CD38-mediated \( \text{Ca}^{2+} \) Signaling Contributes to Angiotensin II-induced Activation of Hepatic Stellate Cells

ATTENUATION OF HEPATIC FIBROSIS BY CD38 ABLATION

Received for publication, October 14, 2009. Published, JBC Papers in Press, November 12, 2009, DOI 10.1074/jbc.M109.076216

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CD38 is a type II glycoprotein that is responsible for the synthesis and hydrolysis of cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), \( \text{Ca}^{2+} \)-mobilizing second messengers. The activation of hepatic stellate cells (HSCs) is a critical event in hepatic fibrosis because these cells are the main producers of extracellular matrix proteins in the liver. Recent evidence indicates that the renin-angiotensin system plays a major role in liver fibrosis. In this study, we showed that angiotensin II (Ang II) evoked long lasting \( \text{Ca}^{2+} \) rises and induced NAADP or cADPR productions via CD38 in HSCs. Inositol 1,4,5-trisphosphate as well as NAADP-induced initial \( \text{Ca}^{2+} \) transients were prerequisite for the production of cADPR, which was responsible for later sustained \( \text{Ca}^{2+} \) rises in the Ang II-treated HSCs. Ang II-mediated inositol 1,4,5-trisphosphate- and NAADP-stimulated \( \text{Ca}^{2+} \) signals cross-talked in a dependent manner with each other. We also demonstrated that CD38 plays an important role in Ang II-induced proliferation and overproduction of extracellular matrix proteins in HSCs, which were reduced by an antagonistic cADPR analog, 8-bromo-cADPR, or in CD38−/− HSCs. Moreover, we presented evidence to implicate CD38 in the bile duct ligation-induced liver fibrogenesis; infiltration of inflammatory cells and expressions of \( \alpha \)-smooth muscle actin, transforming growth factor-\( \beta \)1, collagen \( \alpha I(1) \), and fibronectin were reduced in CD38−/− mice compared with those in CD38+/+ mice. These results demonstrate that CD38-mediated \( \text{Ca}^{2+} \) signals contribute to liver fibrosis via HSCs activation, suggesting that intervention of CD38 activation may help prevent hepatic fibrosis.

Hepatic fibrosis represents a major medical problem worldwide with significant morbidity and mortality. Chronic stimuli like alcohol consumption, viral infection, cholestasis, or metabolic disorders result in the deposition of scar tissue and the development of cirrhosis. Hepatic stellate cells (HSCs) are the major players during liver fibrogenesis (1). Upon liver injury, normally quiescent HSCs become activated, undergo profound morphological changes, and transdifferentiate into myofibroblast-like cells. This process is termed “HSC activation,” in which de novo expression of \( \alpha \)-smooth muscle actin (SMA), enhanced cell proliferation, and excessive production of extracellular matrix (ECM) are the most characteristic features (2). HSCs are activated by a variety of hormones or growth factors, including angiotensin II (Ang II) (3–6). There is evidence that the renin-angiotensin system components are up-regulated in HSCs isolated from human cirrhotic livers and in cultured HSCs (7). Moreover, previous studies have revealed that an activated HSC expresses Ang II type 1 receptor (7), and blockade of Ang II type 1 receptor has been shown to attenuate hepatic fibrosis in an animal model (8). Furthermore, therapeutic efficacy of an Ang II type 1 receptor blocker in a patient with nonalcoholic steatohepatitis has also been reported (9).

Cells possess multiple \( \text{Ca}^{2+} \) stores and multiple \( \text{Ca}^{2+} \)-mobilizing messenger molecules (10–13). These include inositol 1,4,5-trisphosphate (IP₃), cyclic adenosine diphosphoribose (cADPR), and nicotinic acid adenine dinucleotide phosphate (NAADP). It is generally accepted that \( \text{Ca}^{2+} \) stores for IP₃ and cADPR are located in endoplasmic reticulum (ER) in most mammalian cells, whereas \( \text{Ca}^{2+} \) stores for NAADP are localized in acidic organelles (11–13). The IP₃ receptor (IP₃R) and receptor for cADPR (ryanodine receptor) have been well characterized (13, 14); however, one isoform of two-pore channels (TPC2) has recently been proposed as a receptor for NAADP (15). Intriguingly, the receptor for NAADP has a property of desensitization with a high concentration of NAADP (16). The multifunctional ectoenzyme CD38 synthesizes cADPR and NAADP (17). Recently, CD38 was identified as a key regulator of HSC activation (18). However, the precise mechanism underlying the association between CD38 and HSC activation in liver fibrosis remains to be resolved.

In this study, we studied whether Ang II-mediated CD38 activation can induce an increase of intracellular \( \text{Ca}^{2+} \) concentration (\([\text{Ca}^{2+}]_{i}\)) in HSCs via NAADP and/or cADPR production, ECM protein accumulation, and proliferation. We also attempted to elucidate the mechanism of CD38 involvement in Ang II-induced HSC activation and evaluated preventive potential of genetic ablation of CD38 in the development and growth factor \( \beta 1 \); ECM, extracellular matrix; ER, endoplasmic reticulum; Col-I, collagen \( \alpha I(1) \); Br, bromo.
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Isolation of Mouse Stellate Cells—Mouse hepatic stellate cells were isolated as described previously (8). In brief, livers of 6- to 8-week-old male mice (20–25 g body mass) were perfused first with a Ca\(^{2+}\)/Mg\(^{2+}\)-free solution (137 mM NaCl, 5.4 mM KCl, 0.6 mM NaH\(_2\)PO\(_4\)/2H\(_2\)O, 0.8 mM Na\(_2\)HPO\(_4\)/12H\(_2\)O, 10 mM Hepes, 0.5 mM EGTA, 4.2 mM NaHCO\(_3\), and 5 mM glucose, pH 7.4) at 37 °C and next with 0.015% collagenase A (Roche Applied Science) and 0.1% Pronase E (Roche Applied Science) solution (137 mM NaCl, 5.4 mM KCl, 0.6 mM NaH\(_2\)PO\(_4\)/2H\(_2\)O, 0.8 mM Na\(_2\)HPO\(_4\)/12H\(_2\)O, 3.8 mM CaCl\(_2\), 10 mM Hepes, 4.2 mM NaHCO\(_3\), pH 7.4) at 37 °C. The digested liver was excised, dispersed in Ca\(^{2+}\)/Mg\(^{2+}\)-free solution, and filtered through gauzes. Residual hepatocytes were removed twice by a low speed centrifugation (50 × g, 4 °C, 2 min). The nonparenchymal cells were pelleted by centrifugation (450 × g, 4 °C, 10 min). A stellate cell-enriched fraction was obtained by the use of centrifugation with a triple-layered (9, 11, and 17%) Nycodenz cushion (Sigma-Aldrich, 1,400 g/lane) supplemented with 10% fetal calf serum (Invitrogen) and antibiotics. After plating, the culture medium was changed every other day.

\[^{3}H\]Thymidine Incorporation Assay—DNA synthesis was measured by the incorporation of methyl-\[^{3}H\]thymidine (Amersham Biosciences) as described previously (19). Before measuring the incorporation, HSCs were pretreated with 8-Br-cADPR or losartan for 30 min and then incubated with Ang II for 24 h. HSCs were then incubated in the same medium with 1.0 µCi/ml \[^{3}H\]thymidine for 6 h at 37 °C. The cells were then washed once with phosphate-buffered saline treated with ice-cold 5% trichloroacetic acid at 4 °C for 15 min, and then washed twice in 5% trichloroacetic acid. The acid-insoluble material was dissolved in 2 × NaOH at room temperature and counted for radioactivity by liquid scintillation counting. All experiments were performed in triplicate.

Immunoblotting—Whole-cell extracts and liver extracts were prepared as described previously (19). Proteins (20 µg/lane) were resolved on 8 or 12% SDS-PAGE gel and transferred to polyvinylidine difluoride membranes (GE Healthcare). Antibodies against α-SMA (DakoCytomation, Carpinteria, CA), TGF-β1, collagen αI(1) (Col-I), and fibronectin (Santa Cruz Biotechnology, Santa Cruz, CA) were used. Horse-radish peroxide-conjugated secondary antibodies (Santa Cruz Biotechnology) were used and visualized using enhanced...
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Chemiluminescence. All immunoreactive signals were analyzed by densitometric scanning (Fuji Photo Film Co., Tokyo, Japan).

**Measurement of [Ca\textsuperscript{2+}]**—The measurement method is described. Changes of [Ca\textsuperscript{2+}] in HSCs were determined as described previously (20). HSCs grown to near confluence were made quiescent by serum deprivation overnight at 37 °C. Serum-starved cells were incubated with 5 μM Fluoro-3 AM (Molecular Probes) in Hank’s balanced salt solution (2 mM CaCl\textsubscript{2}, 145 mM NaCl, 5 mM KCl, 1 mM MgCl\textsubscript{2}, 5 mM D-glucose, and 20 mM HEPES, pH 7.3) at 37 °C for 40 min. The cells were washed three times with Hank’s balanced salt solution. Changes of [Ca\textsuperscript{2+}], were determined at 488 nm excitation/530 nm emission by air-cooled argon laser system. The emitted fluorescence at 530 nm was collected using a photomultiplier. The image was scanned using a confocal microscope (Nikon). For the calculation of [Ca\textsuperscript{2+}], the method of Tsien et al. (21) was used with the following equation: \[ K_d (F - F_{	ext{min}})/F_{\text{max}} - F, \] where \( K_d \) is 450 nm for fluo-3, and \( F \) is the observed fluorescence level. Each tracing was calibrated for the maximal intensity (\( F_{\text{max}} \)) by addition of ionomycin (8 μM) and for the minimal intensity (\( F_{\text{min}} \)) by addition of EGTA 50 mM at the end of each measurement.

**Measurement of Intracellular cADPR Concentration**—The sample extraction was performed as described previously (20, 21). cADPR was measured by some modification of the cycling method described previously (20). Briefly, cells were treated with 0.3 ml of 0.6 M perchloric acid under sonication. Precipitates were removed by centrifugation at 20,000 × g for 10 min. Perchloric acid was removed by mixing the aqueous sample with a solution containing 3 volumes of 1,1,2-trichlorotrifluoroethane to 1 volume of tri-n-octylamine. After centrifugation for 10 min at 1,500 × g, the aqueous layer was collected and neutralized with 20 mM sodium phosphate pH 8.0. To remove all contaminating nucleotides, the samples were incubated with the following hydrolytic enzymes overnight at 37 °C: 0.44 unit/ml nucleotide pyrophosphatase, 0.0625 units/ml NAD glycohydrolase, and 2.5 mM unit/ml nucleotide pyrophosphatase, 12.5 units/ml alkaline phosphatase, 0.0625 units/ml Aplysia ADP-ribosyl cyclase, 30 mM nicotinamide, and 100 mM MgCl\textsubscript{2} in 20 mM sodium phosphate buffer, pH 8.0. Enzymes to convert cADPR to NAADP, 0.0625 units/ml NaADP glycohydrolase, and 20 mM HEPES, pH 7.3) at 37 °C for 40 min. The cells were washed three times with Hank’s balanced salt solution. Changes of [Ca\textsuperscript{2+}], were determined at 488 nm excitation/530 nm emission by air-cooled argon laser system. The emitted fluorescence at 530 nm was collected using a photomultiplier. The image was scanned using a confocal microscope (Nikon). For the calculation of [Ca\textsuperscript{2+}], the method of Tsien et al. (21) was used with the following equation: \[ K_d (F - F_{	ext{min}})/F_{\text{max}} - F, \] where \( K_d \) is 450 nm for fluo-3, and \( F \) is the observed fluorescence level. Each tracing was calibrated for the maximal intensity (\( F_{\text{max}} \)) by addition of ionomycin (8 μM) and for the minimal intensity (\( F_{\text{min}} \)) by addition of EGTA 50 mM at the end of each measurement.

**Measurement of Intracellular NAADP Concentration**—The level of NAADP was measured using a cyclic enzymatic assay as described previously (21).

**Measurement of IP\textsubscript{3}**—Intracellular IP\textsubscript{3} was measured as described previously (22). Briefly, HSCs were incubated with

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**FIGURE 2. Ang II-evoked [Ca\textsuperscript{2+}] rise is mediated by IP\textsubscript{3}/NAADP/cADPR.** A, increases of [Ca\textsuperscript{2+}], by Ang II in CD38\textsuperscript{−/−} HSCs, B, the later phase of [Ca\textsuperscript{2+}], was abolished in CD38\textsuperscript{−/−} HSCs by Ang II. C, Ang II-mediated sustained increase of [Ca\textsuperscript{2+}], increases were inhibited by pretreatment with 8-Br-cADPR (100 μM). D and E, pretreatment of HSCs with NAADP (100 μM) and XeC (2 μM) completely blocked Ang II-induced [Ca\textsuperscript{2+}], F, changes in [Ca\textsuperscript{2+}], at 80 and 150 s, *p < 0.001 versus basal [Ca\textsuperscript{2+}], #p < 0.05 versus Ang II. Values are means ± S.E. of three independent experiments. The arrow indicates the time of Ang II addition.

**Histological Analysis**—Formalin-fixed liver tissues were decalcified in EDTA for 5–7 days, dehydrated, and embedded in paraffin. Serial sections (4 μm) were stained with hematoxylin-eosin. The extent of inflammation was evaluated on glass slides, deparaffinized with xylene, and rehydrated with graded ethanol. Endogenous peroxidase was blocked with 3% hydrogen peroxide for 20 min, and slides were rinsed with phosphate-buffered saline. After protein blocking, slides were incubated overnight at 4 °C with a primary antibody for α-SMA...
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(1:100; DakoCytomation). The stained sections were then incubated with biotinylated secondary antibody (DakoCytomation), and the immune complexes were detected using an ABC kit according to manufacturer’s instructions. The sections were finally counterstained with hematoxylin before mounting. The area of positive staining was determined by computerized image analysis (LUZEX F, Nikon).

Statistical Analysis—Data represent means ± S.E. of at least three separate experiments. Statistical comparisons were performed using one-way analysis of variance followed by Scheffe’s test. Statistical significance of difference between groups was determined using Student’s t test. Differences were considered significant if the p value was <0.05.

RESULTS

Ang II-mediated cADPR Production by CD38 Is Essential in HSC Activation, Proliferation, and ECM Protein Accumulation—Because α-SMA is a sensitive marker of activated HSCs in situ, it has increasingly been used as an early indicator of fibrogenic activity in human liver disease, even before ECM accumulates (23). To elucidate a possibility of an association between CD38 and HSC activation in liver fibrosis, we compared the α-SMA expression in HSCs isolated from CD38+/- and CD38-/- mice after 48 h of treatment with Ang II. As seen in Fig. 1, A and B, the increase of α-SMA protein in CD38-/- HSCs in response to Ang II was consistently lower than in CD38+/- HSCs. Treatment of HSCs with Ang II stimulated proliferative responses as demonstrated by an increase in [3H]thymidine uptake (Fig. 1C). Pretreatment of HSCs for 30 min with 8-Br-cADPR, an antagonist analog of cADPR, significantly inhibited Ang II-induced DNA synthesis. We also confirmed the inhibitory effect of losartan, an Ang II type 1 receptor blocker, on HSCs proliferation. Ang II treatment up-regulated TGF-β1 and Col-I levels and that these up-regulations were abrogated by pretreatment of HSCs with 8-Br-cADPR or losartan (Fig. 1, D and E).

CD38 Ablation Attenuates Ang II-Induced Sustained Intracellular Ca2+ Increases in HSCs—To explore the role of CD38 in Ang II-mediated Ca2+ signaling in HSCs, we compared Ca2+ signaling in HSCs from CD38+/+ and CD38-/- mice. Ang II evoked sustained intracellular Ca2+ increase in CD38+/+ HSCs; however, the later phase of sustained Ca2+ signal was not detected in the Ang II-treated HSCs from CD38-/- mice (Fig. 2, A and B). Pretreatment of HSCs with 8-Br-cADPR blocked the sustained Ca2+ increase, similar to that shown in

CD38+/+ HSCs (Fig. 2C), suggesting that Ang II-mediated long lasting Ca2+ signaling in HSCs is due to the action of cADPR. Pretreatment of HSCs with high concentration of NAADP or XeC, an IP3R blocker, led to a complete blockade of the Ang II-induced intracellular Ca2+ response, including initial Ca2+ transient in HSCs (Fig. 2, D and E). Thus, the Ang II-mediated Ca2+ signal in HSCs consists of two phases of Ca2+ signals; initial Ca2+ transient is dependent on both IP3 and NAADP, whereas later sustained Ca2+ signal on cADPR (Fig. 2F).

Ang II Stimulates cADPR Production following NAADP Production in HSCs—To elucidate whether cADPR and NAADP are produced in Ang II-treated HSCs, we measured intracellular cADPR or NAADP concentrations before and after treatment of HSCs with Ang II. As shown in Fig. 1A, NAADP was produced ~10 s earlier than cADPR with its initial peak at 10 s following Ang II treatment, and the levels of NAADP and cADPR were sustained with similar patterns until 120 s. Moreover, exogenous NAADP evoked cADPR production directly in HSCs, whereas exogenous cADPR did not induce NAADP production (Fig. 3B), indicating that NAADP-mediated initial Ca2+ increase is required for cADPR production. Ang II-mediated cADPR production was blocked by pretreatment of the cells with high concentrations of NAADP, XeC, and losartan, but not by the Ca2+-free condition (Fig. 3C). Therefore, it is highly likely that exogenous NAADP could induce a Ca2+ signal similar to that of Ang II-mediated Ca2+ increase in HSCs. Indeed, NAADP was found to elicit a Ca2+ increase similar to
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Characterization of the Ang II-induced Initial Phase of Ca\(^{2+}\) Signals in HSCs—Figs. 2 and 3 show that NAADP and IP\(_3\) contributed to initial phase of Ca\(^{2+}\) increase and cADPR formation in the Ang II-treated HSCs. When the Ca\(^{2+}\) pool in the ER was emptied by inhibiting the sarcoplasmic/endoplasmic reticulum Ca\(^{2+}\) ATPase pumps with thapsigargin, the Ang II-induced Ca\(^{2+}\) increase was not detected (Fig. 4A). In addition, bafilomycin A1, which is known to block the vacuolar H\(^+\) ATPase (24), also completely prevented Ang II-mediated Ca\(^{2+}\) increases (Fig. 4B). Fig. 4, C and D show that the NAADP-evoked Ca\(^{2+}\) response was blocked by pretreatment of the cells with thapsigargin as well as XeC. These findings suggest that the NAADP-mediated Ca\(^{2+}\) signal requires IP\(_3\)-induced Ca\(^{2+}\) release from ER, and the IP\(_3\)-mediated Ca\(^{2+}\) signal requires NAADP-induced Ca\(^{2+}\) release from the acidic store. Therefore, we tested the effect of a high concentration of NAADP on Ang II-mediated intracellular IP\(_3\) production and the effect of IP\(_3\)R blocker on NAADP formation induced by Ang II. After pre-treatment with a high concentration of NAADP for 30 min, Ang II was unable to induce intracellular IP\(_3\) increase (Fig. 4E). Furthermore, in the presence of IP\(_3\)R blocker, Ang II did not induce NAADP production in HSCs (Fig. 4F). These data indicate that Ang II-mediated IP\(_3\) production can be affected by NAADP-induced Ca\(^{2+}\) increase and that Ang II-mediated NAADP production depends also on IP\(_3\)-mediated Ca\(^{2+}\) release in HSCs. Taken together, these results suggest that NAADP and IP\(_3\)-elicited Ca\(^{2+}\) releases from the acidic organelles and ER Ca\(^{2+}\) stores, respectively, have a cross-talk in the Ang II-mediated Ca\(^{2+}\) signals in HSCs.

Liver Fibrosis Is Markedly Attenuated in CD38 Knockout Mice after BDL—Because blockade of the Ang II receptor has been previously reported to attenuate BDL-induced hepatic fibrosis and our present findings showed that Ang II receptor signaling in HSCs was mediated by CD38, we examined the role of CD38 played in liver fibrosis, by applying the BDL-induced fibrosis model in CD38\(^{+/+}\) and CD38\(^{-/-}\) mice. Following BDL for 4 weeks, the degree of liver injury was attenuated in CD38\(^{-/-}\) mice, as demonstrated by lower serum liver enzymes levels (Table 1) and lower hepatocytes necrosis and inflammatory cell infiltration than those in CD38\(^{+/+}\) mice (Fig. 5A). Because \(\alpha\)-SMA is primarily responsible for the overproduction of ECM at pathogenic conditions and also indicates HSC activation, we investigated the activation of HSCs by assessing the expression of \(\alpha\)-SMA after BDL. Fig. 5B illustrates the expression of hepatic \(\alpha\)-SMA protein in sham and BDL livers of CD38\(^{+/+}\) and CD38\(^{-/-}\) mice. Compared with sham controls, \(\alpha\)-SMA expression in the BDL-CD38\(^{+/+}\) mice liver was markedly increased, suggesting the activation of hepatic myofibroblasts following BDL-induced injury. However, the induction of \(\alpha\)-SMA in the ligated CD38\(^{-/-}\) mice liver was largely blocked (Fig. 5B). This was validated by Western blot analysis of \(\alpha\)-SMA protein (Fig. 5, C and D). Hepatic expression of TGF\(\alpha\)-1, Col-I, and fibronectin in BDL-CD38\(^{-/-}\) mice was significantly attenuated compared

Table 1

| Enzyme  | Sham CD38\(^{+/+}\) | Sham CD38\(^{-/-}\) | BDL CD38\(^{+/+}\) | BDL CD38\(^{-/-}\) |
|---------|-------------------|-------------------|-------------------|-------------------|
| ALT (U/L) | 30.56 ± 6.43     | 30.64 ± 3.33     | 347.98 ± 66.94    | 290.20 ± 34.11   |
| AST (U/L) | 72.81 ± 10.56    | 76.96 ± 6.74     | 282.07 ± 58.20    | 180.10 ± 43.68   |
| ALP (U/L) | 50.00 ± 3.96     | 48.92 ± 5.94     | 141.64 ± 13.68    | 110.07 ± 7.90    |

\(p < 0.05\) compared with the sham-operated CD38\(^{+/+}\) mice group.

\(p < 0.05\) compared with the bile duct-ligated CD38\(^{+/+}\) mice group. Values are means ± S.E.
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A recent study documented CD38 as a regulator of activation and effector functions of HSCs (18). CD38-immunopositive HSCs were found to increase following the progression of hepatic fibrosis, which was shown in liver biopsies from patients with chronic liver disease (25). CD38 is one of the ADP-ribosyl cyclases, a family of multifunctional enzymes that are seemingly ubiquitous in eukaryotic cells, and ADP-ribosyl cyclases play a key role in several physiological processes, including cell proliferation, muscle contraction, stem cell regeneration, and hormone secretion (17). We previously reported activation mechanisms of Ang II-mediated ADP-ribosyl cyclase in mouse mesangial cells or adult rat cardiomyocytes (19, 26) and also showed that ADP-ribosyl cyclase inhibitions could provide a protective effect on chronic renal and cardiac failure (27, 28). In the present study, we proposed a link between Ang II and CD38 activation during liver fibrosis and presented evidence that Ang II induces fibrogenic actions through CD38 activation in activated HSCs. Our present results showed that the profibrogenic effect of Ang II is associated with an increased concentration of TGF-β1. Previous studies have demonstrated that Ang II induces the expression of TGF-β1 (29, 30) and that TGF-β1 has powerful fibrogenic action. By using cADPR antagonistic analogs, several laboratories have shown that cADPR regulates numerous Ca^{2+} signaling pathways in plants, invertebrates, and vertebrates (13, 31). Our present data revealed that treatment of HSCs with 8-Br-cADPR inhibited Ang II-mediated expression of TGF-β1, Col-I deposition, and cell proliferation. Furthermore, Ang II-mediated sustained Ca^{2+} increase was not observed in CD38−/− HSCs as well as in HSCs pretreated with 8-Br-cADPR, indicating that CD38-mediated Ca^{2+} signals regulate HSC activation, proliferation, and ECM accumulation. The present study identified an Ang II-evoked Ca^{2+} signaling in HSCs with a complex pattern of Ca^{2+} spiking induced by NAADP and IP_3. The Ang II-mediated initial phase of Ca^{2+} increase was abolished completely by either a high concentration of NAADP (100 μM), which is used to desensitize NAADP receptors (32) or by XeC, an IP_3R blocker. Furthermore, NAADP (100 nM)-induced Ca^{2+} signals as well as Ang II-induced NAADP production were blocked by XeC pretreatment. These data together with the

FIGURE 5. Genetic ablation of CD38 attenuates BDL-induced liver fibrosis. A, hematoxylin and eosin staining of liver specimens. BDL-induced necrosis and inflammatory cell infiltration in the liver of CD38−/− and CD38+/+ mice (original magnification ×400). Quantification of inflammatory cells in liver specimens. 25 fields (×400) were studied in each liver sample. *p < 0.01 versus sham-operated CD38+/+ mice liver. # p < 0.05 versus bile duct-ligated CD38−/− mice liver. B, immunohistochemical examination of α-SMA expression in livers of CD38−/− and CD38+/+ mice (original magnification ×200 on upper panel and ×400 on lower panel). C, comparison of expression of α-SMA, TGF-β1, Col-I, and fibronectin in the livers from CD38−/− and CD38+/+ mice following BDL. D, relative expression of each protein compared with β-actin. *p < 0.01 versus sham-operated CD38−/− mice. # p < 0.05 versus bile duct-ligated CD38−/− mice. Values are means ± S.E. of three independent experiments.

with that in CD38+/+ mice (Fig. 5, C and D). These results indicate that CD38 is an important mediator in liver fibrosis.

DISCUSSION

A recent study documented CD38 as a regulator of activation and effector functions of HSCs (18). CD38-immunopositive HSCs were found to increase following the progression of hepatic fibrosis, which was shown in liver biopsies from
result that NAADP-induced Ca\(^{2+}\) increase was abolished by pretreatment with thapsigargin indicate that NAADP-induced Ca\(^{2+}\) signals require ER Ca\(^{2+}\) stores and IP\(_3\) production. Inter-

FIGURE 6. Proposed schematic model of CD38 activation mechanism in HSCs; in HSCs, Ang II binds to the Ang II type 1 receptor and signals to activate CD38 and PLC. Activated CD38 produces NAADP, which, in turn, stimulates Ca\(^{2+}\) release from acidic organelles. IP\(_3\), a product of PLC, also evokes Ca\(^{2+}\) release from ER. Activations of CD38 and PLC are most likely interdependent on each Ca\(^{2+}\) signals, which, in turn, stimulate cADPR production. The numbers denote sequence of events. ES, endosome; LS, lysosome; IP\(_3\), IP\(_3\) receptor; PLC, phospholipase C; RyR, ryanodine receptor.

CD38\(^{−/−}\). These in vivo results indicate that CD38 plays a crucial role in liver fibrosis.

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