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Review Article

Functional roles of gut bacteria imbalance in cholangiopathies☆

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ABSTRACT

Cholangiopathies are caused by bile duct damage or inflammation followed by cholestasis leading to liver fibrosis. Bile duct epithelial cells, cholangiocytes, are a primary target for cholangiopathies. Ductular reaction is often observed in cholangiopathies and the proliferation of cholangiocytes is associated with ductular reaction and liver fibrogenesis. Accumulating evidence suggests that patients with cholangiopathies have different gut bacterial profiles from healthy individuals, indicating the association between gut microbiota and cholangiopathies. Bile acids are produced by hepatocytes and modified by gut bacteria. Bile acids regulate cholangiocyte proliferation but effects vary depending on the type of bile acids. Recent studies suggest that therapies targeting gut bacteria, such as antibiotics administration and gut bacteria depletion or therapies using gut bacteria-associated bile acids, such as ursodeoxycholic acid (UDCA) administration, may be useful for treatments of cholangiopathies, although data are controversial depending on animal models or cohorts. This review summarizes current understandings of functional roles of gut bacterial imbalance and strategies for treatments of cholangiopathies targeting gut bacteria.

1. Introduction

Cholangiopathies, such as primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and biliary atresia (BA), are characterized by bile duct damage or inflammation leading to cholestasis and bile duct hyperplasia.[1,2] Ductular reactions and liver fibrosis are also commonly identified in cholangiopathies.[3–5] Cholangiocytes are bile duct epithelia cells and the primary target for therapies of cholangiopathies. Previous studies have demonstrated that cholangiocytes are heterogeneous since isolated cholangiocytes from small (<15 μm diameter) and large (>15 μm diameter) bile ducts have different cell size and morphology.[6,7] Cholangiocytes also show heterogeneity in protein expressions as well as functions between small and large cholangiocytes.[8,9]

Bile acids are the main component of bile and have an important role in the digestion and the absorption of fats and fat-soluble vitamins in small intestine.[10,11] Although bile acids are produced mainly by hepatocytes, cholangiocytes express numbers of channels and transporters on both apical and basolateral domains and contribute to the modification of bile via secretion and absorption through these membrane proteins.[12,13] Bile acids are transferred into intestine through bile ducts and are further modified by gut bacteria before absorption.[14–16] Bile acids are transferred back into the liver and are recycled in this enterohepatic circulation of bile acids. Accumulating evidence suggests that gut bacteria and their contribution to bile acid modification may play a key role for pathophysiology of cholangiopathies. This review summarizes the current understandings of functional roles of gut bacteria imbalance in cholestatic liver diseases.

2. Association between gut bacteria and cholangiopathies

2.1. Intestinal permeability and cholangiocyte senescence

It is well known that PSC is strongly associated with inflammatory bowel disease (IBD) and 70–80% of patients have both

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conditions (PSC-IBD).[1,17,18] Previous studies have identified increased intestinal permeability in patients with IBD and it could be associated with the genotype in a family tree.[19–21] Increased intestinal permeability may be responsible for the association between PSC and IBD but could also play a key role in pathophysiology of liver diseases. Increased intestinal permeability elevates gut bacteria-derived molecules in enterohepatic circulation such as endotoxin or lipopolysaccharide (LPS), which is a strong inducer for inflammatory responses, leading to liver damage.[22,23] For example, chronic alcohol drinking elevates intestinal permeability in rats, and acute binge drinking increases serum endotoxin and bacterial deoxyribonucleic acid (DNA) levels in humans, leading to alcoholic hepatitis.[24,25] For cholangiopathies, Tornai *et al.*[26] have analyzed sera of 67 PSC patients and 153 healthy individuals and have shown that antibodies against F-actin or gliadin are positive in higher percentage of PSC patients, suggesting higher mucosal immune responses compared to healthy individuals. Feld *et al.*[27] have analyzed 86 PBC patients and 155 healthy individuals by lactulose-mannitol test and have shown that PBC patients have increased small bowel permeability. Sasatomi *et al.*[28] have observed that endotoxin is accumulated in cholangiocytes in liver specimens from 30 PBC patients and 7 PSC patients.

LPS stimulation increases proinflammatory cytokine production, interleukin-6 (IL-6), which induces biliary and liver inflammation, in cholangiocytes *in vitro*.[29] LPS also disrupts tight junctions in cholangiocyte monolayers and this may lead to bile leakage into the liver parenchyma causing liver damage.[30] Another important aspect is cellular senescence in cholangiocytes during cholangiopathies. Cholangiocyte senescence is characteristic in cholangiopathies and cholangiocytes in liver specimen from PBC or PSC patients express senescence-associated secretory phenotype (SASP) markers, such as IL-6 and C-C motif chemokine ligand 2 (CCL2).[31] Senescent cholangiocytes produce various cytokines with chemotactic activities and this may contribute to pathogenesis of cholangiopathies.[32] Elevated cellular senescence and SASP marker production in cholangiocytes were also observed in animal models of cholestatic liver injuries.[33] As LPS induces cellular senescence in cholangiocytes *in vitro*,[31] elevated intestinal permeability and serum levels of LPS may play a key role of pathophysiology of cholangiopathies via cholangiocyte senescence.

2.2. Altered gut bacteria profiles

Association of gut bacteria with hepatobiliary inflammation has been introduced in 1990s. Lichtman *et al.*[34] have demonstrated that small bowel bacterial overgrowth created by jejunal self-filling blind loops induces liver inflammation and damage in rats. The authors have also shown that bacterial overgrowth in rats elevates bile duct mass and portal liver fibrosis in the liver, and metronidazole (MTZ) treatment attenuates those effects caused by overgrown gut bacteria.[35]

Sequencing analyses of the V3–V4 region in the 16S ribosomal ribonucleic acid (rRNA) using stool samples identify species of gut bacteria in patients. Kummen *et al.*[36] have analyzed 85 stool samples collected from PSC patients and shown that PSC patients have different gut bacteria profiles from 263 healthy individuals with marked increase of *Veillonella* genus. Another study has also analyzed 52 PSC patients and shown that PSC patients have decreased gut microbiota diversity and overgrowth of *Enterococcus*, *Fusobacterium*, and *Lactobacillus* genera compared to 52 healthy individuals.[37] Torres *et al.*[38] have compared 15 PSC-IBD patients and 15 IBD patients and shown that PSC-IBD patients have different gut microbiota composition with enrichment in *Ruminococcus* and *Fusobacterium* genera. High-performance liquid chromatography-tandem mass spectrometry also showed decreased bile acid pool in stool samples of PSC-IBD compared to those of IBD patients in this study. Bajer *et al.*[39] have analyzed stool samples from 43 PSC patients with or without IBD and 31 healthy controls, and have found that PSC patients have lower bacterial diversity and overgrowth of *Rothia*, *Enterococcus*, *Streptococcus*, and *Veillonella* genera regardless of IBD compared to healthy controls. For PBC, Lv *et al.*[40] have analyzed 42 early-stage PBC patients and have found that potentially beneficial species, such as *Bacteroides egerthii* and *Ruminococcus*, are depleted but some opportunistic pathogens including *Veillonella* and *Streptococcus* are enriched in PBC patients compared to 30 healthy individuals. Tang *et al.*[41] have compared gut bacteria between 60 PBC patients and 80 matched healthy individuals and have found that PBC patients have decreased microbial diversity and increased abundance of some genera, such as *Streptococcus* and *Veillonella*, showing correlation with PBC. These findings suggest that specific gut bacteria compositions, such as *Veillonella* and *Streptococcus* genera, and bacteria imbalance between beneficial and harmful bacteria in the intestinal tract are associated with PBC and PSC, and these bacteria may play a key role in pathophysiology of cholangiopathies. Table 1 shows gut bacteria that have been identified in patients and may be associated with cholangiopathies, and Fig. 1 summarizes contributions of gut bacteria to pathogenesis of cholangiopathies.

3. Bile acids and cholangiocyte proliferation

The balance and composition of the human gut microbiota can be altered by diet or diseases. The composition and levels of bile acids in bile can be altered by numbers, composition, or activities of gut bacteria.[42] A previous study has demonstrated that patients with cirrhosis have altered gut bacteria profiles as well as low bile acid levels and altered bile acid composition compared to healthy individuals.[43] Primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), are synthesized in hepatocytes.[44] These primary bile acids are conjugated with taurine or glycine to produce conjugated bile acids including taurocholic acid (TCA) and glycocholic acid (GCA).[15] Bile acids are transferred into intestine and then gut bacteria convert them to produce other bile acids such as deoxycholic acid (DCA) and ursodeoxycholic acid (UDCA) (Fig. 2).[45,46]

Cholangiocytes are quiescent at normal liver conditions but can be activated and start proliferation at disease conditions, leading to ductular reactions and bile duct hyperplasia regulated by various mediators, such as hormones, neurotransmitters, and bile acids.[47,48] Proliferation of cholangiocytes during disease conditions is associated with liver fibrosis via the activation of hepatic stellate cells (HSCs) and/or portal myofibroblasts.[4,49] Cholestasis is obstructed or impaired bile flow caused by bile duct inflammation or damage and is characteristic in cholangiopathies. In experimental cholestasis models, bile duct ligation (BDL), which is a surgical ligation of extrahepatic common bile duct, is commonly used to mimic cholestasis in rodents.[50,51] Zhang *et al.*[52] have demonstrated that BDL increases bile acid concentrations in the liver and serum, and it also changes compositions of bile acids in mice.

Bile acids, such as CDCA, TCA, DCA, and glycochenodeoxycholic acid (GCDCA), cause inflammatory responses and apoptosis in hepatocytes.[53,54] On the other hand, bile acids including TCA and taurolithocholic acid (TLCA) induce cell proliferation of cholangiocytes, leading to elevated bile duct mass *in vivo*.[55,56] However, effects on cholangiocyte proliferation may differ depending on bile acids. UDCA or tauroursodeoxycholic acid (TUDCA) feeding for BDL rats inhibited cholangiocyte proliferation and secretion *in vivo*.[57] Further studies are required to elucidate the specific bile acids that activate/inhibit cholangiocytes and how they lead to the pathogenesis of cholangiopathies.

Table 1
Identified gut bacteria in patients with cholangiopathies.

Diseases	Control groups	Increased abundance	Decreased abundance	References
PSC	Healthy	*Veillonella*	*Succinivibrio* *Desulfovibrio* *Phascolarctobacterium* *Coprococcus*	36
PSC	Healthy	*Enterococcus* *Fusobacterium* *Lactobacillus* *Morganella* *Streptococcus*	*Anaerostipes*	37
PSC-IBD	IBD	*Fusobacteriaceae* *Fusobacterium* *Ruminococcus*	*Dorea* *Veillonella* *Lachnospira* *Roseburia* *Blautia*	38
PSC and PSC-IBD	Healthy	*Rothia* *Enterococcus* *Streptococcus* *Clostridium* *Veillonella* *Haemophilus*	*Coprococcus*	39
PBC	Healthy	*Veillonella* *Bifidobacterium* *Klebsiella* *Neisseria*	*Bacteroides eggerthii* *Hallella* *Ruminococcus* *Megamonas*	40
PBC	Healthy	*Klebsiella* *Lactobacillus* *Closridium* *Pseudomonas* *Haemophilus* *Streptococcus* *Veillonella* *Enterobacteriaceae*	*Oscillospira* *Faecalibacterium* *Sutterella* *Bacteroides*	41

Abbreviations: PSC, primary sclerosing cholangitis; IBD, inflammatory bowel disease; PBC, primary biliary cholangitis.

4. Therapies of cholangiopathies targeting gut bacteria or bile acids

4.1. Antibiotics

As described above, daily MTZ therapy to inhibit small bowel bacterial overgrowth decreased bile duct mass and liver fibrosis during blind loops-induced liver injury in rats,[35] indicating that the management of gut bacteria using antibiotics could contribute to therapies of live diseases.[58] Tabibian *et al.*[59] have performed a pilot study using 35 PSC patients with vancomycin or MTZ for 12 weeks. Both vancomycin and MTZ administrations decreased serum levels of alkaline phosphatase (ALP) in treated patients. Other pilot studies have also demonstrated that oral vancomycin or minocycline administration decreases ALP activity in PSC patients.[60,61] Jee *et al.*[62] fed adult mice a diet supplemented with sulfamethoxazole and trimethoprim during pregnancy, and injected rhesus rotavirus into pups within 24 h of life to induce experimental BA. In this model, maternal exposure to these antibiotics decreased bile duct obstruction and improved survival rates of pups. Although these studies suggest that the inhibition of growth of gut bacteria by antibiotics may be effective to improve liver conditions during cholangiopathies, limited numbers of studies are available to date and further studies are required for the clarifications.

4.2. Probiotics

Some bacteria or bacteria-derived molecules could be beneficial for human health. Administration of probiotics is another strategy for treatments of liver diseases.[63,64] For cholangiopathies, a case report showed that a 13-year old boy suffering from PSC associated with undetermined colitis was treated with the combination of prednisolone, salazosulfapyridine, and probiotic *Lactobacillus casei*. Two weeks later, symptoms and laboratory tests including ALP were improved.[65] Another pilot study administered 14 PSC patients with placebo or multispecies probiotic Ecologic 641, which consists of four *Lactobacillus* and two *Bifidobacillus* strains, for 3 months.[66] In this study, there were no changes or differences in liver conditions observed between patients with placebo or probiotics. Studies for probiotic treatments for cholangiopathies are limited to date and further studies are required to identify effective strains and understand whether the administration of probiotics is effective or it needs to be combined with steroid or other drugs.

4.3. Supplementation of bile acids

Some bile acids including UDCA are modified by gut bacteria and they inhibit cholangiocyte proliferation as described above, and hence supplementation of these bile acids may have therapeutic effects on cholangiopathies. UDCA was introduced as a therapeutic drug for PBC and was approved by the Food and Drug Administration (FDA) in 1997.[67–69] As described above, Tang *et al.*[41] have analyzed gut bacteria of 60 PBC patients and have identified PBC-associated genera. The authors performed 6-month administration of UDCA for patients and demonstrated that UDCA decreased abundance of those PBC-related genera as well as ALP, showing improved liver conditions in patients.[41] Hatano *et al.*[70] generated knockdown (kd) mice (*Vil2*$^{kd/kd}$ mice) for ezrin that is a cytoskeletal cross linker protein, and these mice showed cholestasis and liver damage mimicking human cholestatic liver injuries.

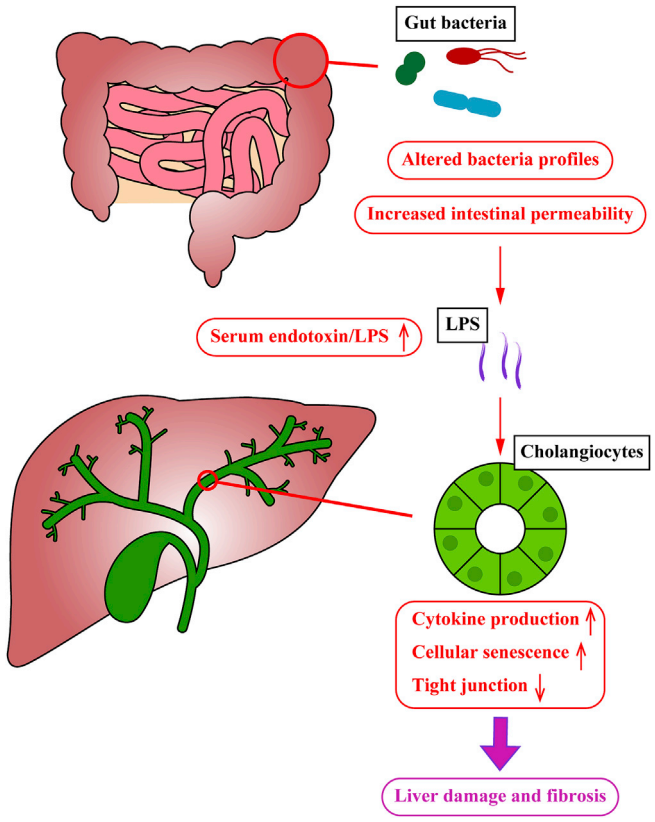

Fig. 1. Contributions of gut bacteria to pathogenesis of cholangiopathies. Altered gut bacteria profiles and increased intestinal permeability have been observed in PSC and PBC patients. Increased permeability elevates serum levels of endotoxin or LPS, and LPS stimulation increases proinflammatory cytokines and chemokine production and cellular senescence in cholangiocytes. LPS also disrupts tight junctions of cholangiocyte monolayers leading to liver inflammation, damage, and fibrosis during cholangiopathies. Abbreviations: PSC, primary sclerosing cholangitis; PBC, primary biliary cholangitis; LPS, lipopolysaccharide.

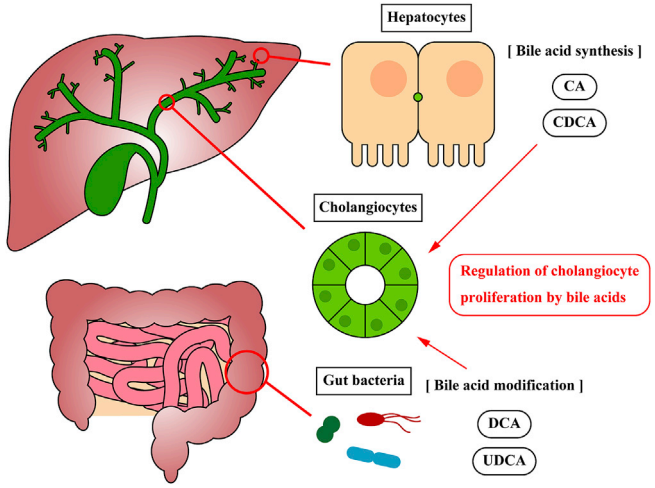

Fig. 2. Bile acid synthesis and modification. In enterohepatic circulation, primary bile acids CA and CDCA are synthesized by hepatocytes in the liver. These bile acids are transferred into the intestine through bile ducts, and DCA and UDCA are produced as metabolic byproducts of intestinal bacteria. Bile acids are then transferred back into the liver for the recycle. Cholangiocytes are bile duct epithelial cells and cholangiocyte proliferation is associated with bile duct hyperplasia and liver fibrosis during cholestatic liver injuries. Bile acids regulate cholangiocyte proliferation but effects vary depending on bile acids. Abbreviations: CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; UDCA, ursodeoxycholic acid.

In this model, the administration of UDCA decreased gene expression of type I collagen and transforming growth factor-β1 (TGF-β1) in the liver, leading to attenuated liver damage and fibrosis in *Vil2*$^{kd/kd}$ mice.[71]

Although administration of UDCA is clinically approved for PBC, its effects are still controversial for PSC to date.[72] Lindor *et al.*[73] have performed long-term high-dose UDCA administration for PSC patients. In this study, 76 PSC patients were administered with UDCA (28–30 mg/kg/day) and 74 patients took placebo for 6 years. During therapy, the UDCA group showed improved serum tests such as lower ALP compared to the placebo group, but in long-term follow-up period, the UDCA group showed higher rates for death and liver transplantation, indicating that UDCA treatments did not improve survival rates or hazard ratio of adverse events.[73] PSC is a progressive disorder and there are four stages depending on conditions.[1,74] Imam *et al.*[75] have shown that UDCA administration (28–30 mg/kg/day) for PSC patients increases risks of adverse events for patients in early stages (stage 1 or 2) but not in later stages (stage 3–4) during follow-up period (7.5 years). The combination of UDCA with antibiotics may be more effective on PSC. A previous study has administered 41 PSC patients with UDCA (15 mg/kg/day) and placebo, and 39 PSC patients with UDCA and MTZ for 36 months.[76] In this trial, the UDCA/MTZ group showed decreased serum ALP levels and better liver conditions compared to the UDCA/placebo group. These studies suggest that some bile acids, especially UDCA, may improve liver conditions during cholangiopathies by regulating cholangiocyte proliferation and/or fibrogenesis in the liver even though further studies are required to establish effective methodology for dose and duration for human patients.

4.4. Withdrawal or depletion of bile acids

Wunsch *et al.*[77] administered 26 PSC patients with UDCA (10–15 mg/kg/day) for at least 12 months. After treatments, UDCA administration was withdrawn for 3 months. In this trial, UDCA withdrawal significantly decreased serum levels of some bile acids, not only UDCA but also TUDCA and glycoursodeoxycholic acid (GUDCA), and increased some other bile acids, such as TCA. UDCA withdrawal improved serum biochemistry tests including ALP, and also patients' quality of life evaluated by questionnaires.[77]

Some bile acids, such as TCA, induce apoptosis in hepatocytes and proliferation in cholangiocytes as described above. Therefore, the depletion of bile acids may lead to improved liver conditions by inhibiting hepatocyte damage and bile duct hyperplasia. The depletion of bile acids by external bile drainage in bile duct-incannulated rats decreased cholangiocyte proliferation and bile duct mass, and TCA supplementation restored bile duct proliferation *in vivo*.[78] Feeding a diet supplemented with bile acid sequestrant, colesevelam, decreased liver inflammation, damage, and fibrosis as well as bile duct hyperplasia in *Mdr2*$^{-/-}$ mice, which are a mouse model of human PSC.[79] These studies implicate that withdrawal or depletion of some specific bile acids may be another therapeutic approach to manage cholangiocyte proliferation during cholestatic liver injuries.

4.5. Germ-free animals

It is still unclear whether the modification of bile acids by gut bacteria, such as the production of UDCA, promotes cholangiopathies or protects the liver from bile duct damage. In experimental cholestasis models, data are controversial and not conclusive to date. Tabibian *et al.*[80] have analyzed germ-free and conventionally housed *Mdr2*$^{-/-}$ mice. In this study, germ-free mice showed more severe serum biochemical conditions including ALP as well as exacerbated ductular reaction, liver fibrosis, and cellular

senescence in cholangiocytes compared to control mice. UDCA treatments for cultured cholangiocytes decreased cellular senescence caused by H_2O_2 *in vitro*, but DCA treatments showed no effects.[80] These findings suggest that bile acid modification and production of UDCA by gut bacteria protect the liver from damage and the supplementation of UDCA may be beneficial for PSC patients with impaired bacterial functions and bile acid modification.

NOD.c3c4 mice develop liver conditions of autoimmune biliary disease and have been introduced as a mouse model of human PBC.[81] Schrumpf *et al.*[82] have compared germ-free and conventionally housed NOD.c3c4 mice and have found that germ-free mice show slightly improved liver conditions including mild portal inflammation and reduced immune cell infiltration around portal area. Although effects of bacteria depletion in germ-free animals may differ between animal models of PSC and PBC, current studies are limited and further studies are required to understand functional roles of gut bacteria.

5. Conclusions and future perspectives

Current studies present the association of gut bacteria with cholestatic liver injuries. The related findings, however, are still controversial and further studies are required to elucidate functional roles of gut bacteria and their activities during cholangiopathies. UDCA is approved as a therapeutic drug for PBC, but its effects are controversial for PSC.[72] Depletion of gut bacteria exacerbated liver conditions in PSC model *Mdr2*$^{-/-}$ mice, but improved in PBC model NOD.c3c4 mice.[80,82] Pathophysiology and functions of cholangiocytes as well as bile acids in PSC and PBC are still unclear, and it is possible that same bile acids show different effects or have different functional roles depending on cholangiopathies. Different experimental conditions, animal models, or doses of bile acids could produce controversial results.

Cholangiopathies are associated with not only cholangiocytes but also other liver cells such as liver macrophages or HSCs.[33,83] UDCA treatments inhibited proinflammatory cytokine production in activated RAW 264.7 macrophage cell line *in vitro*.[84] Another study has demonstrated that UDCA treatments decrease the expression of TGF-β1 in cultured rat HSCs *in vitro*.[85] Although current studies to elucidate the association of gut bacteria with these liver cells during cholestatic liver injuries are limited, the regulation of those cells by specific bile acids, such as UDCA, could play a key role for pathophysiology of cholangiopathies.

In conclusion, the balance and activities of gut bacteria play a key role in pathogenesis of cholangiopathies, and they could be a potential therapeutic target for cholestatic liver injuries.

Authors' contributions

K. Sato: study concept and design; drafting of the manuscript. F. Meng, G. Fava, S. Glaser: critical revision of the manuscript. G. Alpini: study concept and design; critical revision of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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