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Expression and function of the bile acid receptor TGR5 in Kupffer cells

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ABSTRACT

Kupffer cells are resident macrophages in the liver and play a central role in the hepatic response to injury. Bile acids can impair macrophage function leading to decreased cytokine release. TGR5 is a novel, membrane-bound bile acid receptor, and it has been suggested that the immunosuppressive effect of bile acids can be mediated by TGR5. However, the function of TGR5 in Kupffer cells has not been studied and a direct link between TGR5 and cytokine production in macrophages has not been established. The present study demonstrates that TGR5 is localized in the plasma membrane of isolated Kupffer cells and is responsive to bile acids. Furthermore, bile acids inhibited LPS-induced cytokine expression in Kupffer cells via TGR5-cAMP dependent pathways. TGR5-immunoreactivity in Kupffer cells was increased in rat livers following bile-duct ligation, suggesting that TGR5 may play a protective role in obstructive cholestasis preventing excessive cytokine production thereby reducing liver injury.

Bile acids are essential for dietary lipid absorption and cholesterol excretion. It was recognized only recently that bile acids are signaling molecules with endocrine functions [1]. After uptake into the cell bile acids can activate mitogen-activated protein kinase pathways [2–4] as well as nuclear hormone receptors such as the farnesoid-X-receptor [5,6]. Recently, the first membrane-bound bile acid receptor TGR5 (M-BAR, Gpbar-1) has been described [7,8]. This receptor is activated by extracellular bile acids resulting in an increase in intracellular cyclic AMP (cAMP) content [7,8]. TGR5 mRNA is expressed in many tissues, including liver [7–10]. Our previous study demonstrated localization of TGR5 in sinusoidal endothelial cells (SEC) of rat liver. In isolated SEC stimulation of TGR5 with taurolithocholate (TLC) and taurocholate (TC) increased intracellular cAMP levels and led to an activation of protein kinase A, phosphorylation of endothelial nitric oxide synthase as well as increased nitric oxide production [11]. Furthermore, activation of TGR5 in SEC resulted in an enhanced serine phosphorylation of the CD95 receptor [11], which may trigger the internalization of the receptor from the plasma membrane thereby preventing CD95-induced apoptosis [12].

TGR5 has also been localized in Kupffer cells (KC) of rat liver [11], however the function of TGR5 in KC has not been investigated. KC represent the major source of inflammatory cytokines in the liver [13]. Bile acids can alter Kupffer cell function, affecting both cytokine production as well as phagocytic activity [14–18]. Hydrophobic bile acids, such as lithocholic acid and deoxycholic acid are potent activators of TGR5 [19] and studies with isolated alveolar macrophages have suggested that the suppressive effects of bile acids on cytokine production may be mediated by TGR5 [7], however, direct evidence for the link between TGR5 and cytokine expression was not provided. The purpose of the present study was to determine the role of TGR5 in KC.

Materials and methods

Materials. Cell culture media were from Gibco (Invitrogen, Karlsruhe, Germany). Penicillin/streptomycin were from Biochrom (Berlin, Germany). Foetal calf serum was from PAA (Coelbe, Germany). Taurocholate, taurolithocholate, oleanoloic acid were from Sigma–Aldrich (Taufkirchen, Germany). The TGR5 agonist BR27 was a gift from the Bayer AG (Leverkusen, Germany).

Antibodies. Polyclonal antibodies were raised in guinea pigs (M38) and rabbits (K36) against amino acids 306–329 from the C-terminus of rat TGR5 [11]. The antibodies anti-CD163 (ED2) (Serotec, Duesseldorf, Germany), anti-cytokeratin-19 (Progen, Heidelberg, Germany), anti-GFAP (Chemicon, Hampshire, UK) were used according the manufacturer's instructions.

Isolation of Kupffer cells (KC) and hepatic stellate cells (HSC). KC were isolated from Wistar rats by collagenase-pronase perfusion, separation by a single Nycodenz gradient and centrifugal elutriation [20]. HSC were isolated from Wistar rats using enzymatic

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digestion of liver tissue and enrichment of cells by a single density gradient (8% Nycodenz) [21]. KC and HSC were cultured in RPMI and DMEM medium, respectively, containing 10% FCS and 1% penicillin/streptomycin.

Quantitative realtime PCR. Total RNA was isolated using the RNA extraction kit (Qiagen, Hilden, Germany). After DNA-digestion, 1 μg RNA was reverse transcribed with the QuantiTect Kit (Qiagen). Quantitative realtime PCR was carried out over 40 cycles on an AB7500 (Applied Biosystems, Foster City, CA, USA) using SYBR Green as reporter dye. Data were produced in duplicates for each gene. Mean values of cycle numbers of the target gene (cytokines) were subtracted from the mean of cycle numbers of the housekeeping gene (HPRT) for the respective sample. These values taken to the power of 2 are the mRNA expression of the target genes in relation to HPRT expression. Primers were as follows: HPRT-for: 5′-tgctcgagatgtcatgaagga-3′; HPRT-rev: 5′-cagagggccacaatgtgatg-3′; IL-1α-for: 5′-gatgacctggaggccatagc-3′; IL-1α-rev: 5′-tctgggttggatggtctcttc-3′; IL-1β-for: 5′-agacagctgcactgcaggct-3′; IL-1β-rev: 5′-cagcacgaggcatttttgtt-3′; IL-6-for: 5′-agacttccagccagttgcct-3′; IL-6-rev: 5′-ggcagtggctgtcaacaaca-3′; TNF-α-for: 5′-tgatcggtcccaacaaggag-3′; TNF-α-rev: 5′-tgatgagagggagcccattt-3′.

Measurement of cAMP. After incubation with the respective substance, cells were washed and harvested with 120 μl of Tris–EDTA buffer (0.05 M Tris, pH 7.5; 4 mM EDTA), incubated at 80 °C (2 min) and centrifuged at 12,000*g* (1 min). The supernatant was analyzed using the cyclic AMP(^{3}H) assay from Amersham Biosciences (Freiburg, Germany). Aliquots from each sample were used for protein determination and the total amount of cAMP (pmol)/mg protein was calculated. Relative increase in intracellular cAMP per sample was determined in relation to untreated controls.

Immunofluorescence and confocal laser scanning microscopy. Cells grown on glass coverslips were fixed in 100% methanol (5 min, −20 °C). Cryosections (5 μm) of perfused rat livers were prepared with a Leica cryotome, air-dried and fixed in methanol. Immunofluorescence was carried out as described [11]. The primary antibody dilutions were: M38 1:200, CD163 1:50, CK19 1:50, GFAP 1:200. Fluorescein or cyanine-3 conjugated secondary antibodies (Dianova, Hamburg, Germany) were diluted 1:100 and 1:500, respectively. Immunostained samples were analyzed by a Zeiss LSM 510 META confocal microscope.

Gel electrophoresis and immunoblotting. Cells were lysed as described [11]. Equal protein amounts were separated by SDS–PAGE and blotted onto PVDF membranes. Proteins were visualized by enhanced chemiluminescence (Perkin Elmer, Waltham, MA, USA). Primary antibodies M38 and K36 were diluted 1:5000.

Reporter gene assay. HEK293 cells were co-transfected with a cAMP-sensitive reporter gene construct, rat TGR5-YFP and a *Renilla* expression vector using LipofectAMINE (Invitrogen), as described [11]. The pEYFP-N1 plasmid was used instead of the TGR5-YFP plasmid in control experiments. Cell lysis and luciferase assays were performed using the dual-luciferase kit (Promega, Madison, WI, USA). Transfection efficiency was monitored through co-transfection of a *Renilla* expression vector (Promega) and luciferase activity was normalized to transfection efficiency.

Bile-duct ligation. Following anesthesia (ketamine (80 mg/kg), xylazine (12 mg/kg)) male Sprague–Dawley rats (250–300 g) underwent double ligature of the proximal common bile-duct or sham operation. After three days animals were anesthetized, livers were rinsed with ice-cold saline through the portal vein, removed, frozen in liquid nitrogen and stored at −80 °C. The study protocols were approved by the animal welfare committee Düsseldorf, Germany.

Statistics. Results from a least three independent experiments are expressed as means ± SEM. Results were analyzed using the two-sided Student's *t*-test and a p value < 0.05 was considered statistically significant.

Results

Localization of TGR5 in rat liver

Immunolocalization of TGR5 was studied in rat livers using an antibody directed against the C-terminus of rat TGR5 [11]. TGR5-immunoreactivity was detected in sinusoidal endothelial cells and in Kupffer cells (KC); the latter was shown by the co-localization of the TGR5 specific staining with the CD163 (ED2) fluorescence pattern, a common marker protein of KC (Fig. 1A–C). Furthermore, TGR5 was localized in cytokeratin-19 (CK19)-expressing bile-ducts [22], as demonstrated by the co-localization of the TGR5 staining with the CK19-immunoreactivity (Fig. 1D–I). No TGR5 staining was detected in quiescent hepatic stellate cells, which were visualized by immunofluorescence detection of the glial fibrillary acidic protein (GFAP) (Fig. 1J–L).

Ligation of the common bile-duct serves as a model for obstructive cholestasis with increased serum bile acid concentrations and induction of proinflammatory cytokines [23,24]. Both, elevated bile acid and cytokine levels contribute to the hepatic injury observed in this model [24,25]. Immunofluorescence staining of TGR5 in bile-duct-ligated rats showed a strong increase in the TGR5-immunoreactivity in CD163-positive Kupffer cells as compared to sham-operated control animals, while TGR5 staining in SEC remained unchanged (Fig. 2).

Functional characterization of TGR5 in transfected HEK293

The activity of rat TGR5 was analyzed in HEK293 cells which were co-transfected with the rat TGR5-YFP cDNA as well as a cAMP-responsive reporter gene construct as described in materials and methods. Luciferase activity served as a measure for the intracellular cAMP elevation following stimulation of TGR5 with bile acids. Both taurolithocholate (TLC) and taurocholate (TC) increased luciferase activity dose-dependently and concentrations of 0.1 μM TLC and 0.25 μM TC (Fig. 2A and B; $n = 4$) were sufficient to raise luciferase activity significantly. In the presence of physiological albumin concentrations (4 g/dl) 2-fold higher bile acid concentrations were required, in order to significantly elevate cAMP production as measured by luciferase activity (Fig. 2C–D). However, these bile acid concentrations (0.25 μM for TLC and 0.5 μM for TC; $n = 3$) are in the range of portal venous bile acid levels observed in humans and rats [26–28], which vary between 14 and 43 μM [26].

Localization and function of TGR5 in isolated Kupffer cells

In isolated KC TGR5 was localized in the plasma membrane and in some intracellular compartments (Fig. 3A). Both TGR5 antibodies (M38 and K36) detected a broad band around 70 kDa on Western blot analysis, which is in line with previous data on TGR5 obtained with isolated sinusoidal endothelial cells (Fig. 3B) [11]. Deglycosylation with PNGase F reduced the molecular mass of TGR5 from isolated SEC to 40 kDa [11], which represents the predicted molecular mass of the receptor.

Functional activity of TGR5 in KC was demonstrated by the stimulation of KC with taurolithocholate and taurocholate (25 μM each, 4 min), which led to a 1.7-fold and 1.5-fold increase in intracellular cAMP levels, respectively (Fig. 3C).

KC represent the major source of inflammatory cytokines in the liver [13]. In isolated KC lipopolysaccharide (LPS, 1 ng/ml; 3 h) induced an increase of mRNA levels of the cytokines IL-1α, IL-1β, IL-6 and TNF-α by 129 ± 24, 206 ± 36, 283 ± 49, and 447 ± 101-fold ($n = 19$, mean ± SEM), respectively, compared to controls. Stimulation of KC with TLC (50 μM) for 1 h prior to LPS addition reduced the LPS-induced upregulation of IL-1α,

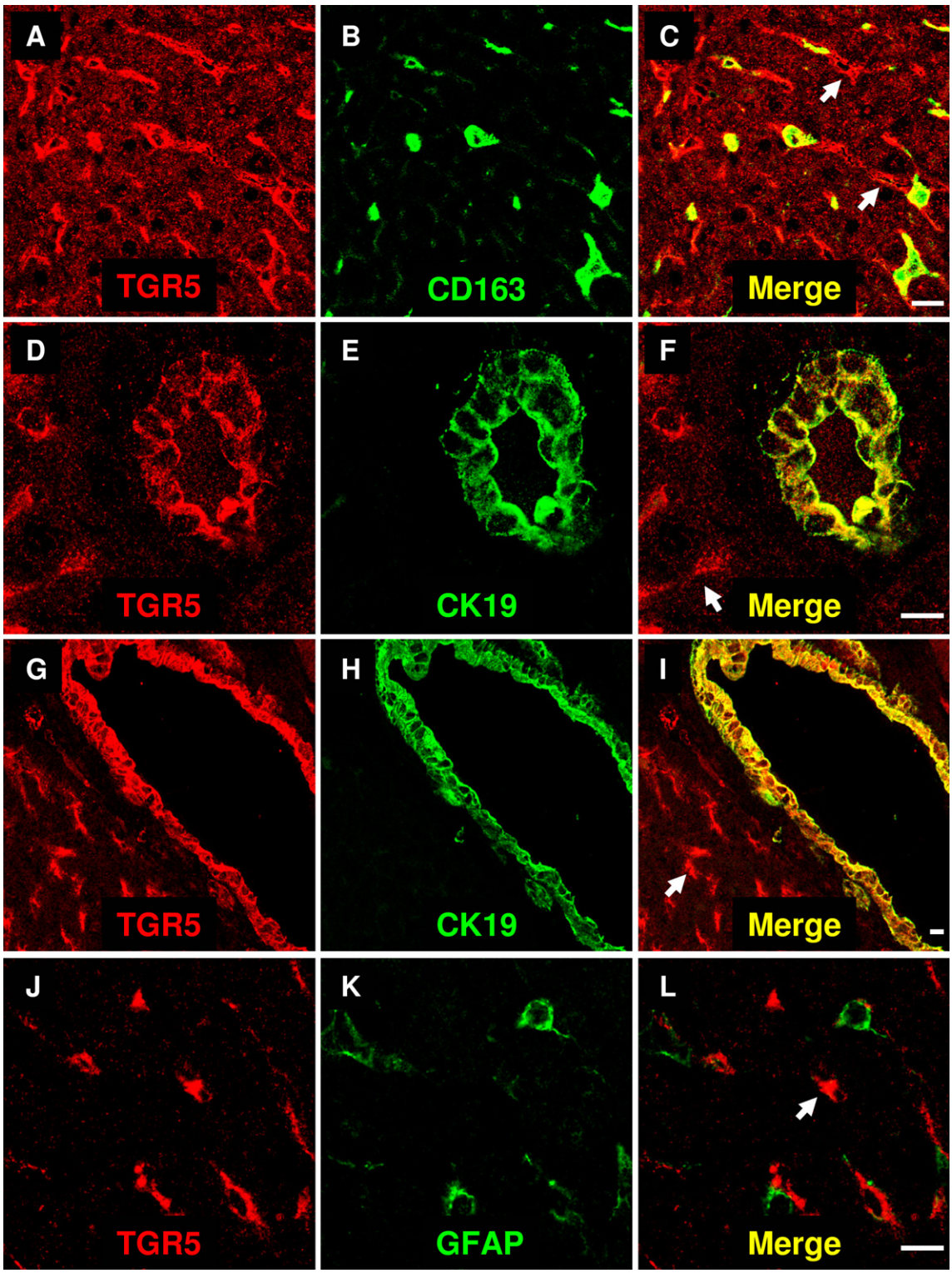

Fig. 1. Immunofluorescence staining of TGR5 in rat liver. TGR5 was detected in sinusoidal endothelial cells (arrows in C, F, I, L) as well as in Kupffer cells, as demonstrated by co-localization of the TGR5-immunoreactivity (A) with the staining obtained from the anti-CD163 (ED2) antibody (B), which is a marker protein for Kupffer cells. The fluorescence pattern of the CK19 (E,H) antibody, which was used to detect cholangiocytes, also co-localized with the TGR5-immunofluorescence (F,I), indicating that TGR5 is expressed in bile-ducts. Quiescent hepatic stellate cells were stained using an antibody against GFAP and showed no TGR5 specific fluorescence. Bars = 10 μM.

IL-1β, IL-6, and TNF-α significantly by 55%, 58%, 42% and 49%, respectively ($n = 10$; Fig. 4D). Dibutyryl cAMP (100 μM) significantly decreased cytokine expression after LPS for all studied cytokines (reduction by 77%, 73%, 68% and 71% for IL-1α, IL-1β, IL-6 and TNF-α ($n = 6$)), indicating that the observed effects may be mediated via cAMP. Oleanoloic acid (10 μM), which has recently been identified as TGR5 agonist [29], also significantly inhibited LPS-induced cytokine expression of IL-1α, IL-1β, IL-6 and TNF-α by 41%, 37%, 43% and 34%, respectively ($n = 6$). A second TGR5 specific agonist (BR27) lowered LPS-induced cytokine expression by 64%, 64%, 67%, and 67% for IL-1α, IL-1β, IL-6 and TNF-α mRNA, respectively, which is comparable to the effects

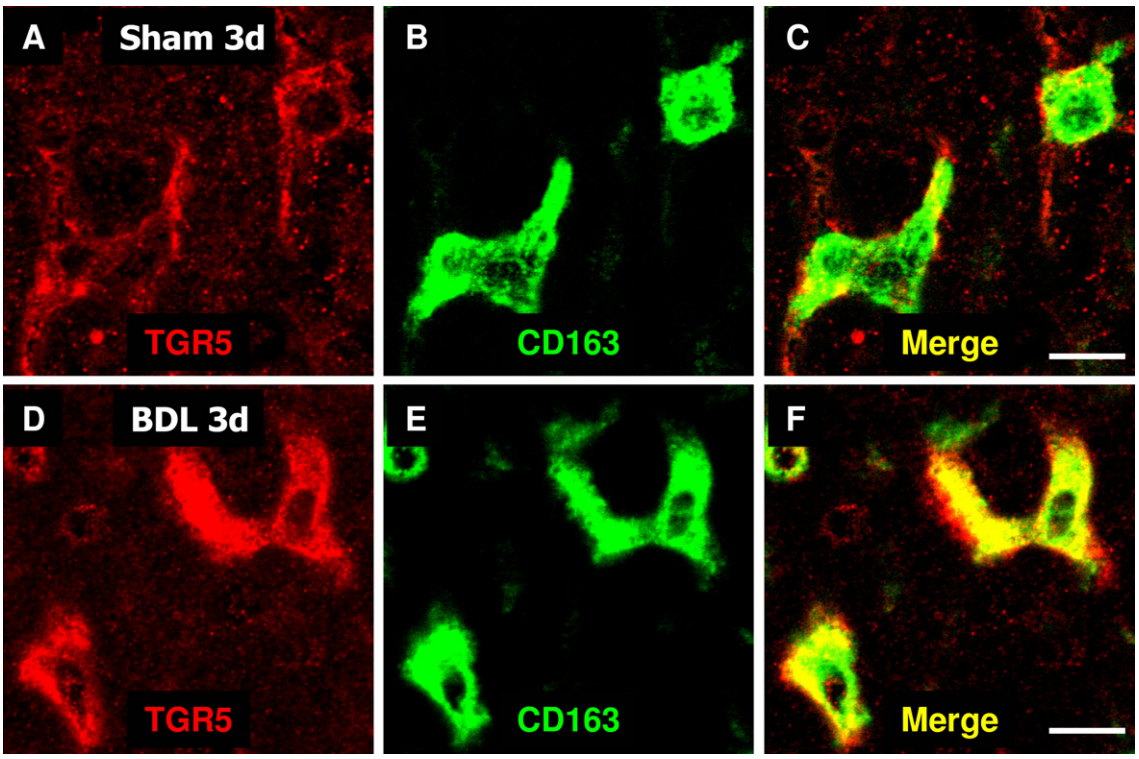

Fig. 2. Bile-duct ligation led to an upregulation of TGR5-immunoreactivity in Kupffer cells. Immunofluorescence staining of TGR5 in liver sections from sham-operated (A–C) and bile-duct-ligated rats (D–F). An antibody directed against CD163 (ED2) was used to detect Kupffer cells (green fluorescence in B,E). The fluorescence pattern from the anti-TGR5 antiserum M38 (red fluorescence in A,D) co-localized with the anti-CD163 fluorescence pattern (C,F). Bile-duct ligation for 3 days led to a strong increase in the TGR5-immunoreactivity in CD163-positive Kupffer cells (D,F), while the TGR5 staining of sinusoidal endothelial cells was unchanged. Bars = 10 μm.

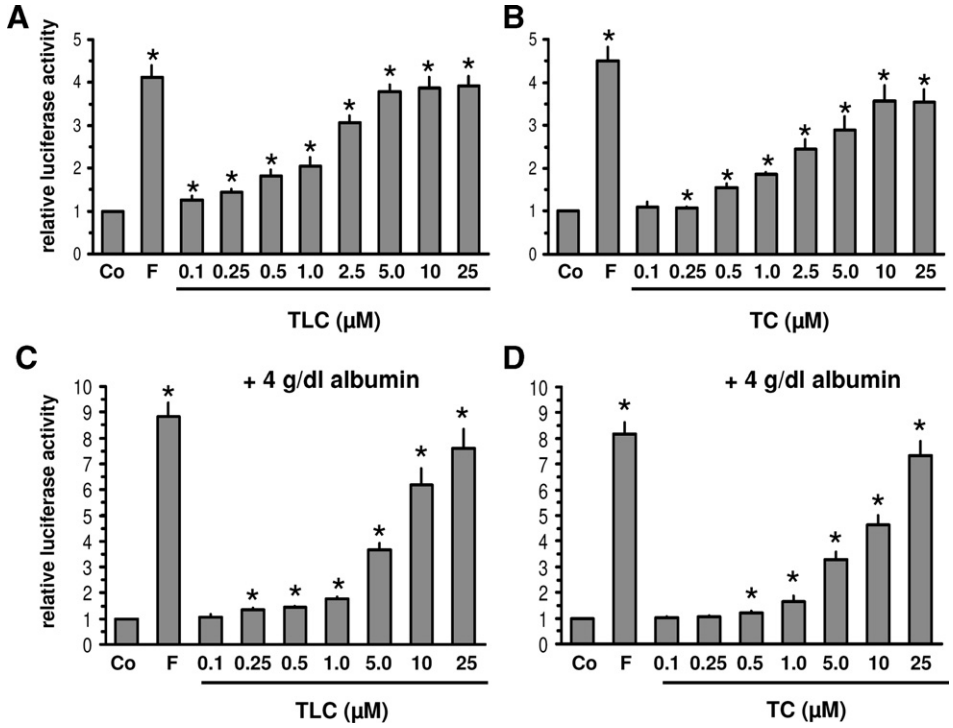

Fig. 3. TGR5 activation by bile acids was measured in TGR5-transfected HEK293 cells using a reporter gene plasmid, which contained 5 cAMP responsive elements in front of the luciferase gene. Luciferase activity served as a measure for rise in intracellular cAMP following activation of TGR5. Forskolin (F) stimulated cAMP production independent of TGR5 and was used as positive control. Taurolithocholate (TLC) and taurocholate (TC) induced cAMP production in a dose-dependent manner in the absence (A,B; $n = 4$) or presence (C,D; $n = 3$) of physiological concentrations of albumin. Results are expressed as means ± SEM, stars indicate significant differences ($p < 0.05$).

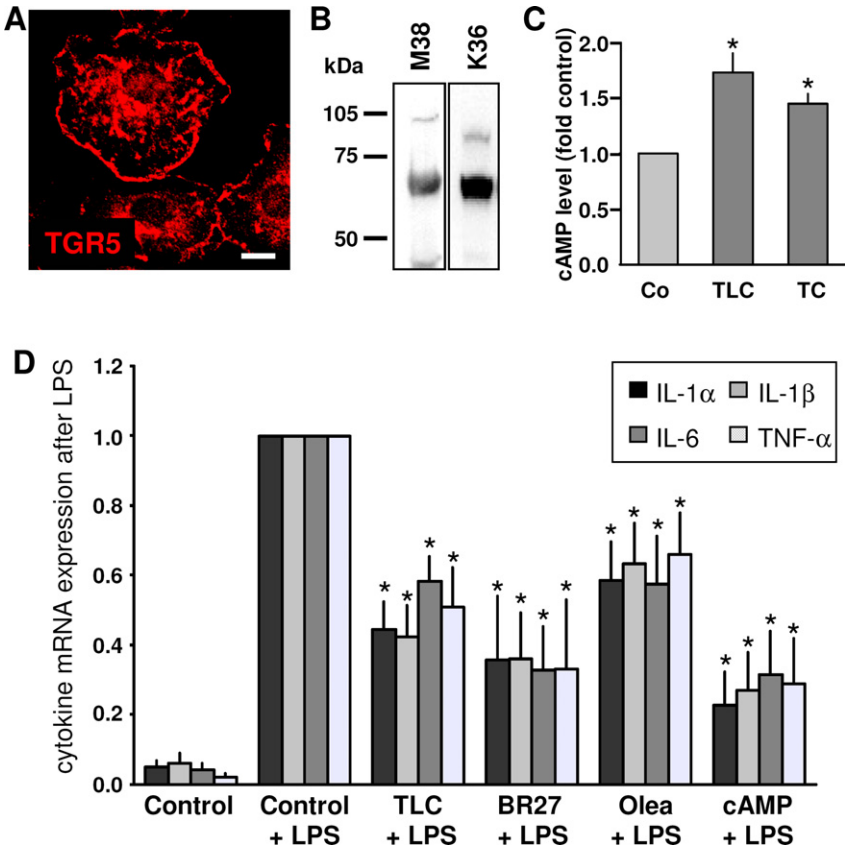

Fig. 4. Expression and function of TGR5 in isolated Kupffer cells from rat liver. (A) Immunofluorescence staining of isolated Kupffer cells (KC) localized TGR5 (M38 antiserum) both in the plasma membrane as well as in some intracellular compartments. (B) Both anti-TGR5 antisera (K36, M38) detected a broad band around 70 kDa on Western blotting experiments from isolated KC. (C) Stimulation of KC with bile acids (25 μM) led to a significant increase in intracellular cyclic AMP content ($n = 3$). (D) Stimulation of TGR5 reduces cytokine mRNA expression following LPS treatment in isolated KC. Cytokine mRNA expression was measured by quantitative PCR. Cells were stimulated with taurolithocholate (TLC; 50 μM; $n = 10$), TGR5 specific agonists (BR27, oleanoloic acid (Olea); 10 μM each; $n = 6$) or cAMP (100 μM; $n = 6$) for 1 h, followed by addition of lipopolysaccharide (LPS, 1 ng/ml) and incubation over 3 h. The increase in cytokine mRNA expression following LPS treatment in control cells was set to 1.0. TLC, cAMP and both TGR5 agonists significantly suppressed the increased expression of IL-1α, IL-1β, IL-6, and TNF-α following LPS treatment. Data are means ± SEM; bar = 10 μM; stars indicate a significant decrease as compared to LPS stimulated controls ($p < 0.05$).

observed by TLC ($n = 6$) (Fig. 4D). These data indicate that the effect of bile acids on LPS-induced cytokine expression is mediated through activation of TGR5 and subsequent production of cAMP.

Discussion

As shown previously, the bile acid receptor TGR5 is expressed in sinusoidal endothelial cells (SEC), where bile acids induced expression of eNOS mRNA and increased NO production [11]. Expression of TGR5 was also found in Kupffer cells (KC) [11], but the functional significance of TGR5 in KC remained unclear to date. The present study addressed this issue and demonstrates that TGR5 is localized in the plasma membrane of isolated KC and is activated in response to bile acids leading to increased cAMP production, establishing a direct link between TGR5 and the immunomodulatory effect of hydrophobic bile acids.

TGR5 was detected in KC of rat liver as well as in isolated cells by immunofluorescence staining. Quiescent hepatic stellate cells did not show any TGR5-immuoreactivity in situ or after isolation (Supplementary Fig. 1). A strong expression of TGR5 was also found in cholangiocytes of CK19-positive bile-ducts. It is likely that TGR5 mediates the elevation of intracellular cAMP, which was observed by Alpini and colleagues in isolated rat cholangiocytes after stimulation with taurocholate (TC) and taurolithocholate (TLC) [30,31].

Since the available data on TGR5 agonists have been determined with human TGR5 [7,8,19] and significant interspecies differences in regulation of key enzymes in bile acid homeostasis, such as the CYP7A1 gene [32] exist, the potency of different bile acids to activate rat TGR5 was determined in HEK293 cells transfected with rat TGR5-YFP and a cAMP luciferase reporter construct. Whereas no increase in luciferase activity was observed in vector transfected control cells after stimulation with bile acids, both, TLC and TC dose-dependently induced intracellular cAMP production in cells transfected with rat TGR5. These effects were similar to those observed with human TGR5 [7,8,19] and were also detected in the presence of physiological albumin concentrations, suggesting that TGR5 may be functionally active under physiological conditions.

In isolated KC, 2.5-fold higher bile acid concentrations were required to induce a significant elevation of cAMP as compared to our previous study on isolated SEC [11]. This reduced responsiveness of KC toward bile acids may be attributed to the different distribution of TGR5 between intracellular compartments and the plasma membrane in KC and SEC. While in SEC most of the TGR5 protein was detected in the plasma membrane [11], in KC TGR5 was localized in both, the plasma membrane and intracellular compartments, where the receptor may not be accessible to bile acids.

To determine whether the suppressive effect of bile acids on cytokine expression of KC was mediated by TGR5-dependent pathways we incubated isolated KC with dibutyryl cAMP and forskolin (data not shown), which increases cAMP through activation of the adenylate cyclase, prior to stimulation with lipopolysaccharide (LPS). Both substances significantly decreased LPS-induced cytokine expression, indicating that this downregulation of cytokine mRNA in isolated KC is dependent on cAMP. Oleanoloic acid was

recently identified as agonist for human TGR5 with a potency comparable to that of TLC [29]. When tested on HEK293 cells transfected with rat TGR5 and the cAMP reporter construct, oleanoloic acid also activated rat TGR5 but to a lesser extent than TLC at similar doses (10 and 25 μM of oleanoloic acid increased luciferase activity significantly by 2.5 ± 0.33- and 2.14 ± 0.21-fold, respectively, while TLC (10 μM) in the same experiment raised luciferase activity 5.28 ± 0.14-fold ($n = 3$)). Oleanoloic acid significantly reduced the rise in cytokine expression in KC but to a lesser extent than TLC (50 μM). This may be attributed to the lower potency of oleanoloic acid on TGR5 as compared to TLC. The compound BR27 was identified as TGR5 agonist using CHO cells transfected with both, TGR5 as well as the cAMP reporter plasmid and screening of a chemical library for potential TGR5 agonists. Cyclic AMP production after stimulation with BR27 was only observed in TGR5-transfected cells but not in control cells. In isolated KC, BR27 (10 μM) lowered LPS-induced expression of IL-1α, IL-1β, IL-6, and TNF-α expression to a similar extent as TLC. These findings suggest, that bile acids inhibit LPS-induced expression of IL-1α, IL-1β, IL-6, and TNF-α mRNA via TGR5-cAMP dependent mechanisms.

Bile-duct ligation in rats leads to impaired bile flow and a rise in cytokine production [23,33]. TGR5-immunoreactivity was increased in KC of bile-duct-ligated rats as compared to sham-operated control animals. This elevation of the TGR5 staining was not observed in SEC and therefore seems to be specific for KC. The increase of TGR5 in KC is likely to be caused by upregulation of the receptor, but other mechanisms such as enhanced phagocytosis of TGR5-expressing cells (e.g. SEC) may contribute to the increased TGR5-immunoreactivity in KC after BDL. Stimulation of TGR5 in KC may prevent excessive cytokine production in obstructive cholestasis thereby alleviating liver injury. This hypothesis is supported by the former observations that oral administration of bile acids successfully reduced endotoxin-related complications following surgery in patients with obstructive jaundice [34–36]. This beneficial effect of bile acids was attributed to inhibition of endotoxin-induced TNF-α production [37]. In order to define the role of TGR5 in Kupffer cells under cholestatic conditions and in the presence of lipopolysaccharide further *in vivo* studies, using TGR5 knockout mice are needed.

In summary, we have demonstrated expression, localization and functional activity of TGR5 in Kupffer cells. Furthermore, a direct link between cytokine production and TGR5 has been established, supporting the idea that TGR5 plays an important role for macrophage function.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.04.171.

References

[1] S.M. Houten, M. Watanabe, J. Auwerx, Endocrine functions of bile acids, EMBO J. 25 (2006) 1419–1425.
[2] D. Häussinger, A.K. Kurz, M. Wettstein, D. Graf, D.S. vom, F. Schliess, Involvement of integrins and Src in tauroursodeoxycholate-induced and swelling-induced choleresis, Gastroenterology 124 (2003) 1476–1487.
[3] A.K. Kurz, D. Graf, M. Schmitt, S. vom Dahl, D. Häussinger, Tauroursodesoxycholate-induced choleresis involves p38(MAPK) activation and translocation of the bile salt export pump in rats, Gastroenterology 121 (2001) 407–419.
[4] F. Schliess, A.K. Kurz, S. vom Dahl, D. Häussinger, Mitogen-activated protein kinases mediate the stimulation of bile acid secretion by tauroursodeoxycholate in rat liver, Gastroenterology 113 (1997) 1306–1314.
[5] M. Makishima, A.Y. Okamoto, J.J. Repa, H. Tu, R.M. Learned, A. Luk, M.V. Hull, K.D. Lustig, D.J. Mangelsdorf, B. Shan, Identification of a nuclear receptor for bile acids, Science 284 (1999) 1362–1365.
[6] D.J. Parks, S.G. Blanchard, R.K. Bledsoe, G. Chandra, T.G. Consler, S.A. Kliewer, J.B. Stimmel, T.M. Willson, A.M. Zavacki, D.D. Moore, J.M. Lehmann, Bile acids: natural ligands for an orphan nuclear receptor, Science 284 (1999) 1365–1368.
[7] Y. Kawamata, R. Fujii, M. Hosoya, M. Harada, H. Yoshida, M. Miwa, S. Fukusumi, Y. Habata, T. Itoh, Y. Shintani, S. Hinuma, Y. Fujisawa, M. Fujino, A G protein-coupled receptor responsive to bile acids, J. Biol. Chem. 278 (2003) 9435–9440.
[8] T. Maruyama, Y. Miyamoto, T. Nakamura, Y. Tamai, H. Okada, E. Sugiyama, T. Nakamura, H. Itadani, K. Tanaka, Identification of membrane-type receptor for bile acids (M-BAR), Biochem. Biophys. Res. Commun. 298 (2002) 714–719.
[9] T. Maruyama, K. Tanaka, J. Suzuki, H. Miyoshi, N. Harada, T. Nakamura, Y. Miyamoto, A. Kanatani, Y. Tamai, Targeted disruption of G protein-coupled bile acid receptor 1 (Gpbar1/M-Bar) in mice, J. Endocrinol. 191 (2006) 197–205.
[10] G. Vassileva, A. Golovko, L. Markowitz, S.J. Abbondanzo, M. Zeng, S. Yang, L. Hoos, G. Tetzloff, D. Levitan, N.J. Murgolo, K. Keane, H.R. Davis Jr., J. Hedrick, E.L. Gustafson, Targeted deletion of Gpbar1 protects mice from cholesterol gallstone formation, Biochem. J. 398 (2006) 423–430.
[11] V. Keitel, R. Reinehr, P. Gatsios, C. Rupprecht, B. Gorg, O. Selbach, D. Haussinger, R. Kubitz, The G-protein coupled bile salt receptor TGR5 is expressed in liver sinusoidal endothelial cells, Hepatology 45 (2007) 695–704.
[12] R. Reinehr, D. Haussinger, Inhibition of bile salt-induced apoptosis by cyclic AMP involves serine/threonine phosphorylation of CD95, Gastroenterology 126 (2004) 249–262.
[13] G. Ramadori, T. Armbrust, Cytokines in the liver, Eur. J. Gastroenterol. Hepatol. 13 (2001) 777–784.
[14] Y. Calmus, J. Guechot, P. Podevin, M.T. Bonnefis, J. Giboudeau, R. Poupon, Differential effects of chenodeoxycholic and ursodeoxycholic acids on interleukin 1, interleukin 6 and tumor necrosis factor-alpha production by monocytes, Hepatology 16 (1992) 719–723.
[15] M. Funaoka, M. Komatsu, I. Toyoshima, K. Mikami, T. Ono, T. Hoshino, J. Kato, T. Kuramitsu, T. Ishii, O. Masamune, Tauroursodeoxycholic acid enhances phagocytosis of the cultured rat Kupffer cell, J. Gastroenterol. Hepatol. 14 (1999) 652–658.
[16] R.M. Minter, M.H. Fan, J. Sun, A. Niederbichler, K. Ipaktchi, S. Arbabi, M.R. Hemmila, D.G. Remick, S.C. Wang, G.L. Su, Altered Kupffer cell function in biliary obstruction, Surgery 138 (2005) 236–245.
[17] C.E. Scott-Conner, J.B. Grogan, The pathophysiology of biliary obstruction and its effect on phagocytic and immune function, J. Surg. Res. 57 (1994) 316–336.
[18] J.J. Sung, M.Y. Go, Reversible Kupffer cell suppression in biliary obstruction is caused by hydrophobic bile acids, J. Hepatol. 30 (1999) 413–418.
[19] H. Sato, A. Macchiarulo, C. Thomas, A. Gioiello, M. Une, A.F. Hofmann, R. Saladin, K. Schoonjans, R. Pellicciari, J. Auwerx, Novel potent and selective bile acid derivatives as TGR5 agonists: biological screening, structure–activity relationships, and molecular modeling studies, J. Med. Chem. (2008).
[20] J.G. Bode, T. Peters-Regehr, R. Kubitz, D. Häussinger, Expression of glutamine synthetase in macrophages, J. Histochem. Cytochem. 48 (2000) 415–422.
[21] H.F. Hendriks, W.A. Verhoofstad, A. Brouwer, A.M. de Leeuw, D.L. Knook, Perisinusoidal fat-storing cells are the main vitamin A storage sites in rat liver, Exp. Cell Res. 160 (1985) 138–149.
[22] S. Glaser, H. Francis, S. Demorrow, G. LeSage, G. Fava, M. Marzioni, J. Venter, G. Alpini, Heterogeneity of the intrahepatic biliary epithelium world, J. Gastroenterol. 12 (2006) 3523–3536.
[23] M.H. Bemelmans, D.J. Gouma, J.W. Greve, W.A. Buurman, Cytokines tumor necrosis factor and interleukin-6 in experimental biliary obstruction in mice, Hepatology 15 (1992) 1132–1136.
[24] M. Plebani, M.P. Panozzo, D. Basso, P.M. De, R. Biasin, D. Infantolino, Cytokines and the progression of liver damage in experimental bile duct ligation, Clin. Exp. Pharmacol. Physiol. 26 (1999) 358–363.
[25] H. Jaeschke, G.J. Gores, A.I. Cederbaum, J.A. Hinson, D. Pessayre, J.J. Lemasters, Mechanisms of hepatotoxicity, Toxicol. Sci. 65 (2002) 166–176.
[26] B. Angelin, I. Bjorkhem, K. Einarsson, S. Ewerth, Hepatic uptake of bile acids in man. Fasting and postprandial concentrations of individual bile acids in portal venous and systemic blood serum, J. Clin. Invest. 70 (1982) 724–731.
[27] K. Einarsson, G. Alvelius, C.G. Hillebrant, E. Reihner, I. Bjorkhem, Concentration of unsulfated lithocholic acid in portal and systemic venous plasma: evidence that lithocholic acid does not down regulate the hepatic cholesterol 7 alpha-hydroxylase activity in gallstone patients, Biochim. Biophys. Acta 1317 (1996) 19–26.
[28] K. Fukushima, H. Ichimiya, H. Higashijima, H. Yamashita, S. Kuroki, K. Chijiiwa, M. Tanaka, Regulation of bile acid synthesis in the rat: relationship between hepatic cholesterol 7 alpha-hydroxylase activity and portal bile acids, J. Lipid Res. 36 (1995) 315–321.
[29] H. Sato, C. Genet, A. Strehle, C. Thomas, A. Lobstein, A. Wagner, C. Mioskowski, J. Auwerx, R. Saladin, Anti-hyperglycemic activity of a TGR5 agonist isolated from *Olea europaea*, Biochem. Biophys. Res. Commun. 362 (2007) 793–798.
[30] G. Alpini, S. Glaser, W. Robertson, J.L. Phinizy, R.E. Rodgers, A. Caligiuri, G. LeSage, Bile acids stimulate proliferative and secretory events in large but not small cholangiocytes, Am. J. Physiol. 273 (1997) G518–G529.
[31] G. Alpini, S. Glaser, D. Alvaro, Y. Ueno, M. Marzioni, H. Francis, L. Baiocchi, T. Stati, B. Barbaro, J.L. Phinizy, J. Mauldin, G. LeSage, Bile acid depletion and

repletion regulate cholangiocyte growth and secretion by a phosphatidylinositol 3-kinase-dependent pathway in rats, Gastroenterology 123 (2002) 1226–1237.
[32] B. Goodwin, M.A. Watson, H. Kim, J. Miao, J.K. Kemper, S.A. Kliewer, Differential regulation of rat and human CYP7A1 by the nuclear oxysterol receptor liver X receptor-alpha, Mol. Endocrinol. 17 (2003) 386–394.
[33] M.E. Sewnath, P.T. van der, F.J. ten Kate, C.J. van Noorden, D.J. Gouma, Interleukin-1 receptor type I gene-deficient bile duct-ligated mice are partially protected against endotoxin, Hepatology 35 (2002) 149–158.
[34] C.J. Cahill, Prevention of postoperative renal failure in patients with obstructive jaundice—the role of bile salts, Br. J. Surg. 70 (1983) 590–595.
[35] J.A. Pain, M.E. Bailey, Prevention of endotoxaemia in obstructive jaundice—a comparative study of bile salts, HPB Surg. 1 (1988) 21–27.
[36] J.A. Pain, C.J. Cahill, J.M. Gilbert, C.D. Johnson, J.E. Trapnell, M.E. Bailey, Prevention of postoperative renal dysfunction in patients with obstructive jaundice: a multicentre study of bile salts and lactulose, Br. J. Surg. 78 (1991) 467–469.
[37] J.W. Greve, D.J. Gouma, W.A. Buurman, Bile acids inhibit endotoxin-induced release of tumor necrosis factor by monocytes: an in vitro study, Hepatology 10 (1989) 454–458.