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# Fatty Acid Binding Protein 7 Regulates Phagocytosis and Cytokine Production in Kupffer Cells during Liver Injury

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Kupffer cells (KCs) are involved in the progression of liver diseases such as hepatitis and liver cancer. Several members of the fatty acid binding proteins (FABPs) are expressed by tissue macrophages, and FABP7 is localized only in KCs. To clarify the role of FABP7 in the regulation of KC function, we evaluated pathological changes of *Fabp7* knockout mice during carbon tetrachloride-induced liver injury. During liver injury in *Fabp7* knockout mice, serum liver enzymes were increased, cytokine expression (tumor necrosis factor-α, monocyte chemoattractant protein-1, and transforming growth factor-β) was decreased in the liver, and the number of KCs in the liver necrotic area was significantly decreased. Interestingly, in the FABP7-deficient KCs, phagocytosis of apoptotic cells was impaired, and expression of the scavenger receptor CD36 was markedly decreased. In chronic liver injury, *Fabp7* knockout mice showed less fibrogenic response to carbon tetrachloride compared with wild-type mice. Taken together, FABP7 is involved in the liver injury process through its regulation of KC phagocytic activity and cytokine production. Such modulation of KC function by FABP7 may provide a novel therapeutic approach to the treatment of liver diseases. *(Am J Pathol 2014, 184: 2505–2515; http://dx.doi.org/10.1016/j.ajpath.2014.05.015)*

Macrophages play a central role in inflammatory reactions by producing pro- and anti-inflammatory cytokines as well as by phagocytizing microorganisms. Recently, it has become evident that adipose tissue macrophages and liver macrophages are important not only in local inflammation and tissue damage but also in systemic diseases bound to metabolic abnormalities.[1,2] Kupffer cells (KCs) are liver-resident macrophages that constitute approximately 70% to 80% of all macrophages in the body. KCs play an important role in normal physiology and homeostasis and participate in the acute and chronic response of the liver to toxic compounds. Activated KCs vigorously scavenge damaged hepatocytes while they release inflammatory and growth control mediators. This activation appears to modulate acute hepatocyte injury and chronic liver responses, including hepatic fibrosis. These specialized liver macrophages are primarily exposed to gut-derived antigens, and they orchestrate inflammatory processes in diseases such as alcoholic and nonalcoholic liver disease.[3,4] Although it seems that the role of KCs should be protective by removing gut-derived pathogens, studies have found that they contribute to the promotion of liver inflammation.[5,6] Therefore, KCs can play both deteriorative and ameliorative roles in the pathogenesis of liver diseases according to their ambient conditions. Unraveling the controlling mechanism of the KC activation is essential for understanding the mechanism of the liver injury.

Fatty acid binding proteins (FABPs) can bind different FAs and their derivatives with distinct binding preferences and act as their intracellular chaperones.[7] FABPs have been reported to govern the transcriptional activity of peroxisome proliferator-activated receptors by targeting the ligands to nucleus[8] and to be involved in modulating macrophage function.[9,10] FABPs

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are expressed in various macrophages with distinct patterns for the individual FABPs. For example, FABP4 is highly expressed in the activated macrophages of atherosclerotic lesions,[11,12] whereas FABP5 is found in atretic ovarian follicles[13] and alveolar macrophages express FABP1.[14] Such differential expression of FABP subtypes might reflect the fact that macrophages adopt different regulatory systems to adjust to tissue-specific lipid environment. Macrophage-specific FABP4 deficiency protects against atherosclerosis in apolipoprotein E-deficient mice,[11] and pharmacological inhibition of FABP4 protects mice against atherosclerosis and compromises inflammatory responses in macrophages.[15] Moreover, it has been reported that macrophage FABP5 deficiency suppresses atherosclerosis in low-density lipoprotein receptor-null mice.[16] Among different macrophages, FABP7 is expressed only by KCs,[17] suggesting its regulatory role by controlling lipid homeostasis in KCs. However, the contribution of FABP7 to the functional regulation of KCs remains unknown.

In this study, to explore the role of FABP7 in the process of acute liver injury and liver fibrosis, we examined the phenotype of *Fabp7*-knockout (KO) mice after carbon tetrachloride ($CCl_4$) or D-galactosamine (D-GalN)/lipopolysaccharide (LPS) treatment, both of which have been frequently used by many researchers because the important role of KCs in the clearance of dead cells and cytokine production has been well defined.[6,18–20] We found that FABP7 regulates the phagocytosis of apoptotic cells and the production of cytokines, including fibrogenic factors in KCs.

## Materials and Methods

### Animals

We used 8- to 11-week-old male wild-type (WT) and *Fabp7*-KO[21] C57BL/6 mice. Liver injury was induced by i.p. injection of $CCl_4$ (1 μL/g body weight, dilution 1:3 in olive oil), once for the acute injury model and 16 i.p. injections (twice per week for 8 weeks) for the chronic fibrosis model. A suspension of liposome-encapsulated clodronate (Clo-lipo) prepared as described previously[22] was administrated by i.p. injection for the depletion of KCs. D-GalN (500 mg/kg body weight; Wako, Osaka, Japan) and LPS (5 μg/kg body weight; Sigma-Aldrich, St. Louis, MO) were administrated by i.p. injection. Mice were given i.p. injection of 2 mL of thioglycolate, and the peritoneal macrophages were harvested 4 days after injection. All experimental protocols were reviewed by the Ethics Committee for Animal Experimentation of Yamaguchi University School of Medicine and were performed according to the Guidelines for Animal Experimentation of the Yamaguchi University School of Medicine and under the law and notification requirements of the Japanese government.

### Enrichment and Isolation of KCs from Mice

KCs were isolated according to the previously reported method[23,24] with some modifications. Briefly, mice livers were perfused initially by 0.03% collagenase (Wako). After perfusion, the liver was minced and incubated with 0.03% collagenase for 5 minutes at 37°C. The cell suspension was centrifuged at 50 × *g* for 3 minutes, and the supernatant was centrifuged at 650 × *g* for 5 minutes at 4°C. The pellet was used as KC-enriched fraction. The viability (propidium iodide) of KC-enriched fraction was 90%, and the purity of KCs ($F4/80^+$) was approximately 50% by fluorescence-activated cell sorting analysis (data not shown). For further isolation of KCs, KC-enriched fraction was loaded on a 25% to 50% Percoll gradient and centrifuged at 1500 × *g* for 15 minutes at 4°C. The layer of KCs was collected and used for experiments. The final purity of KCs was >80% and the viability was >90% (data not shown).

### Cell Culture and *FABP7* Gene Transduction

Murine macrophage-like cell line J774 was obtained from Yamaguchi University Center for Gene Research. J774 cells were maintained by passage in RPMI 1640 medium (Life Technologies, Carlsbad, CA) supplemented with 10% heat-inactivated fetal bovine serum (Thermo Fisher Scientific, Waltham, MA), 100 U/mL penicillin, 100 μg/mL streptomycin (Sigma-Aldrich), and 2 mmol/L L-glutamine (Life Technologies). J774 cells were transfected with either pcDNA3-FABP7 expression construct (FABP7/J774) or empty pcDNA3 vector (MOCK) by using Lipofectamine 2000 (Life Technologies). For transient expression, cells were analyzed 24 to 48 hours after transfection. For stable expression, the transfected clones were selected with 0.1 mg/mL G418 (Sigma-Aldrich) for 2 weeks.

### Phagocytosis Assays

Apoptotic cells and necrotic cells were prepared according to the method previously reported[25] and were used as target cells for the phagocytosis assay. Briefly, thymocytes harvested from mice aged 4 to 7 weeks were incubated with 10 mmol/L dexamethasone for 5 hours in RPMI 1640 medium that contained 10% fetal bovine serum. Necrosis was induced by incubating the cells at 56°C for 10 minutes and was confirmed by trypan blue staining. Apoptotic thymocytes were labeled with 2 μmol/L 5-chloromethylfluorescein diacetate (CellTracker Green CMFDA; Life Technologies). CMFDA-labeled apoptotic thymocytes were intravenously injected into mice through the portal vein. Ten minutes after injection, the five largest lobes (five sections) of the liver were harvested, fixed with 4% paraformaldehyde, and stained for F4/80. CMFDA-positive thymocytes enclosed by $F4/80^+$ KC cytoplasm were counted. The total number of ingested apoptotic thymocytes in 50 microscopic fields for five sections from different liver lobes was quantified as the phagocytic activity of each mouse. For the *in vitro* phagocytosis assay, $1 \times 10^5$ FABP7/J774 cells per well and $4 \times 10^6$ CMFDA-labeled apoptotic thymocytes cells per well were co-cultured for 120 minutes in a 24-well plate. After incubation, apoptotic thymocytes were removed by washing twice with phosphate-buffered saline (PBS) to break weak