

# Biological Significance and Prognostic Role of Opsonic Activity for Kupffer Cell Phagocytosis in Experimental Liver Injuries and Partially Hepatectomized Patients

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## Abstract

This study investigated the biological and clinical significance of bioassayable opsonic activity measured using primary cultures of rat Kupffer cells.

Rats with CCl<sub>4</sub>-induced cirrhosis exhibited an increase in the opsonic index, leading to a rise in the phagocytic index, but in the rats with severely advanced cirrhosis, the phagocytic index was decreased despite a high opsonic index.

In the rats with galactosamine-induced fulminant hepatitis, both of the indexes were remarkably decreased. These findings suggested that the increase in the opsonic activity associated with liver injury was a compensatory response to maintain the host defense, and its remarkable decrease indicated a failure of the compensatory mechanism in the reticuloendothelial system (RES).

Based on the experimental results, the opsonic index in 35 partially hepatectomized patients were analyzed. Three distinct types of responses in the opsonic index after hepatectomy were observed. Group A consisted of patients without remarkable changes in the opsonic index; Group B was comprised of those with marked increases in the opsonic index, which was followed by the subsequent normalization; Group C consisted of patients with decreases in the opsonic index after surgery. Each of these three groups exhibited a different clinical course. Group A patients had an uncomplicated course; Group B patients developed infectious complications and high fever; and group C patients had a critical course, with a mortality rate of 60%.

Thus, the results of this study suggest that the opsonic index is a reliable indicator of the outcomes of the partially hepatectomized patients.

## Introduction

Since the liver accounts for more than 80% of the reticuloendothelial system (RES), hepatic resection may lead to RES depression, thereby impairing the host defense. This concept has

**Key words:** Opsonic activity, Opsonic index, Phagocytic index, Reticuloendothelial system, Kupffer cells.

**索引語:** オプソニン活性, オプソニン指数, 貪食率, 細網内皮系, クッファー細胞.

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been supported by the evidence that partially hepatectomized patients frequently develop sepsis and endotoxemia, resulting in respiratory insufficiency, disseminated intravascular coagulopathy, renal failure, and multiple organ failure<sup>4,5,11,12,13</sup>).

Humoral opsonic activity plays an important role in RES phagocytosis. *Saba et al.* speculated that the depression of the reticuloendothelial systemic host defense seen after sepsis, burn, and trauma is due to a reduction in the level of the opsonic protein, fibronectin<sup>8,9,10,15,16,19</sup>.

We previously reported a newly devised measurement of a bioassayable opsonic activity, which was affected by unknown opsonin-like factors, and found that the opsonic activity was increased after partial hepatectomy in rats; this increase appeared to be a compensatory response to maintain the host defense<sup>2</sup>).

The present study evaluated the biological significance of bioassayable opsonic activity determined by our method, with respect to RES function in various degrees of experimentally induced-liver injuries. Moreover, based on the experimental results, we investigated the role of the opsonic activity as a prognostic indicator in the partially hepatectomized patients.

### Materials and Methods

Male Wistar rats weighing 200 to 250 gm each were used. Cirrhosis was induced by intraperitoneal injection of an equally mixed solution of CCl<sub>4</sub> (Sigma, ST Louis, Mo.) and olive oil (3 ml/kg body weight) twice a week. RES function in the cirrhotic rats were measured 3, 9, and 13 weeks after the initial injection. Hepatitis was induced by the single intravenous injection of 2 g/kg body weight of galactosamine hydrochloride (Sigma). RES function was determined ten hours after the injection.

Opsonic activity was determined using a primary culture of Kupffer cells prepared from normal rat liver<sup>20</sup> as described previously<sup>1,2</sup>). In brief,  $5 \times 10^5$  Kupffer cells per dish (Falcon 3001; Falcon Labware Division, Becton Dickinson & Co., Oxnard Calif) were cultured as a monolayer in a humidified incubator at 37°C under 5% CO<sub>2</sub> in air. The culture medium was Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (Gibco Laboratories, Grand Island, N.Y.). Twenty-four hours after the initial plating, the medium was changed to the serum-free MEM, and the cultures were used for the assay. Plasma was obtained from the abdominal aorta of the animals or peripheral vein of patients using a heparinized syringe. Control plasma was taken from five normal rats or five healthy adults. <sup>51</sup>Cr-endotoxin at a dose of 100 μg was administered to the culture-added test or control plasma at a final concentration of 33%. One hour later, the cells were washed thoroughly with phosphate-buffered saline, then desquamated with a rubber policeman and harvested. Opsonic activity was expressed as an opsonic index (O.I) according to the following formula:

$$O.I = \frac{\text{Radioactivity in the cultured cells with test plasma}}{\text{Radioactivity in cells with control plasma}}$$

The phagocytic index (P.I) was determined by the disappearance rate calculated from the slope of logarithm of plasma radioactivity vs. time after the injection of <sup>51</sup>Cr-labeled endotoxin.

$$P.I = \log C_1 - \log C_2 / T_2 - T_1$$

where  $C_1$  and  $C_2$  represent the radioactivity in the peripheral blood at times  $T_1$  and  $T_2$ , respectively.  $^{51}\text{Cr}$ -endotoxin at a dose of 4 mg/kg was injected via the tail vein, and blood was taken from the femoral vein at intervals of 1, 3, 5, 10 and 20 min. The method of binding  $\text{Na}_2\text{CrO}_4$  ( $^{51}\text{Cr}$ ) to endotoxin (*Escherichia coli* lipopolysaccharide 026:B6; Difco Laboratories, Detroit, Mich.) was based on the principle of *Braude et al*<sup>3</sup>. The ratio of  $^{51}\text{Cr}$  to endotoxin was 1 mCi/100 mg.

#### Clinical study

The opsonic activity and the level of plasma fibronectin were measured in 35 partially hepatectomized patients (24 males and 11 females), consisting of 32 with hepatoma, 2 with bile duct cancer and 1 with hemangioma. Their ages ranged from 41 to 68 years with a mean of 56 years. The opsonic activity was determined preoperatively, and on the 1st, 4th, 7th and 14th post-operative days. Plasma fibronectin was measured using an in vitro turbidimetric assay (Boehringer-Manheim kit). Statistical analysis was performed by Student's t test and Chi's square test.

### Results

Table 1 demonstrates the development of liver cirrhosis in  $\text{CCl}_4$ -injected rats and the corresponding RES function. O.I at each time examined was significantly increased to 150% of the control. While, P.I varied according to the development of the liver cirrhosis. At 3 weeks P.I was increased to 150% of the control, but at 9 weeks, it was within normal limits. In contrast, P.I at 13 weeks was decreased to 63% of the control despite the high opsonic activity.

In the rats with galactosamine-induced fulminant hepatitis, both of the O.I and P.I were remarkably decreased as shown in Table 2.

Figure 1 shows the changes in O.I of 35 partially hepatectomized patients. The patients could be classified into three groups: Group A ( $n=18$ ) consisted of patients without the remarkable changes in O.I (Fig. 1-A). Group B ( $n=12$ ) was comprised of patients with marked increases in O.I greater than 1.5, with subsequent gradual normalization (Fig. 1-B), and group C ( $n=5$ ) consisted of patients with decreases in O.I to a level less than 0.7 (Fig. 1-C).

The clinical features and course in each group are shown in Table 3. There were no signifi-

**Table 1.** Development of liver cirrhosis and RES function

	period of injection		
	3 weeks (8)	9 weeks (8)	13 weeks (8)
nodular change of the liver	—	+	++
splenomegaly	—	+	+
ascites	—	—	+
opsonic index	1.45 ± 0.13*	1.42 ± 0.20*	1.48 ± 0.38*
phagocytic index	0.155 ± 0.003*	0.103 ± 0.018	0.065 ± 0.008*

Values are expressed as mean ± SEM. \*;  $p < 0.01$ , compared with values of saline-injected controls (O.I;  $1.0 \pm 0.2$ , P.I;  $0.103 \pm 0.015$ ). Numbers in parenthesis indicate numbers of animals.

**Table 2.** RES function in the rats with galactosamine-induced hepatitis

	O.I	P.I
galn (10)	0.32±0.07*	0.064±0.014*
control (8)	1.0 ±0.2	0.103±0.015

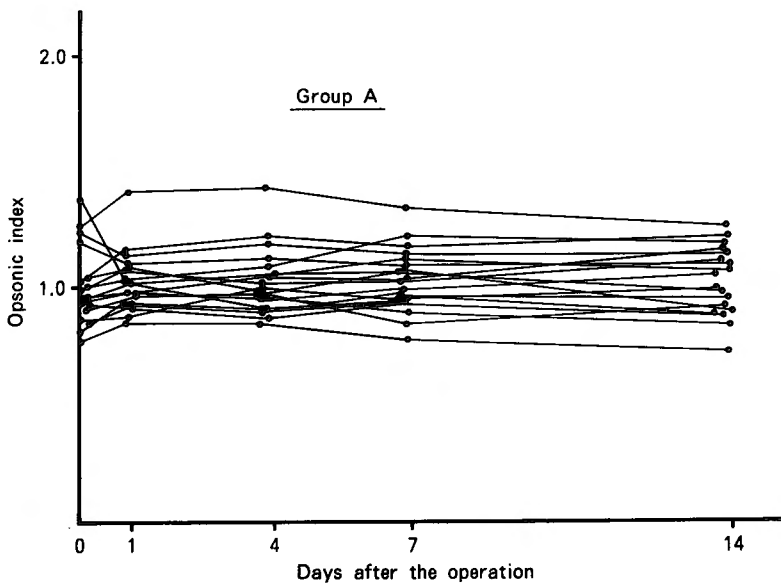
Values are expressed as mean±SEM. \*;  $p<0.01$ , compared with values of saline-injected controls. Numbers in parenthesis indicate numbers of animals.

**Table 3.** Clinical features and outcome of each group

	group A	group B	group C
No. of cases	18	12	5
Age	55.1± 8.0	57.5± 7.8	61.5± 6.5
GOT (IU/l)	66±26.5	70±36.2	70±45.3
GPT (IU/l)	60±18.3	62±22.6	65±24.2
Bilirubin (mg/dl)	0.9± 0.1	1.0± 0.2	1.0± 0.2
Albumin (g/dl)	3.8± 0.3	3.6± 0.2	3.6± 0.3
Prothrombin time (second)	12.1± 1.1	12.5± 1.3	12.5± 1.3
Postoperative fever (days) <sup>a)</sup>	7.5± 3.3	14.5± 5.3*	15.1± 5.5*
Operative mortality (%)	0	0	60*

Values are expressed as mean±SEM. <sup>a)</sup>; Postoperative fever is defined as body temperature higher than 37.5°C within 1 month after the operation. \*;  $p<0.01$ , compared with value of group A

cant differences in the various measurements to assess preoperative liver function among these three groups. All the patients in group A had a normal postoperative course without unusual

**Fig. 1A.**

fever or infectious complications. In contrast, the patients in group B experienced fever and various infectious complications such as pneumonia and intraabdominal abscess, which subsided with normalization of O.I. The patients in group C had a poor postoperative course, as indicated by their high mortality rate. Three of 5 patients in this group developed sepsis, resulting in

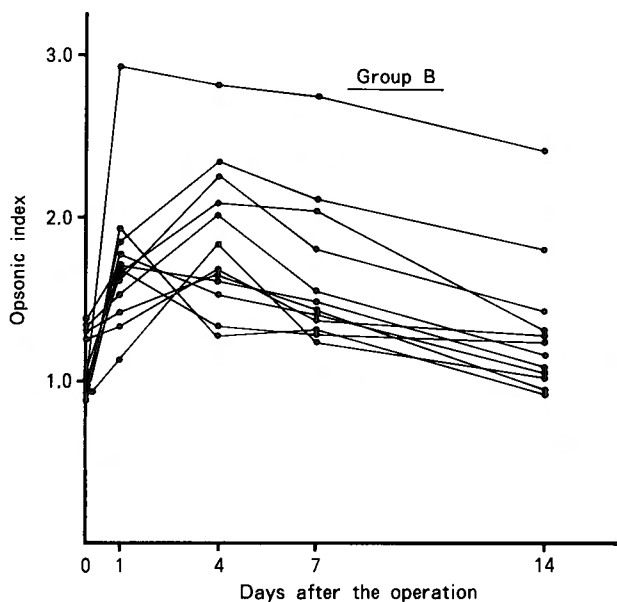


Fig. 1B.

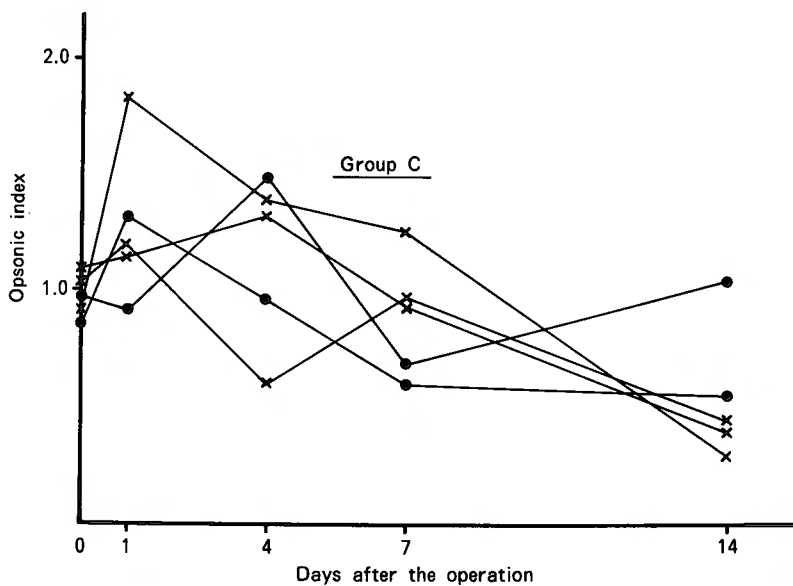


Fig. 1C.

Fig. 1-A, B, and C. Postoperative changes in the O.I. of partially hepatectomized patients. O—O; living cases, x—x; deceased cases within 1 month after the operation.

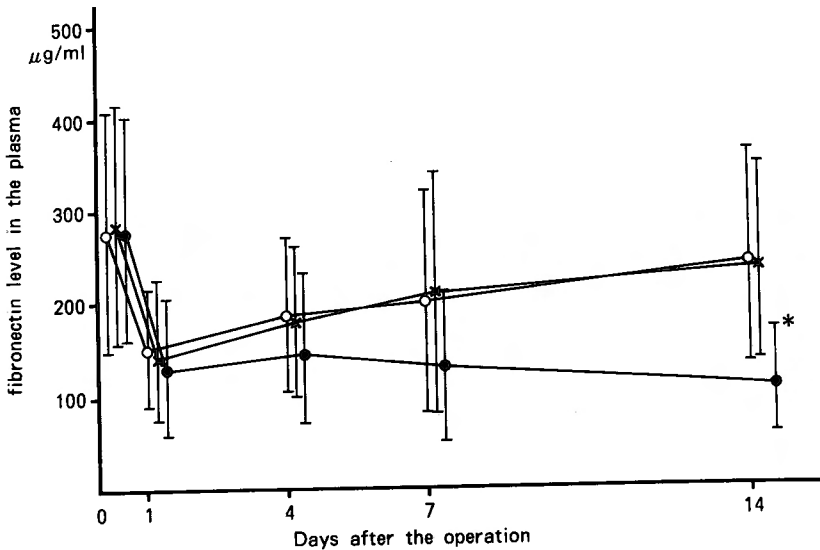


Fig. 2. Postoperative changes in fibronectin level of the plasma of partially hepatectomized patients. ○—○; Group A, ×—×; Group B, ●—●; Group C. a):  $p < 0.01$ , compared with the value of group A and B on the same postoperative day.

multiple organ failure (liver failure, renal failure and respiratory insufficiency).

The changes in plasma fibronectin levels after hepatectomies are shown in Fig. 2. In groups A and B, the levels were initially decreased, and then returned to the preoperative levels within 1 to 3 weeks. In contrast, the group C patients exhibited no recovery of plasma fibronectin levels during this period.

### Discussion

Since the RES plays an important role in clearing and inactivating bacteria, endotoxin, and non-bacterial particles<sup>14)</sup>, depression of the RES frequently lead to severe complications<sup>4,5,11,12,13,19)</sup>. Thus, it is extremely important to assess RES function in liver injuries.

Previously, we reported that RES function in partially hepatectomized rats varies with the changes in the plasma opsonic activity according to the period after hepatectomy<sup>2)</sup>.

In the present study, to further investigate the response of RES in liver injuries and to evaluate the clinical significance of the opsonic activity, RES function in experimental liver injuries was studied. In  $\text{CCl}_4$ -injected rats, O.I was increased at each time examined. While P.I was varied according to the severity of liver cirrhosis. Three weeks after the beginning of  $\text{CCl}_4$  treatment, the rats showed an increase in P.I, concomitant with a rise of O.I. On the other hand, P.I at 9 weeks was not increased despite the high opsonic activity. Furthermore, P.I was remarkably decreased at 13 weeks when the rats had severe liver cirrhosis. Thus, the enhancement in the O.I may be a homeostatic response necessary for maintaining normal RES phagocytic function, and would appear to be indispensable in preventing RES depression. However, in the rats with severe liver injuries, it failed to stimulate the phagocytic activity.

A possible explanation for the above discrepancy between P.I and O.I is that in advanced liver cirrhosis, Kupffer cell phagocytosis, which mostly depends on energy from glycolysis<sup>17)</sup>, may be impaired. This is supported by our unpublished data that an inhibitor of Embden-Meyerhof pathway, NaF, suppressed the phagocytosis of cultured Kupffer cells even when a high concentration of opsonin was added to the culture. On the other hand, in the rats with galactosamine-induced fulminant hepatitis, 50% of which were died within 3 days, compensatory enhancement of the opsonic activity did not occurred.

These results suggest that an increase in the opsonic activity represents a compensatory response, and its decrease implies a failure of the compensatory mechanism, indicating compromised liver damage.

Among patients who underwent partial hepatectomy, three distinct types of the postoperative changes in O.I were observed. The increases in the O.I, as typically seen in group B patients, may be a homeostatic response similar to that seen in experimental liver injury. Such posthepatectomy O.I enhancement may be an expression of the functional reserve of the RES. In addition, the negligible postoperative changes in the O.I, as seen in group A patients, implies minimal impairment of the intrinsic reticuloendothelial systemic host defense. Similarly, the remarkable decrease in the O.I observed in group C patients indicates a failure of the compensatory mechanism, as observed in galactosamine induced hepatic rats, thus leading to compromised RES function and a poor postoperative course. Such a decrease in the O.I may result from an impairment in the ability to produce opsonin-like substances and/or excessive increase in their consumption. Obviously, a decrease in the plasma opsonic activity is not always the main cause of the many postoperative complications seen in group C patients, but it may certainly aggravate the situation.

The delayed recovery of plasma fibronectin levels suggests an unfavorable postoperative prognosis, which was coincident with the findings of *Saba et al*<sup>8,9,10,16)</sup>. They reported that the bioassayable opsonin measured with liver slices and <sup>125</sup>I-gelatinized lipid emulsion is derived from plasma fibronectin<sup>9)</sup>. However, the opsonic activity measured with the present method did not correlate with the plasma fibronectin level. This discrepancy between the plasma fibronectin levels and the opsonic activity is at least partly due to the phagocytizable materials used, because plasma fibronectin opsonizes fibrin, microaggregates, collagenous debris, and various gelatinized particles<sup>6,18,20,21)</sup>, but not endotoxin. This indicates that the opsonic activity was affected by unknown humoral factors. Further studies on the characterization of the humoral factors regulating the opsonic activity are now in progress.

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## 実験的肝障害及び肝部分切除患者におけるクッパー 細胞貪食能に關与するオプソニン活性の意義

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有 井 滋 樹

本研究は、著者の開発した方法により測定されたオプソニン活性の生物学的かつ臨床的意義を検討したものである。

四塩化炭素による肝硬変作製過程のラットでは、in vivo における貪食率は極く早期には上昇し、肝硬変が形成され、進行するに従い、低下した。一方、オプソニン活性は、常に有意に高値を示した。肝障害におけるこのようなオプソニン活性の上昇は、生体防御上の合目的な反応であることが示唆された。また、致死モデルであるガラクトサミン肝炎ラットでは、オプソニン活性は著明に低下し、これは、合目的適応反応の破綻と考えられた。

つぎに、肝切除35症例のオプソニン活性を測定した結果、術後の変動形態が3群に分類され、各々、特徴的な術後経過をとった。

A群：オプソニン活性の変動は軽微で、術後経過良好であった。

B群：オプソニン活性は有意に高値を示した群で、発熱や軽度の感染がみられた。

C群：オプソニン活性が低下した群で、5例中3例が多臓器障害にて死亡した。

以上の如く、実験結果、及び臨床成績から本測定法によるオプソニン活性は、肝障害度や肝切除後の経過の有意な指標になるものと推察された。