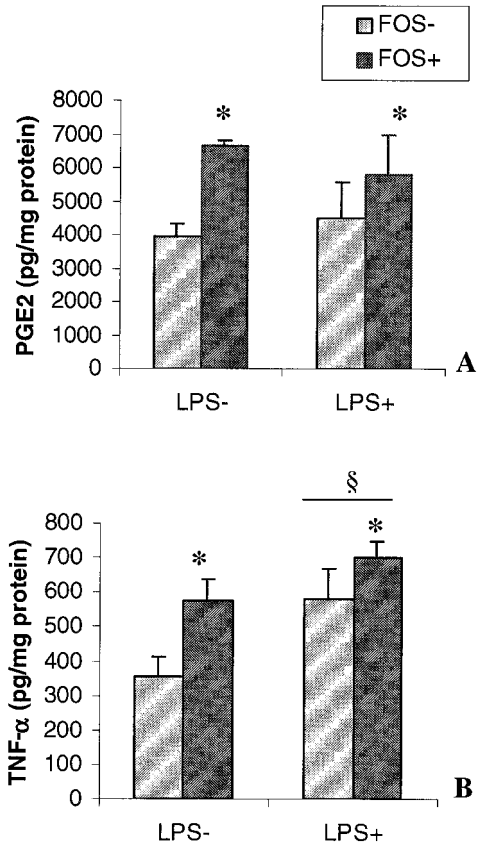

FIGURE 5 PGE_2 (*A*) and TNF-α (*B*) secretion by PCLS obtained from rats fed a control diet (−FOS) or a diet supplemented with FOS (+FOS). PCLS were incubated for 20 h in the presence or absence of LPS. Values are means ± SEM ($n \geq 3$). *Significant effect of FOS treatment; §significant effect of LPS treatment in vitro ($P < 0.05$, two-way ANOVA performed on logarithmic values).

liver (31,32). The administration of LPS at a high dose, mimicking infections, produces hypertriglyceridemia by decreasing the activity of lipoprotein lipase, thus slowing the clearance of triglyceride-rich lipoproteins (22). In the present study, triglyceridemia progressively increased within 4 h and was sustained at similar higher levels in both groups until 8 h after LPS challenge, reaching higher levels in FOS-fed rats after 24 h.

High doses of endotoxin cause liver injury that frequently leads to multiple organ failure but the mechanism involved in this pathology is not completely understood. The major benefit of FOS shown here was the improvement in hepatic lesions, i.e., the hepatic necrosis confirmed by ALT levels in the serum was reduced by FOS supplementation to the diet. However, we suggest that some measures that were modified by LPS, which were not reversed, and were even promoted by FOS supplementation, could play a role in the "protection" against infection. This was the case for the higher triglyceridemia and the lower glycemia in FOS-fed rats vs. controls 24 h after LPS. On the one hand, triglyceride-rich lipoproteins could play a role as "LPS" scavenger and protect against endotoxin-induced death in mice (33). On the other hand, the lower glycemia after LPS could also be construed as a "positive" outcome because it may reflect higher metabolic and phagocytic activity of Kupffer cells, which use glucose as a major energy source (30). Despite those putatively positive effects, some markers, such as a higher TNF-α and the subsequent lower body temperature observed in FOS-fed rats, attest to the limitation of positive effects of FOS in our protocol because TNF-α can take part in extrahepatic tissue alterations after LPS.

Several reports demonstrated that mice fed a diet enriched with FOS had increased activities of natural killer cells and phagocytes such as cecal and colonic macrophages or the number of Peyer's patches (1,2). In the present study, the phagocytic activity of the liver (without any septic stimulus) was increased with FOS supplementation in the diet. In addition, TNF-α and PGE_2 secretion by the liver tissue increased after dietary FOS. In vivo, TNF-α and PGE_2 concentrations in the serum after LPS administration were also overall higher in FOS-treated rats. This would mean that higher Kupffer cell phagocytic activity through FOS supplementation, which is accompanied by increased secretion capacity of mediators such as TNF-α or PGE_2, improves LPS clearance in the liver tissue. Of note, serum PGE_2 levels 8 h after LPS/NaCl challenge were higher than after 24 h in all groups. Membrane stores of free arachidonic acid, whose availability is the rate-limiting step in the synthesis of prostaglandins, were probably consumed after 24 h (34). Moreover, prostaglandins are rapidly and extensively degraded upon the first liver passage (35). The effect of dietary FOS on PGE_2 released in the serum was more pronounced 8 h after LPS injection than after 24 h. Could higher PGE_2 release, mainly observed after 8 h, be involved in the liver cytoprotection offered by FOS treatment, as suggested by the lower serum ALT activity 24 h after LPS injection? Indeed, the hepatoprotective effect of PGE_2 was demonstrated in a model of LPS-induced injury through attenuation of cytokine release from Kupffer cells (35). Consistent with this hypothesis, a study reported that in vivo administration of PGE_2 significantly protected mice against liver injury after *Escherichia coli* infection (18). However, in our study, the higher secretion of immunosuppressive PGE_2 was not correlated with a lower TNF-α release. This would suggest that LPS-induced liver injury is not necessarily related to a higher TNF-α production. A similar phenomenon was reported by Yang et al. (36), i.e., both higher serum ALT activity and lower TNF-α concentration were observed in female obese rats 24 h after LPS administration compared with male rats.

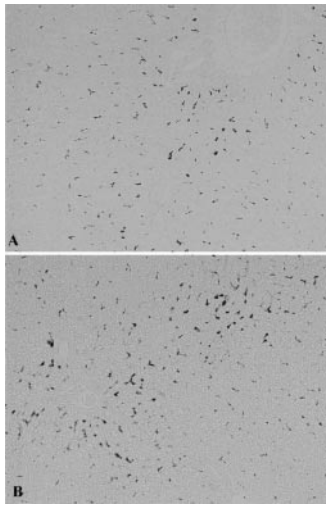

FIGURE 6 Distribution of colloidal carbon in the liver 20 min after injection into the portal vein of rats fed a control diet (*A*) or a diet supplemented with FOS (*B*). Colloidal carbon is taken up mainly by Kupffer cells (colored black). Original magnification X200.

In conclusion, even though an effect of fermentable carbohydrate on gut immunity was described recently (2), this is the first study to show that dietary FOS has consequences for intrahepatic immune cells, by promoting phagocytic activity and the release of both immunostimulating and immunosuppressive substances in response to LPS. This would have consequences for liver tissue injury, which might be less important after FOS treatment. The precise mechanism remains to be established, but we propose that higher PGE_2 release could play a role in the hepatoprotective effect of FOS.

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