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Extracellular ATP induces P2X7 receptor activation in mouse Kupffer cells, leading to release of IL-1β, HMGB1, and PGE2, decreased MHC class I expression and necrotic cell death

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ABSTRACT

Kupffer cells, which are resident macrophages in liver, can produce various cytokines and chemokines that induce hepatitis and liver fibrosis. It is suggested that extracellular ATP-induced activation of macrophage P2X7 receptor plays an important role in inflammation via release of pro-inflammatory mediators, but the role of P2X7 receptor in Kupffer cells remains unclear. Here, we show that activation of P2X7 receptor in Kupffer cells causes multiple inflammatory responses, using the clonal mouse Kupffer cell line (KUP5) that we previously established. Treatment of LPS-primed Kupffer cells with 3 mM ATP induced Ca^{2+} influx, non-selective large pore formation, activation of MAPK, cell lysis, IL-1β release, prostaglandin E2 (PGE2) release, high mobility group box1 (HMGB1) release, and major histocompatibility complex (MHC) class I shedding. These events were significantly suppressed by pretreatment with P2X7 antagonist A438079, indicating involvement of P2X7 receptor activation in these inflammatory responses. Our results suggest that extracellular ATP-induced activation of P2X7 receptor of Kupffer cells plays multiple roles in the inflammatory response in liver. P2X7 receptor might be a new therapeutic target for treatment of liver diseases.

1. Introduction

Kupffer cells/liver macrophages are involved in immune response in the liver via phagocytosis, antigen-presenting ability and production of cytokines, which induce inflammation, necrosis, regeneration, and fibrogenesis [1]. They also participate in the acute and chronic responses of the liver to toxic agents, leading to hepatic damage through the release of various molecules [2–4], including pro-inflammatory cytokines [5]. In liver disease, Kupffer cells produce both pro-inflammatory and pro-fibrogenic factors [6–8]. Liver cirrhosis is an increasing public health issue associated with alcoholic hepatitis, non-alcoholic steatohepatitis, and infection with viruses such as hepatitis C virus and hepatitis B virus. Virus-associated liver damage is attributed to immune-mediated mechanisms. In the inflammatory microenvironment caused by infections, infiltration of immune cells and secretion of inflammatory cytokines may result in liver damage, and may indirectly induce cell death. Reconstruction of damaged liver cells induces hepatic fibrosis, and progression of hepatic fibrosis eventually leads to liver cirrhosis, which can be considered as a wound-healing response to chronic inflammation [9]. Thus, cirrhosis is a consequence of the interaction between liver damage and tissue repair processes.

P2X7 receptor is the seventh member of the P2X receptor subfamily, and is expressed in immune cells, such as monocytes/macrophages, T cells, and dendritic cells [10]. P2X7 receptor

Abbreviations: $[Ca^{2+}]i$, cytosolic Ca^{2+} concentration; EtBr, ethidium bromide; HMGB1, high mobility group box 1; LDH, lactate dehydrogenase; MHC, major histocompatibility complex; PGE2, prostaglandin E2.

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has two unique features among the P2X receptors, i.e., it requires high concentrations (millimolar range) of ATP to be activated and its C-terminus is over 200 aa longer than that of any other family member [11]. P2X7 receptor is up-regulated in response to lipopolysaccharide (LPS) and other inflammatory stimuli [10]. Activation of P2X7 receptor induces Ca^{2+} influx, non-selective large pore formation (allowing the passage of hydrophilic molecules of up to 900 Da in size), MHC class I shedding, CD62 ligand shedding, membrane blebbing, fibrosis, caspase-1 activation, IL-1β release, PGE2 release, and cell death [10–14].

Though it is suggested that P2X7 receptor contributes to liver injury in acetaminophen hepatotoxicity and carbon tetrachloride-mediated steatohepatitis [15,16], the involvement of P2X7 receptor of Kupffer cells has not been established. Since activation of P2X7 receptor in macrophages causes inflammation, cell death, and fibrosis, we hypothesized that activation of P2X7 receptor in liver macrophages/Kupffer cells would contribute to liver cirrhosis. Therefore, the objective of this study was to define the role of P2X7 receptor activation in Kupffer cells in inflammation. To address this question, we focused on P2X7 receptor-mediated inflammatory functions, such as necrotic cell death, release of IL-1β, HMGB1 and PGE2, and MHC class I shedding. Our findings indicate that activation of P2X7 receptor in Kupffer cells has multiple roles in inflammation, cell death, and tissue repair in liver.

2. Materials & methods

2.1. Cell culture

Kupffer cells were isolated from mixed primary culture of C57BL/6 mouse liver cells as described, and immortalized by retroviral transduction of human c-myc [17]. The resulting clonal Kupffer cell line (KUP5) was cultured in D-MEM (high glucose type, Wako Pure Chemical, Osaka, Japan) supplemented with 10% heat-inactivated FBS (Life Technologies), 100 U/mL penicillin, 100 μg/mL streptomycin, 10 μg/mL insulin and 100 μM 2-mercaptoethanol at 37 °C in the presence of 5% CO_2. As required, cells were pre-incubated for 4 h with 1 μg/mL LPS.

2.2. Mobilization of intracellular calcium

Cells were loaded with the Ca^{2+}-sensitive fluorescent dye Fluo-4AM (Invitrogen, Carlsbad, CA) for 30 min at 37 °C, washed twice with Ca^{2+}-free buffer, and suspended in HBSS-HEPES buffer. Samples were analyzed using a fluorescence spectrometer (F-2500, Hitachi) with laser excitation at 495 nm and emission at 518 nm.

2.3. Analysis of pore formation

Cells were re-suspended in HBSS-HEPES buffer at 1.0×10^5 cells/mL and incubated with various concentrations of ATP (Sigma, St. Louis, MO) and 25 μM ethidium bromide (EtBr) at 37 °C. After incubation, each sample was analyzed using a flow cytometer (FACSCaliber cytometer, Becton, Dickinson and Co., Franklin Lakes, NJ) with laser excitation at 488 nm. Ethidium fluorescence was examined using an FL-2 filter.

2.4. Immunoblotting

Culture supernatant or an equal amount of cell lysate was mixed with 2× sample buffer (50% glycerin, 2% SDS, 125 mM Tris, 10 mM DTT) and incubated at 95 °C for 10 min. Aliquots were subjected to 10% SDS-PAGE and bands were transferred to PVDF membranes. The blots were incubated for 2 h at room temperature in TBST (0.1% Tween-20, 10 mM Tris–HCl, 0.1 M NaCl) containing 1% bovine serum albumin (BSA), then further incubated overnight at 4 °C with phosphor-p44/p42 MAPK (Thr202/Tyr204) rabbit monoclonal antibody (1:1000) (Cell Signaling Technology, Inc., Beverly, MA), p44/p42 MAP kinase antibody (1:1000) (Cell Signaling Technology), phosphor-p38 MAPK (Thr180/Thr182) rabbit monoclonal antibody (1:1000) (Cell Signaling Technology), p38α MAP kinase mouse monoclonal antibody (1:1000) (Cell Signaling Technology), anti-HMGB1 antibody (1:1000) (Cell Signaling Technology) or COX-2 (Cell Signaling Technology) to confirm equal loading. Blots were washed with TBST, incubated with goat horseradish peroxidase (HRP)-conjugated anti-rabbit IgG antibody (1:20,000) (Cell Signaling Technology) or goat horseradish peroxidase (HRP)-conjugated anti-mouse IgG antibody (1:20,000) (Santa Cruz Biotechnology) for 1.5 h, and washed again with TBST. Specific proteins were visualized by using ImmunoStar®LD (Wako) Western blotting detection reagents.

2.5. Quantification of lactate dehydrogenase (LDH) release

Release of LDH into the cell culture supernatant was quantified with a Cytotoxicity Detection Kit (Roche Applied Science, Penzberg, Germany), according to the supplied instructions. The cells were incubated in a 96-well plate at 37 °C for the indicated times with ATP in HBSS-HEPES buffer. At the end of incubation, supernatants were collected and the LDH content was measured. LDH release is expressed as a percentage of the total content determined by lysing an equal amount of cells with 1% Triton X-100.

2.6. Determination of IL-1β and PGE2

Culture supernatant was harvested, and IL-1β was measured by ELISA, as described previously [18]. PGE2 production was determined with an enzyme immunoassay (EIA) kit (Cayman Chemical) according to the manufacturer's instructions.

2.7. Membrane MHC I quantification

Cells were re-suspended in HBSS-HEPES buffer at 1.0×10^6 cells/mL in 1.5 mL tube and washed with HBSS-HEPES buffer. To identify surface MHC I (H-$2K^b$), cells were stained with PE-conjugated anti-MHC I (H-$2K^b$) for 30 min at 37 °C and then washed with HBSS-HEPES. The fluorescence intensity of these samples were analyzed with a flow cytometer (FACSCaliber cytometer, Becton, Dickinson and Co.).

2.8. Statistics

Values are given as the mean ± SE. Comparison between two values was performed by means of the unpaired Student's t-test. The statistical significance of differences between control and other groups was calculated by using Dunnett's test with the Instat version 3.0 statistical package (GraphPad Software, San Diego, CA, USA). The criterion of significance was set at $P < 0.05$.

3. Results & discussion

It is known that activation of P2X7 receptor induces an increase of intracellular Ca^{2+} ($[Ca^{2+}]_i$). As shown in Fig. 1A, we examined the involvement of ATP in the elevation of $[Ca^{2+}]_i$. When KUP5 cells were stimulated with ATP, they showed an initial peak of $[Ca^{2+}]_i$ followed by a sustained increase. Pretreatment with A438079 (a P2X7 receptor antagonist) (Tocris Bioscience) suppressed the sustained phase, but not the initial peak of $[Ca^{2+}]_i$. These results indicate that the sustained phase of $[Ca^{2+}]_i$ elevation is induced by

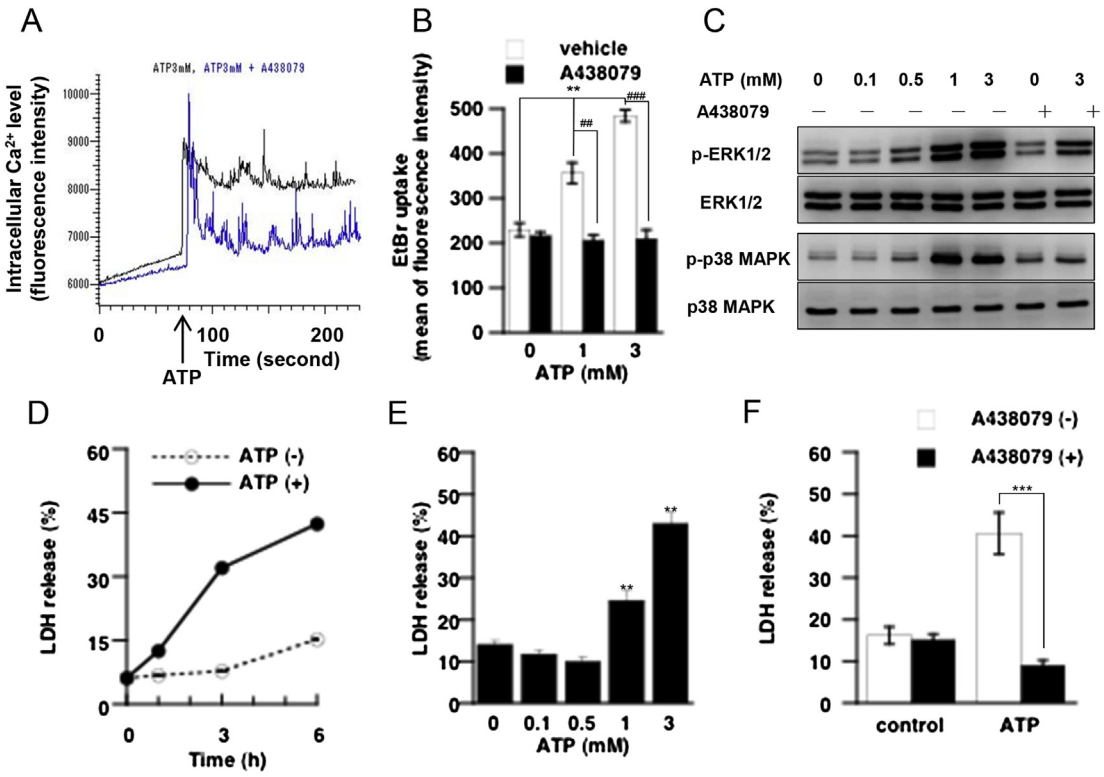

Fig. 1. Induction of Ca^{2+} influx, pore formation, phosphorylation of MAPKs, and cell death by ATP via activation of P2X7 receptor (A) KUP5 cells loaded with Fluo-4 were stimulated with 1 mM ATP in the presence (blue line) or absence (black line) of A438079 (50 μM). The fluorescence was analyzed with a fluorescence spectrometer. (B) Cells were pre-incubated with A438079 (50 μM) for 5 min, and incubated with 1 mM or 3 mM ATP and EtBr (25 μM) in HBSS-HEPES buffer for 5 min. The change in fluorescence intensity of EtBr was analyzed by flow cytometry. (C) Cells were pre-incubated with A438079 (50 μM) for 30 min with various concentrations of ATP. Phosphorylation of ERK1/2 and p38 MAPK was determined by immunoblotting. (D–F) Cells were incubated with 3 mM ATP for the indicated times (D). Cells were incubated with vehicle (control) or various concentrations of ATP (0.1–3 mM) for 6 h (E). Cells were pre-incubated with 50 μM A438079 for 30 min, and incubated with vehicle or 3 mM ATP for 6 h (F). At the end of incubation, supernatants were collected and LDH content was measured. The release of LDH is expressed as a percentage of total content determined by lysing an equal number of cells with 1% Triton X-100. Error bars indicate ± SE (n = 4–8). Significant differences between control and ATP-treated cells are indicated with $^{**}(p < 0.01)$. Significant differences between ATP-treated cells and inhibitor-treated cells are indicated with $^{###}(p < 0.001$ and $^{##}(p < 0.01))$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

activation of P2X7 receptor, but other P2 receptors appear to be involved in the initial peak of $[Ca^{2+}]_i$.

P2X7 receptor forms a large pore permeable to molecules of up to 900 Da, and this leads to cell death [10]. To investigate non-selective large pore formation, we examined the uptake of EtBr into cells by flow cytometry. We found that treatment with ATP induced EtBr uptake into KUP5 cells within 5 min (Fig. 1B). Pre-treatment with A438079 significantly inhibited the ATP-induced EtBr uptake, suggesting that P2X7 receptor activation leads to large pore opening in Kupffer cells (Fig. 1B). These results indicate that extracellular ATP activates P2X7 receptor in Kupffer cells.

The cytoplasmic C-terminal region of P2X7 receptor is essential for activation of the p38 MAPK pathway, which is associated with apoptotic cell death [19]. On the other hand, the importance of the N-terminal region for phosphorylation of ERK1/2 and necrotic cell death has been demonstrated using P2X7 receptor C- and N-terminal mutants [20,21]. We therefore investigated activation of ERK1/2 and p38 MAPK after treatment of KUP5 cells with ATP. High concentrations of ATP induced phosphorylation of ERK1/2 and p38 MAPK (Fig. 1C). Phosphorylation of both ERK1/2 and p38 MAPK peaked at 5 min after ATP stimulation (data not shown). Pretreatment with A438079 suppressed the activation of these MAPKs (Fig. 1C), suggesting that activation of P2X7 receptor mediates activation of both ERK1/2 and p38 MAPK in Kupffer cells.

Activation of P2X7 receptor causes cell death through apoptotic and necrotic mechanisms [10]. In KUP5 cells, a high concentration of ATP induced LDH release in a time-dependent manner (Fig. 1DE), suggesting that ATP-treated Kupffer cells have decreased viability. Pretreatment with A438079 significantly inhibited the release of LDH and the decrease of cell viability (Fig. 1F), indicating that extracellular ATP causes P2X7 receptor-mediated cell death, including necrotic cell death, in Kupffer cells.

IL-1β is a proinflammatory cytokine produced by macrophages in response to inflammatory stimuli. The secretion of IL-1β causes infiltration of pro-inflammatory leukocytes, leading to an inflammatory response. Synthesis of pro-IL-1β is initiated by Toll-like receptor (TLR) agonists, such as LPS [22,23]. Pro-IL-1β is cleaved to generate biologically active mature IL-1 by caspase-1, which is activated within large multi-protein complexes, termed inflammasomes. P2X7 plasma membrane receptor plays the most prominent role in the activation of inflammasomes, leading to release of IL-1β [24]. We have previously reported activation of P2X7 receptor-mediated IL-1β release from KUP5 cells induced by silica nanoparticles [25]. Here, we examined release of IL-1β from LPS-primed KUP5 cells in response to 3 mM ATP (Fig. 2AB). A significant increase of IL-1β production was observed from 1 h, reaching a plateau at 3 h post-treatment. ATP-induced IL-1β production was markedly blocked by pretreatment with A438079 (Fig. 2C), suggesting that activation of P2X7 receptor induces production of IL-1β by Kupffer cells. These results indicated that extracellular ATP induces production of IL-1β through activation of P2X7 receptor in Kupffer cells.

High mobility group box 1 (HMGB1) protein was originally recognized as an intranuclear protein, but extracellular HMGB1

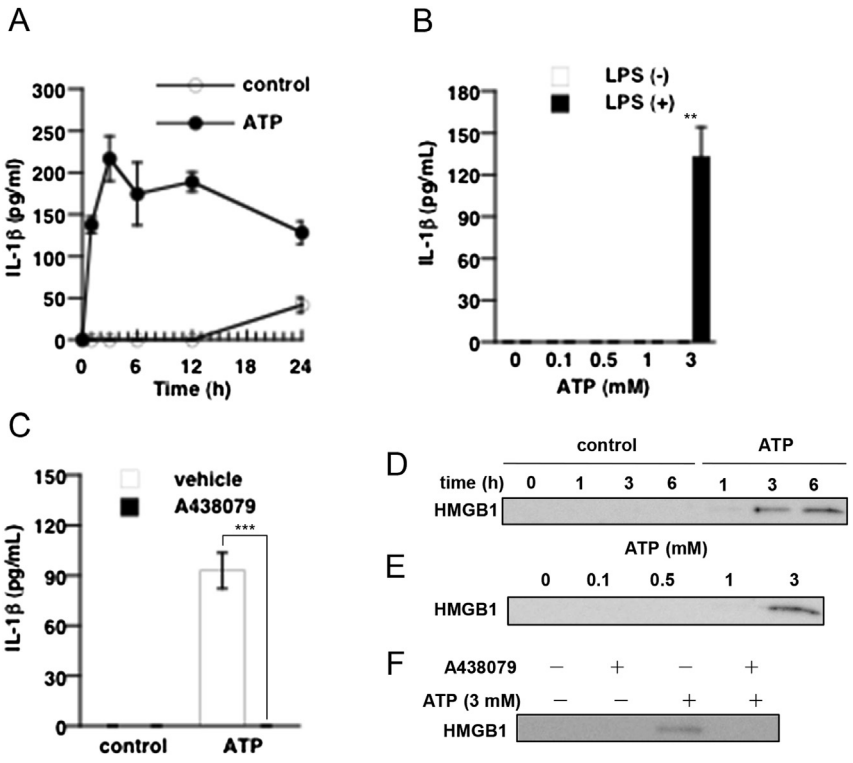

Fig. 2. ATP-induced release of IL-1β and HMGB1 through P2X7 receptor activation (A, D) KUP5 cells were incubated with 3 mM ATP for the indicated times. (B, E) KUP5 cells were incubated with the indicated dose of ATP for 6 h (C, F) Cells were pre-incubated with 50 μM A438079 for 30 min, and then incubated with vehicle (control) or 3 mM ATP for 6 h (A, B, C) The culture supernatant was harvested for determination of IL-1β. Concentrations of IL-1β in the culture supernatants were measured as described in Materials and Methods. Error bars indicate ± SE (n = 4–8). Significant differences between indicated groups are indicated with ***($p < 0.001$) and **($p < 0.01$). (D, E, F) At the end of incubation, supernatants were collected and HMGB1 was detected by immunoblotting as described in Materials and Methods.

functions as a potent pro-inflammatory mediator through activating a wide range of inflammatory responses, including massive production of cytokines, thereby sustaining prolonged inflammation [26]. Prolonged inflammation results in accumulation of tissue damage, leading to cell death. As described above, we confirmed that activation of P2X7 receptor induces necrotic cell death of Kupffer cells. Since HMGB1 can be released during necrotic cell death [26], we examined whether activation of P2X7 receptor induces HMGB1 release from KUP5 cells. Indeed, release of HMGB1 in response to 3 mM ATP started within 3 h, and the amount of extracellular HMGB1 increased in a time-dependent manner (Fig. 2D, E). Pretreatment with A438079 markedly decreased the ATP-induced release of HMGB1 (Fig. 2F). These results indicate that extracellular ATP induces HMGB1 release from Kupffer cells, and also suggest that the release is associated with P2X7 receptor-mediated necrotic cell death.

Prostaglandin E2 (PGE2) is lipid mediator of various physiological responses, and induces inflammation-associated pain and fever. Its synthesis involves a two-step mechanism: signal 1 increases arachidonic acid and signal 2 induces PGE2 synthesis

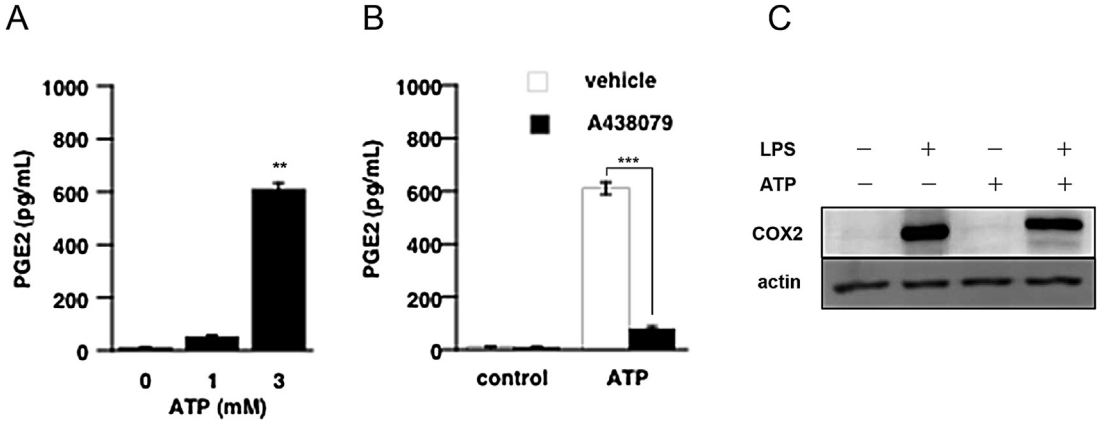

Fig. 3. PGE2 release and COX-2 expression after activation of P2X7 receptor (A, B) KUP5 cells were incubated with the indicated dose of ATP for 3 h (A). Cells were pre-incubated with 50 μM A438079 for 30 min, and incubated with vehicle (control) or 3 mM ATP for 3 h (B). The culture supernatant was harvested for determination of PGE2. Concentrations of PGE2 in culture supernatants were measured by EIA as described in Materials and Methods. Error bars indicate ± SE (n = 4). Significant differences between indicated groups are indicated with ***($p < 0.001$) and **($p < 0.01$). (C) COX-2 was measured by Western blotting in naïve cells or LPS-primed cells subsequently stimulated with ATP for 30 min.

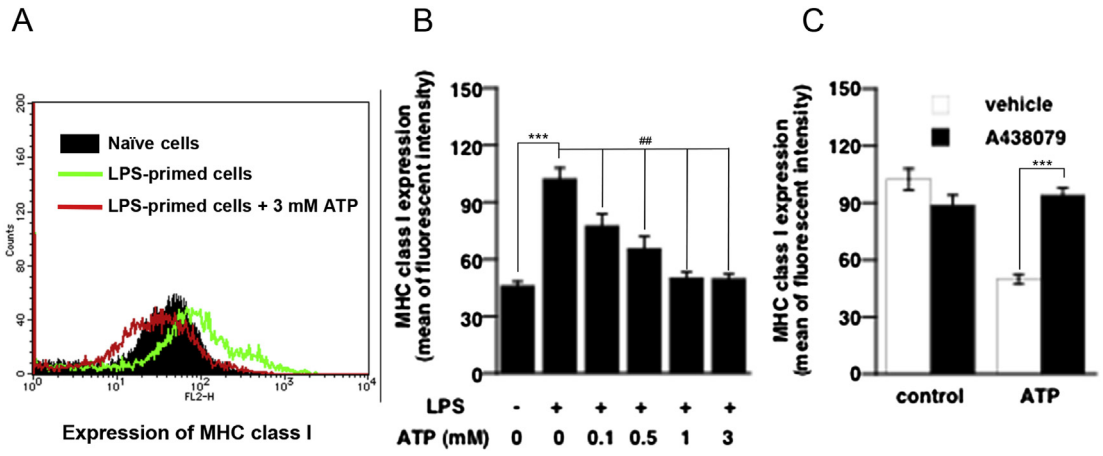

Fig. 4. ATP-induced MHC I decrease via P2X7 receptor. KUP5 cells were stained with PE-conjugated anti-MHC I (H-2K^b) antibody, and the fluorescence intensity was analyzed by flow cytometry. (A) Naïve cells are shown in black histogram. LPS-primed KUP5 cells were treated with vehicle (control; green line) or 3 mM ATP for 30 min (red line). (B) Naïve cells or LPS-primed cells were incubated with the indicated dose of ATP for 30 min. (C) LPS-primed cells were pre-incubated with 50 μM A438079 for 5 min, and then incubated with vehicle (control) or 3 mM ATP for 30 min. Error bars indicate ± SE (n = 4). Significant differences between indicated groups are indicated with $^{***}(p < 0.001)$ and $^{\#\#}(p < 0.01)$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

from arachidonic acid by COX-2. It was recently reported that activation of P2X7 receptor induces PGE2 release without affecting COX-2 expression [27]. Therefore, we examined the effect of ATP on PGE2 release from KUP5 cells. Treatment with 3 mM ATP significantly induced PGE2 release, and this release was abolished by pretreatment with A438079 (Fig. 3A, B). Expression of COX-2 in KUP5 cells was increased by priming with LPS, but was not affected by ATP treatment (Fig. 3C). These results are consistent with previous findings [27], and suggest that extracellular ATP induces PGE2 release through activation of P2X7 receptor without increasing COX-2 expression in Kupffer cells.

Major histocompatibility complex class I (MHC I) molecules are expressed on the surface of almost all cells, and play a role in presenting intracellular peptide antigens to cytotoxic CD8$^+$ T cells. Several studies have found that P2X7 receptor induces shedding of membrane proteins [10]. Recently, it was reported that membrane expression of MHC I is decreased by activation of P2X7 receptor [12]. We examined whether ATP treatment changes the surface expression of MHC I by means of flow cytometry. Priming of KUP5 cells with LPS led to up-regulation of MHC I. We found that treatment with ATP induced a significant and dose-dependent decrease of membrane MHC I expression (Fig. 4A, B). Pretreatment with A438079 suppressed the ATP-induced decrease of surface MHC I expression (Fig. 4C). These results indicate that P2X7 receptor activation leads to decreased plasma membrane MHC I expression. Though the meaning of this phenomenon remains to be fully established, it appears that both LPS and P2X7 receptor modulate Kupffer cell-mediated immune responses.

Overall, our results show that extracellular ATP-induced activation of P2X7 receptor induces secretion of pro-inflammatory molecules such as IL-1β, PGE2, as well as release of intracellular molecules, such as HMGB1, from dead cells. Based on these findings, we suggest that extracellular ATP plays an important role in both acute and chronic inflammation in liver through activation of P2X7 receptor in Kupffer cells. The resulting secretion of inflammatory cytokines and lipid mediators could result in liver damage. Among these compounds, IL-1β initiates an inflammatory response. It is noteworthy that HMGB1, a post-inflammatory mediator that is released during prolonged inflammation, plays a significant role in massive production of cytokines such as IL-1β, suggesting that P2X7 receptor activation-mediated HMGB1 release further enhances the IL-1β-mediated inflammatory response.

Reconstruction of damaged liver cells induces hepatic fibrosis, which may eventually lead to liver cirrhosis [9]. It is reported that P2X7 receptor has a potentially pro-fibrotic function [20]. In accordance with this, we observed that ATP treatment induces fibronectin mRNA expression at 6 h after ATP treatment via activation of P2X7 receptor (Supplementary Data; Fig. S1), though we did not detect increased protein expression of fibronectin under our experimental conditions, because cell death occurred within 6 h after activation of P2X7 receptor. However, if Kupffer cells were exposed to a high concentration of ATP in the liver, expression of fibronectin might be increased, leading to release of fibronectin into the extracellular space as extracellular matrix (ECM), because various growth factors in blood and liver in vivo would attenuate or delay ATP-induced cell death. In conclusion, we have shown here that extracellular ATP induces activation of P2X7 receptor in Kupffer cells, and this leads to activation of multiple inflammatory functions. Taken together, these results indicate that P2X7 receptor-dependent inflammatory responses in Kupffer cells play an important role in liver inflammation, liver damage, and ultimately cirrhosis. P2X7 receptor might be a new therapeutic target for treatment of liver diseases.

Conflict of interest

None.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.02.011.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.02.011.

References

[1] G. Kolios, V. Valatas, E. Kouroumalis, Role of Kupffer cells in the pathogenesis of liver disease, World J. Gastroenterol. 12 (2006) 7413–7420.

[2] D.L. Laskin, A.M. Pilaro, S. Ji, Potential role of activated macrophages in acetaminophen hepatotoxicity. II. Mechanism of macrophage accumulation and activation, Toxicol. Appl. Pharmacol. 86 (1986) 216–226.

[3] S.D. Panuganti, F.D. Khan, C.K. Svensson, Enhanced xenobiotic-induced hepatotoxicity and Kupffer cell activation by restraint-induced stress, J. Pharmacol. Exp. Ther. 318 (2006) 26–34.

[4] V. Thakur, M.R. McMullen, M.T. Pritchard, L.E. Nagy, Regulation of macrophage activation in alcoholic liver disease, J. Gastroenterol. Hepatol. 22 (Suppl. 1) (2007) S53–S56.

[5] C. Liu, Q. Tao, M. Sun, J.Z. Wu, W. Yang, P. Jian, J. Peng, Y. Hu, C. Liu, P. Liu, Kupffer cells are associated with apoptosis, inflammation and fibrotic effects in hepatic fibrosis in rats, Lab. Invest. 90 (2010) 1805–1816.

[6] L. Alric, C. Orfila, N. Carrere, M. Beraud, G. Carrera, J.C. Lepert, M. Duffaut, B. Pipy, J.P. Vinel, Reactive oxygen intermediates and eicosanoid production by Kupffer cells and infiltrated macrophages in acute and chronic liver injury induced in rats by CCl4, Inflamm. Res. 49 (2000) 700–707.

[7] S.L. Friedman, Molecular regulation of hepatic fibrosis, an integrated cellular response to tissue injury, J. Biol. Chem. 275 (2000) 2247–2250.

[8] S.W. Luckey, D.R. Petersen, Activation of Kupffer cells during the course of carbon tetrachloride-induced liver injury and fibrosis in rats, Exp. Mol. Pathol. 71 (2001) 226–240.

[9] J. Lin, J.F. Wu, Q. Zhang, H.W. Zhang, G.W. Cao, Virus-related liver cirrhosis: molecular basis and therapeutic options, World J. Gastroenterol. 20 (2014) 6457–6469.

[10] G. Burnstock, J.M. Boeynaems, Purinergic signalling and immune cells, Purinergic Signal. 10 (2014) 529–564.

[11] A. Surprenant, F. Rassendren, E. Kawashima, R.A. North, G. Buell, The cytolytic P2Z receptor for extracellular ATP identified as a P2X receptor (P2X7), Science 272 (1996) 735–738.

[12] A. Baroja-Mazo, M. Barbera-Cremades, P. Pelegrin, P2X7 receptor activation impairs exogenous MHC class I oligopeptides presentation in antigen presenting cells, PLoS One 8 (2013) e70577.

[13] S.R. Taylor, C.M. Turner, J.I. Elliott, J. McDaid, R. Hewitt, J. Smith, M.C. Pickering, D.L. Whitehouse, H.T. Cook, G. Burnstock, C.D. Pusey, R.J. Unwin, F.W. Tam, P2X7 deficiency attenuates renal injury in experimental glomerulonephritis, J. Am. Soc. Nephrol. 20 (2009) 1275–1281.

[14] J. Li, D. Liu, H.Z. Ke, R.L. Duncan, C.H. Turner, The P2X7 nucleotide receptor mediates skeletal mechanotransduction, J. Biol. Chem. 280 (2005) 42952–42959.

[15] R. Hoque, M.A. Sohail, S. Salhanick, A.F. Malik, A. Ghani, S.C. Robson, W.Z. Mehal, P2X7 receptor-mediated purinergic signaling promotes liver injury in acetaminophen hepatotoxicity in mice, Am. J. Physiol. Gastrointest. Liver Physiol. 302 (2012) G1171–G1179.

[16] S. Chatterjee, R. Rana, J. Corbett, M.B. Kadiiska, J. Goldstein, R.P. Mason, P2X7 receptor-NADPH oxidase axis mediates protein radical formation and Kupffer cell activation in carbon tetrachloride-mediated steatohepatitis in obese mice, Free. Radic. Biol. Med. 52 (2012) 1666–1679.

[17] H. Kitani, C. Sakuma, T. Takenouchi, M. Sato, M. Yoshioka, N. Yamanaka, Establishment of c-myc-immortalized Kupffer cell line from a C57BL/6 mouse strain, Results Immunol. 4 (2014) 68–74.

[18] A. Kawano, M. Tsukimoto, D. Mori, T. Noguchi, H. Harada, T. Takenouchi, H. Kitani, S. Kojima, Regulation of P2X7-dependent inflammatory functions by P2X4 receptor in mouse macrophages, Biochem. Biophys. Res. Commun. 420 (2012) 102–107.

[19] T. Noguchi, K. Ishii, H. Fukutomi, I. Naguro, A. Matsuzawa, K. Takeda, H. Ichijo, Requirement of reactive oxygen species-dependent activation of ASK1-p38 MAPK pathway for extracellular ATP-induced apoptosis in macrophage, J. Biol. Chem. 283 (2008) 7657–7665.

[20] R. Auger, I. Motta, K. Benihoud, D.M. Ojcius, J.M. Kanellopoulos, A role for mitogen-activated protein kinase (Erk1/2) activation and non-selective pore formation in P2X7 receptor-mediated thymocyte death, J. Biol. Chem. 280 (2005) 28142–28151.

[21] J. Amstrup, I. Novak, P2X7 receptor activates extracellular signal-regulated kinases ERK1 and ERK2 independently of Ca^{2+} influx, Biochem. J. 374 (2003) 51–61.

[22] K.A. Hogquist, M.A. Nett, E.R. Unanue, D.D. Chaplin, Interleukin 1 is processed and released during apoptosis, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 8485–8489.

[23] C.A. Dinarello, Blocking IL-1 in systemic inflammation, J. Exp. Med. 201 (2005) 1355–1359.

[24] D. Ferrari, C. Pizzirani, E. Adinolfi, R.M. Lemoli, A. Curti, M. Idzko, E. Panther, F. Di Virgilio, The P2X7 receptor: a key player in IL-1 processing and release, J. Immunol. 176 (2006) 3877–3883.

[25] S. Kojima, Y. Negishi, M. Tsukimoto, T. Takenouchi, H. Kitani, K. Takeda, Purinergic signaling via P2X7 receptor mediates IL-1beta production in Kupffer cells exposed to silica nanoparticle, Toxicology 321 (2014) 13–20.

[26] U. Andersson, D.J. Antoine, K.J. Tracey, The functions of HMGB1 depend on molecular localization and post-translational modifications, J. Intern. Med. 276 (2014) 420–424.

[27] M. Barbera-Cremades, A. Baroja-Mazo, A.I. Gomez, F. Machado, F. Di Virgilio, P. Pelegrin, P2X7 receptor-stimulation causes fever via PGE2 and IL-1beta release, FASEB J. 26 (2012) 2951–2962.