**Slco2a1 deficiency exacerbates experimental colitis via inflammasome activation in macrophages: a possible mechanism of chronic enteropathy associated with SLCO2A1 gene**

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Loss-of-function mutations in the solute carrier organic anion transporter family, member 2a1 gene (**SLCO2A1**), which encodes a prostaglandin (PG) transporter, have been identified as causes of chronic nonspecific multiple ulcers in the small intestine; however, the underlying mechanisms have not been revealed. We, therefore, evaluated the effects of systemic knockout of **Slco2a1** (**Slco2a1**/−/) and conditional knockout in intestinal epithelial cells (**Slco2a1**ΔIEC) and macrophages (**Slco2a1**ΔMP) in mice with dextran sodium sulphate (DSS)-induced acute colitis. **Slco2a1**/−/ mice were more susceptible to DSS-induced colitis than wild-type (WT) mice, but did not spontaneously develop enteritis or colitis. The nucleotide-binding domain, leucine-rich repeats containing family, pyrin domain-containing-3 (NLRP3) inflammasome was more strongly upregulated in colon tissues of **Slco2a1**/−/ mice administered DSS and in macrophages isolated from **Slco2a1**/−/ mice than in the WT counterparts. **Slco2a1**ΔMP, but not **Slco2a1**ΔIEC mice, were more susceptible to DSS-induced colitis than WT mice, partly phenocopying **Slco2a1**/−/ mice. Concentrations of PGE2 in colon tissues and macrophages from **Slco2a1**/−/ mice were significantly higher than those of WT mice. Blockade of inflammasome activation suppressed the exacerbation of colitis. These results indicated that **Slco2a1**-deficiency increases the PGE2 concentration, resulting in NLRP3 inflammasome activation in macrophages, thus exacerbating intestinal inflammation.

Prostaglandin (PG) E2 is the most physiologically abundant eicosanoid biosynthesized from arachidonic acid by cyclooxygenase (COX). PGE2 has important roles in maintaining gut mucosal homeostasis1,2, but also is an essential mediator of the immune response and inflammation in various inflammatory diseases3. Indeed, small intestinal ulcers can be induced by nonsteroidal anti-inflammatory drugs (NSAIDs) treatment through suppressing PGE2 synthesis by inhibition of COX. PGE2 is exported to the extracellular microenvironment by multiple drug resistance-associated protein 4 (MRP4/ABCC4)4 and exerts its effects by binding to a family of G protein-coupled receptors consisting of four subtypes: EP1, EP2, EP3, and EP45. Prostaglandin transporter (PGT), encoded by the solute carrier organic anion transporter family, member 2a1 gene (**SLCO2A1**) mediates cellular uptake of PGE26,7. PGE2 is oxidized intracellularly by 15-ketoprostaglandin dehydrogenase (15-PGDH; encoded by **HPGD**)8,9. Thus, PGT plays an important role in PGE2 metabolism. Umeno et al. recently reported that chronic enteropathy, which

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is characterized by multiple intractable small-intestinal ulcers with manifestations including anaemia and hypoproteinaemia, is caused by autosomal recessive loss-of-function mutations in SLCO2A1. PGE, concentrations in patients with this genetic background should theoretically be higher than those in patients without this mutation because of lower PGE, metabolism. In fact, concentrations of PGE major urinary metabolites, which are stable metabolites derived from PGE, in patients with chronic enteropathy, so-called chronic enteropathy associated with SLCO2A1 gene (CEAS), have been shown to be higher than those in patients with Crohn’s disease. Thus, given the protective effect of PGE, on mucosal injury, PGE, seems to exert contrasting effects on the intestinal mucosa, and the mechanisms underlying enteritis development under SLCO2A1 deficiency have not been clarified.

In the present study, we evaluated a potential role of SLCO2A1 in intestinal homeostasis using systemic as well as conditional Slco2a1-knockout mice. Deletion of Slco2a1 did not cause alteration of epithelial structure and spontaneous enteritis. However, SLCO2A1 protected against gut inflammation in an experimental colitis model. Further, we report a possible mechanism: increased PGE because of spontaneous enteritis. However, Slco2a1 protected against gut inflammation in an experimental colitis model.

Results

**Slco2a1 deficiency does not result in spontaneous enterocolitis.** To investigate the effect of Slco2a1 deficiency on the intestine, germline Slco2a1-knockout (Slco2a1−/−) mice were evaluated for basal phenotype in comparison with wild-type (WT) mice. There were no significant differences in body weight between Slco2a1−/− and WT mice at any time point evaluated (Fig. 1a). Slco2a1−/− mice did not display any sign of enteritis as assessed by histological examination of ilea and colons at 8, 12, and 24 weeks of age (Fig. 1b,c, and Supplementary Fig. S1a,b). mRNAs levels of Il1b and Tnf, which are pivotal inflammatory cytokines, in the colon were not affected by Slco2a1 knockout, although Il1b mRNA expression in the ileum of Slco2a1−/− mice was decreased (Fig. 1d,e). These results demonstrated that Slco2a1 deficiency per se does not induce intestinal inflammation. Slco2a1 deficiency also did not affect the numbers of goblet cells and Paneth cells, which secrete mucins and antibacterial contents for mucosal barrier function.

**Slco2a1−/− mice are more susceptible to DSS-induced colitis.** To investigate the role of SLCO2A1 in colitis development, we next examined the effects of Slco2a1 deficiency in experimental colitis models of Slco2a1−/− and WT mice. Slco2a1−/− and WT mice were established by administering 3.5% DSS in the drinking water for 7 days. Slco2a1−/− mice

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Figure 1. Systemic Slco2a1 deficiency does not induce spontaneous intestinal inflammation. (a) Body weight changes in WT and Slco2a1−/− mice during 24 weeks (n = 8–22). (b,c) Histological scores for mucosal inflammation in the ileum and colon in 8, 12, and 24-week-old WT and Slco2a1−/− mice. (d,e) mRNA expression levels of Il1b and Tnf in ileum and colon tissues of 8-week-old WT and Slco2a1−/− mice as assessed by RT-qPCR. Data represent the mean ± SEM or median and IQR. Statistical significance was calculated using Student’s t-test, Welch’s t-test, or Mann-Whitney test (*P < 0.05).
lost significantly more weight than did WT mice upon DSS-induced colitis (Fig. 2a). Histopathological examination of colons revealed more severe lesions, stronger neutrophil and lymphocyte infiltration into the mucosal and submucosal areas, and more extensive loss of crypts in \( \text{Slco2a1}^{-/-} \) mice than in WT mice, resulting in higher histological colitis grades (Fig. 2b,c).

To investigate the mechanisms underlying colitis exacerbation in \( \text{Slco2a1}^{-/-} \) mice further, we analysed gene expression profiles in colon tissues of \( \text{Slco2a1}^{-/-} \) and WT mice in the presence/absence of DSS-induced colitis by microarray analysis (Fig. 2d). Gene set enrichment analysis (GSEA) indicated that the most strongly enriched pathways in \( \text{Slco2a1}^{-/-} \) mice with DSS-induced colitis were related to the inflammatory immune response and leukocyte migration/activation (Fig. 2e,f). Comparison of the expression levels of enriched genes in the inflammatory response (Fig. 2g) and cytokine expression (Fig. 2h) revealed that the expression of inflammasome-related

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**Figure 2.** Systemic \( \text{Slco2a1} \) deficiency increases susceptibility to dextran sodium sulphate (DSS)-induced colitis. (a) Changes in body weight during the experimental period. WT \((n = 6) \) and \( \text{Slco2a1}^{-/-} \) \((n = 6) \) mice were administered 3.5% DSS for 7 days. (b,c) Representative H&E-stained colon sections (scale bars: 50 µm) and histological colitis scores for WT and \( \text{Slco2a1}^{-/-} \) mice on experimental day 7. (d-h) Microarray analysis of mRNA expression in the colon. (d) Heatmap showing whole-gene expression profiles for WT and \( \text{Slco2a1}^{-/-} \) mice treated with or without DSS for 7 days. (e) Top-20 enriched GO terms for genes upregulated in \( \text{Slco2a1}^{-/-} \) vs. WT mice treated with DSS as revealed by GSEA. (f) GSEA plots of immune response and positive regulation of immune system processes. (g) Heatmap showing the relative expression levels (red = high, blue = low) of the 40 most strongly upregulated genes involved in the inflammatory response in \( \text{Slco2a1}^{-/-} \) vs. WT mice with colitis. (h) Heatmap of cytokine expression in WT and \( \text{Slco2a1}^{-/-} \) mice with colitis. Data represent the mean ± SEM or median and IQR. Statistical significance was calculated by Student’s \( t \)-test, Welch’s \( t \)-test, or Mann-Whitney test (*\( P < 0.05 \), **\( P < 0.01 \)).
genes, such as Il1b, Il18, P2rx7, and macrophage-related chemokines, such as Ccl2 and Ccl4, was upregulated in Slco2a1ΔIEC compared to WT mice administered DSS. The proinflammatory cytokine interleukin (IL)-1β, which is cleaved and activated by inflammasome assembly18, and the nucleotide-binding domain, leucine-rich repeats containing family, pyrin domain-containing-3 (NLRP3) inflammasome reportedly play important roles in inflammation in DSS-induced colitis model mice19. Inflammasomes activation is involved in other experimental enteritis/colitis models20,21 and is related to human inflammatory bowel disease (IBD) via genetic and environmental factors22,23.

**IL-1β production induced by inflammasome activation is increased in Slco2a1ΔIEC mice.** We next focused on the role of the NLRP3 inflammasome in the exacerbated colitis in Slco2a1ΔIEC mice. mRNA expression of pro-inflammatory cytokines in colon tissues as assessed by RT-qPCR was increased in Slco2a1ΔIEC compared to WT mice on days 3 and 7 of DSS treatment (Fig. 3a). Although Casp1 mRNA expression was decreased (Fig. 3b), protein levels of pro- and cleaved IL-1β, cleaved caspase-1 (CASP1), and NLRP3 were also significantly elevated in Slco2a1ΔIEC compared to WT mice with colitis (Fig. 3c, Supplementary Fig. S3). These results indicated that NLRP3 inflammasome activation might be associated with the exacerbation of DSS colitis in Slco2a1ΔIEC mice. The NLRP3 inflammasome and subsequent secretion of mature IL-1β by macrophages reportedly are a critical mechanism of intestinal inflammation in the DSS-induced acute colitis mouse model19. Considering this fact together with the microarray data, we next focused on the inflammasome pathway in intestinal macrophages, and investigated whether inflammasome activation in macrophages could exacerbate the intestinal inflammation. To assess the inflammasome status in macrophages with genetic Slco2a1 deletion, we isolated colonic lamina propria (LP) macrophages from WT and Slco2a1ΔIEC mice with DSS-induced colitis. Consistent with the results in vivo, Il1b mRNA expression was significantly increased, whereas Casp1 mRNA expression was significantly decreased in Slco2a1ΔIEC mice (Fig. 3d). Nlrp3 mRNA expression was elevated, but not significantly. In line with the mRNA data for LP macrophages, the protein levels of NLRP3, cleaved caspase-1, and mature IL-1β were significantly increased in bone marrow-derived macrophages (BMDMs) from Slco2a1ΔIEC compared to the levels in BMDMs from WT mice (Fig. 3e, Supplementary Fig. S4). Accordingly, although administration of MCC950, a specific small-molecule inhibitor of NLRP3 inflammasome activity24, did not affect body weight changes (Fig. 3f), it reduced the severity of colitis in Slco2a1ΔIEC mice (Fig. 3g,h), diminished the Il1b and Tnf mRNA levels (Fig. 3i) with suppressed formation of mature IL-1β and cleaved caspase-1 (Fig. 3j, Supplementary Fig. S5), suggesting that the NLRP3 inflammasome contributed to exacerbation of DSS colitis under Slco2a1 deletion.

**Slco2a1 deficiency in macrophages, but not intestinal epithelial cells, alters susceptibility to DSS-induced colitis.** To investigate whether Slco2a1 deficiency in macrophages is associated with exacerbation of colitis, we developed mice with macrophage-specific deletion of Slco2a1 (Lysozyme M-cre;Slco2a1fl/fl, hereafter referred to as Slco2a1ΔMP). On only experimental day 4, Slco2a1ΔMP mice treated with DSS exhibited more severe reductions in body weight than did Slco2a1ΔIEC littermates, although weight loss was not different significantly between the two groups on experimental day 7 (Fig. 4a). Histological inflammatory changes were also significantly increased in Slco2a1ΔMP mice (Fig. 4b,c). On the other hand, mice with intestinal epithelial cell-specific deletion of Slco2a1 (Villin-cre;Slco2a1ΔIEC, hereafter referred to as Slco2a1ΔIEC) showed less body weight reduction and milder intestinal inflammation than did Slco2a1ΔIEC littermates (Fig. 4d–f).

**Increased PGE2 secretion by macrophages in the colon in Slco2a1ΔIEC mice might be a potential mechanism of exacerbation of DSS-induced colitis.** As Slco2a1 encodes a PGT, we next hypothesized that inflammasome activation in macrophages could be caused by alteration of the PGE2 concentration by Slco2a1 deficiency. In colon tissue of DSS colitis mice on day 7, PGE2 concentrations were significantly higher in Slco2a1 ΔIEC mice treated with DSS (Fig. 5b). Furthermore, PGE2 concentrations in colon tissue were significantly higher in Slco2a1ΔMP administered DSS than in Slco2a1ΔIEC littermates (Supplementary Fig. S6a), although mRNA levels of Ptg1, Ptg2, Abcc4, and Hpgd were not different between both mice (Supplementary Fig. S6b). Therefore, we next explored PGE2 production by Slco2a1ΔIEC-deficient macrophages, which were identified as key players in DSS colitis exacerbation. PGE2 concentrations in extracellular fluids of BMDMs after lipopolysaccharide (LPS) stimulation from Slco2a1ΔIEC mice were significantly higher than those in the WT counterparts, although intracellular concentrations did not differ (Fig. 5c,d). mRNA levels of Ptg1, Ptg2, Abcc4, and Hpgd were significantly decreased in LPS macrophages from Slco2a1ΔIEC mice with DSS-induced colitis when compared with their WT counterparts (Fig. 5e). The decreased mRNA expression of these genes and the increased extra-to-intracellular PGE2 concentration ratio in Slco2a1ΔIEC-deficient BMDMs indicated that Slco2a1 deficiency might contribute to increase in PGE2 concentration via inhibition of transport of extracellular PGE2 into the cytoplasm.

The PGE2/EP2 signalling pathway has been recently reported to boost pro-IL-1β production in BMDMs stimulated with LPS, resulting in mature IL-1β production via NLRP3 activation.25 Together with our data, this finding suggests that Slco2a1 deficiency might lead to a decline in PGE2 metabolism and thus, a high concentration of PGE2 around macrophages, subsequently resulting in exacerbated intestinal inflammation via inflammasome activation in macrophages. In support of this hypothesis, although indomethacin treatment led to considerable bodyweight loss in both WT and Slco2a1ΔIEC mice given DSS compared to no treatment (Fig. 5f), Slco2a1ΔIEC mice administered DSS and treated with indomethacin exhibited significantly lower histological colitis scores than WT mice administered DSS and indomethacin, and Slco2a1ΔIEC mice administered DSS alone (Fig. 5g,h). To assess the mechanistic link between Slco2a1 deficiency and inflammasome activation via PGE2 pathway, BMDMs from WT and Slco2a1ΔIEC mice were stimulated with LPS and PGE2/COX inhibitor indomethacin. In line with
Figure 3. NOD-like receptor protein 3 (NLRP3) inflammasome is activated in Slco2a1−/− mice administered dextran sodium sulphate (DSS). (a,b) mRNA levels of inflammatory cytokines (a) and NLRP3 inflammasome-associated molecules (b) in colonic tissues on experimental days 0, 3, and 7 as assessed by RT-qPCR. (c) Western blot assessment of pro- and mature IL-1β, pro- and cleaved caspase-1, and β-Actin in colonic tissues on experimental day 7. β-Actin was used for normalization. (d) mRNA levels of inflammatory cytokines and NLRP3 inflammasome-related genes in LP macrophages isolated from WT and Slco2a1−/− mice treated with 3.5% DSS for 3 days. (e) BMDMs from WT and Slco2a1−/− mice were stimulated with 1 μg/mL of LPS for 4 h and then analysed NLRP3 for inflammasome-related molecules by western blotting. β-Actin was used for normalization. (f) Changes in body weights of WT (n = 6) and Slco2a1−/− (n = 6) mice intraperitoneally treated with MCC950 (NLRP3 inhibitor) or PBS during 3.5% DSS administration. (g,h) Representative H&E-stained ileum and colon sections (scale bars: 50 μm) and histological colitis scores. (i) mRNA levels of Il1b and Tnf in colon tissues from WT and Slco2a1−/− mice on experimental day 7. Data represent mean ± SEM or median and IQR. Statistical significance was calculated by Student’s t-test, Welch’s t-test, or Mann-Whitney test (*P < 0.05, **P < 0.01).
t-test, Welch's t significance was calculated by Student's *-test, or Mann-Whitney test (*P < 0.05, or **P < 0.01).

Figure 4. Slco2a1 deficiency in macrophages alters susceptibility to dextran sodium sulphate (DSS)-induced colitis. (a) Changes in body weight during the experimental period. Slco2a1+/+ (n = 6) and Slco2a1ΔMP (n = 6) mice were administered 3.5% DSS for 7 days. (b,c) Representative H&E-stained colon sections (scale bars: 50 μm) and histological colitis scores for Slco2a1+/+ and Slco2a1ΔMP mice on day 7 after administration of 3.5% DSS. (d) Changes in body weight during the experimental period. Slco2a1+/+ (n = 6) and Slco2a1ΔIEC (n = 6) mice were administered 3.5% DSS for 7 days, and then given normal drinking water for one day. (e,f) Representative H&E-stained colon sections (scale bars: 50 μm) and histological colitis scores for Slco2a1+/+ and Slco2a1ΔIEC mice on experimental day 8. Data represent the mean ± SEM or median and IQR. Statistical significance was calculated by Student's t-test, Welch's t-test, or Mann-Whitney test (*P < 0.05, or **P < 0.01).

Discussion
This study showed that Slco2a1 deficiency led to high concentration of PGE2, metabolic pathway, resulting in exacerbated intestinal inflammation via inflammasome activation in macrophages (Fig. 6), which might be a possible mechanism of CEAS.

Various studies have reported that PGs, which are synthesized from arachidonic acid by COXs, and selective prostanoid receptor agonists exert anti-inflammatory and mucosal protective effects in experimental colitis by inhibiting inflammatory cytokines and inducing mucus secretion in intestinal epithelial cells26,27. Clinical reports of NSAID-induced intestinal mucosal injury and basic evidence of genetic COX deletion- and COX inhibitor-exacerbated colonic injury in several models of colitis28–30 support the beneficial effect of PGE2 on intestinal epithelial cells.

On the other hand, studies reporting on roles of molecules related to PGE2 metabolism in inflammatory conditions are limited. The PGE2 metabolic pathway involves SLCO2A1, which mainly imports PGE2 into the cytoplasm, and the metabolic enzyme 15-PGDH, which oxidizes PGE2 to 15-keto-PGE231. 15-PGDH expression is upregulated by inflammatory cytokines, such as IL-6, in a prostate cancer cell line32. These findings indicate that the PGE2 metabolic pathway also contributes to an altered response to inflammatory stimuli, depending on the cell type. In fact, Hpgd knockdown or pharmacologic inhibition of 15-PGDH increased tissue PGE2 levels and lowered the susceptibility to DSS colitis in mice33.

The present study revealed that germline Slco2a1-deficient mice were more susceptible to DSS-induced colitis than WT mice, but did not spontaneously develop enteritis or colitis. This result was opposite to what we expected based on findings in Hpgd-knockout mice33 and the beneficial effects of PGEs and selective prostanoid receptor agonists in the intestine36,37. Therefore, we conducted an unbiased microarray assay to identify mechanisms for
Figure 5. Concentrations of prostaglandin E₂ (PGE₂) and expression of PGE₂-related genes in colon tissues.

(a) Concentrations of PGE₂ in supernatants from colon explant cultures and colon tissue homogenates after administration of 3.5% DSS for 3 days were measured by ELISA. (b) mRNA levels of Ptgs 1, Ptgs 2, Abcc4, and Hpgd relative to those in WT mice on day 0 in colon tissue on days 3 and 7 after administration of 3.5% DSS. (c) BMDMs were isolated from WT and Slco2a1−/− mice and stimulated with 1 µg/mL of LPS for 4 h. PGE₂ concentrations in supernatants and cell homogenates were determined by ELISA. (d) Extra-to-intracellular PGE₂ concentration ratios. (e) mRNA levels of PGE₂-related genes in LP macrophages isolated from WT and Slco2a1−/− mice treated with 3.5% DSS for 3 days. (f) Changes in body weight during the experimental period. WT (n = 6) and Slco2a1−/− (n = 6) mice were treated with 3.5% DSS and indomethacin (nonselective cyclooxygenase inhibitor, 1 mg/kg) for 7 days. (g,h) Representative H&E-stained colon sections (scale bars: 50 µm) and histological colitis scores for WT and Slco2a1−/− mice on experimental day 7. (i) BMDMs from WT and Slco2a1−/− mice were stimulated with LPS (1 µg/mL) and PGE₂ (1 μM or 10 μM) or indomethacin (10 μM) for 4 h and then analysed IL-1β and caspase-1 by western blotting. Data represent the mean ± SEM or median and IQR. Statistical significance was calculated by Student’s t-test, Welch’s t-test, or Mann-Whitney test (**P < 0.05, ***P < 0.01).
the exacerbation of experimental colitis in Slco2a1-knockout mice. Intriguingly, microarray analysis of colon tissues revealed that inflammasome-related genes, such as Il1b, Il18, and P2rx7, and macrophage-related chemokines, such as Ccl2 and Ccl4, were upregulated in Slco2a1-knockout mice administered DSS when compared with WT mice treated with DSS. The cytokine IL-1β has been implicated as a central mediator of the inflammatory processes in patients with IBD and in experimental colitis34–36. Inactive pro-IL-1β is cleaved by caspase-1, which is activated within the inflammasome, to generate the mature, active form37. The inflammasome is formed by NLRP3, the adaptor protein apoptosis-associated speck-like protein containing a caspase-recruitment domain, and caspase-118. Protein levels of mature IL-1β and cleaved caspase-1 were increased in colon tissues of Slco2a1-knockout mice treated with DSS, which led us to hypothesize that NLRP3 inflammasome activation might contribute to the exacerbation of DSS colitis in Slco2a1-deficient mice. This hypothesis was proven true by administering mice MCC950, a specific inhibitor of the NLRP3 inflammasome24,38, as MCC950 administration reduced the severity of colitis in Slco2a1-deficient mice via suppressing the expression of mature IL-1β and cleaved caspase-1.

Several studies have shown that macrophages are the main site of IL-1β production in the colon in IBD patients39–41. The NLRP3 inflammasome and subsequent secretion of mature IL-1β by macrophages has been reported as a main mechanism of intestinal inflammation in model mice19,25. In line with these findings, LP macrophages and BMDMs isolated from Slco2a1-deficient mice showed stronger inflammasome activation than WT mice. Macrophage-specific, but not intestinal epithelial cell-specific Slco2a1 knockout increased the susceptibility to DSS-induced colitis, partially phenocopying systemic Slco2a1-deficient mice, which indicated that Slco2a1 in macrophages might play an important role in maintaining mucosal homeostasis in conditions of injury.

The present study showed that Slco2a1 deficiency led to an increased PGE2 concentration in colon tissues of Slco2a1-knockout mice. In-vitro experiments using BMDMs corroborated that PGE2 concentrations in extracellular fluids of BMDMs from Slco2a1-deficient mice were higher than those in their WT counterparts, possibly through suppression of PGE2 metabolism. A recent study demonstrated that PGE2/EP2 signalling pathway facilitated pro-IL-1β production in BMDMs stimulated with LPS, resulting in mature IL-1β by the NLRP3 inflammasome activation25. Increased PGE2 production was related with macrophage infiltration in the intestine in mice with DSS-induced colitis42. Taking these findings together, it can be considered that the NLRP3 inflammasome in macrophages was autocrinally activated by the elevated concentrations of PGE2, leading to exacerbation of colitis in Slco2a1-deficient mice. In support of this, exacerbated colitis in mice lacking Slco2a1 was improved by treatment with the COX inhibitor indomethacin as the concentration of PGE2 around macrophages decreased. On the other hand, in WT mice, colitis was worsened by indomethacin administration, which is consistent with finding by Okayama et al.43. These implied that the severity of colitis would depend on the balance between PGE2 mediated anti-inflammatory effect on epithelium and pro-inflammatory effect on activated macrophage. Indomethacin treatment led to considerable body weight loss in both WT and Slco2a1-deficient mice treated with DSS when compared to non-treated animals, but this was presumed to be because of mucosal injury of the stomach or small intestine caused by indomethacin44–45.

Figure 6. Schematic representation of the possible mechanism of gut inflammation induced by DSS under Slco2a1-deficiency. Slco2a1-deficiency under DSS stimulation increases the PGE2 concentration around macrophages by suppressing PGE2 metabolism. The increased PGE2 causes NLRP3 inflammasome activation in macrophages, thus exacerbating gut inflammation.
Our observation that increased PGE\textsubscript{2} levels exacerbated colitis is in contrast to findings in \textit{Hpgd}\textsuperscript{-}deficient mice by Zhang et al.\textsuperscript{33}, who reported that deletion of 15-PGDH increased tissue PGE\textsubscript{2} levels and protected mice from experimental colitis. A possible explanation could be the difference in the dominant locations of \textit{Slco2a1} and 15-PGDH expression. Zhang et al. concluded that the phenotype of \textit{Hpgd}\textsuperscript{-}deficient mice is due to 15-PGDH deletion in colonic epithelial cells, which are the primary site of 15-PGDH expression in the colon.\textsuperscript{46} On the other hand, our study revealed that the phenotype of \textit{Slco2a1}\textsuperscript{-}deficient mice is mainly due to \textit{Slco2a1} deletion in macrophages. In fact, intestinal epithelial-specific \textit{Slco2a1}\textsuperscript{-}deficient mice has lower susceptibility to experimental colitis as with the model in the study by Zhang et al. Although suppressed PGE\textsubscript{2} metabolism in macrophages might contribute to the enhanced PGE\textsubscript{2} concentration, \textit{Slco2a1}\textsuperscript{-}\textit{ΔIEC}\textsuperscript{-} mice partially reproduced the systemic knockout phenotype in the present study. As \textit{Slco2a1} is mainly expressed on vascular endothelial cells in the intestinal tracts of mice\textsuperscript{47} and humans\textsuperscript{42}, a decrease in the clearance of PGE\textsubscript{2} from vascular endothelial cells might also contribute to the increase in the PGE\textsubscript{2} concentration around macrophages in the intestine. Further studies will clarify the role of \textit{Slco2a1} on endothelial cells in intestinal homeostasis.

Although a recent study has demonstrated that \textit{Slco2a1} function is lost in patients with CEAS\textsuperscript{15}, the current study revealed that \textit{Slco2a1}\textsuperscript{-}deficient mice did not spontaneously develop enteritis. This might be because \textit{Slco2a1}/\textit{Slco2a1} has different functions \textit{in vivo} in mice and humans. Patients with CEAS might have a “second-hit factor” other than lack of \textit{Slco2a1}, such as other genetic factors or certain pathogens in the intestinal lumen. Considering the clinical impact, these mice model may not be the best model to analyse CEAS which affects mainly small intestine. Further studies using another enteritis model are needed to clarify the pathogenesis of CEAS.

In summary, \textit{Slco2a1} deficiency increased the PGE\textsubscript{2} concentration around macrophages, possibly by suppressing PGE\textsubscript{2} metabolism, resulting in activation of the NLRP3 inflammasome in macrophages and thus exacerbating intestinal inflammation in an experimental colitis model. Our findings shed some light on the pathogenesis of the intestinal inflammation associated with \textit{Slco2a1} and might provide a novel therapeutic target.

**Methods**

**Animals.** \textit{Slco2a1}\textsuperscript{-}\textit{IEC}\textsuperscript{-} and \textit{Slco2a1}\textsuperscript{+/+} mice on B6 background were obtained from Kanazawa University\textsuperscript{44}. \textit{Lysozyme (Lys)} M\textit{-}cre mice and \textit{Villin (V)}-\textit{cre} mice were obtained from RIKEN Bioresource Centre (RRID:IMSR\_RRBC02302) and Jackson laboratory (B6.Cg-Tg(Vil1-cre)1000Gum/J), respectively. \textit{Slco2a1}\textsuperscript{+/+} mice were crossed with \textit{LysM}-\textit{cre} mice and \textit{V}-\textit{cre} mice to generate mice with specific \textit{Slco2a1} deletion in macrophages (\textit{Slco2a1}\textsuperscript{−}\textit{ΔIEC}\textsuperscript{-} and intestinal epithelial cells (\textit{Slco2a1}\textsuperscript{−}\textit{ΔIEC}\textsuperscript{-}\textit{IEC}\textsuperscript{-}). Sex- and age-matched WT mice (C57BL/6), obtained from Charles River Japan Inc. (Atsugi, Japan), and sex- and age-matched littersmate \textit{LysM}-\textit{cre} ; \textit{V}-\textit{cre} ; \textit{Slco2a1}\textsuperscript{+/+} mice were used as controls for \textit{Slco2a1}\textsuperscript{-}\textit{IEC}\textsuperscript{-} mice and \textit{Slco2a1}\textsuperscript{−}\textit{ΔIEC}\textsuperscript{-} mice, respectively. The mice were kept under constant housing conditions (12-h light/dark cycles and 22 ± 1 °C) and had free access to water and standard diet throughout the experimental period. All experiments were approved by the animal care committee of the Osaka City University Graduate School of Medicine (approval number: 16025). All experiments were conducted in accordance with relevant guidelines/regulations.

**Assessment of basal phenotype of \textit{Slco2a1}\textsuperscript{-}\textit{IEC}\textsuperscript{-} mice.** Body weights of WT and \textit{Slco2a1}\textsuperscript{-}\textit{IEC}\textsuperscript{-} mice were recorded weekly until the age of 24 weeks. At the ages of 8, 12, and 24 weeks, gender-matched mice were sacrificed by CO\textsubscript{2} asphyxiation. Small intestines and colons were immediately collected for evaluation of spontaneous inflammation. The effect of \textit{Slco2a1} knockout is shown in Supplementary Fig. S8.

**Dextran sodium sulphate (DSS)-induced colitis and treatments.** Acute colitis was induced by 3.5% (w/v) DSS (molecular weight, 5000; Fujifilm, Osaka, Japan) added to the drinking water for 7 days. Seven- to nine-week-old gender-matched mice were included in each group. Body weight was recorded daily. Mice were sacrificed on day 3 or 7, and the colons were collected. To inhibit the NLRP3 inflammasome \textit{in vivo}, mice received an intraperitoneal injection of 50 mg/kg MC950 (AlimatoGen Life Sciences, San Diego, CA) (or PBS as a control) every other day from one day before to day 7 of DSS administration. To inhibit PGE\textsubscript{2} production \textit{in vivo}, indomethacin (Nacalai Tesque, Kyoto, Japan) was dissolved in ethanol (10 mg/mL) and was added to the drinking water at 1 mg/kg per day, concurrent with DSS treatment.

**Histopathological scoring of enterocolitis.** Tissues were flushed with PBS and fixed 4% paraformaldehyde phosphate buffer solution for 5 days prior to paraffin embedding. Sections (4 μm) were then cut and stained with haematoxylin and eosin (H&E) and alcian blue/periodic acid-Schiff (AB/PAS) using an Alcian Blue-PAS Stain Kit (ScyTek Laboratories, Logan, UT) following the manufacturer’s instructions. Ileum or colon sections were evaluated using a histological scoring system\textsuperscript{49} or histological grading of colitis\textsuperscript{50,51}. Goblet and Paneth cells were quantified in at least 100 crypts of each mouse.

**Isolation and stimulation of murine macrophages.** LP macrophages were isolated from the mouse colons as described previously\textsuperscript{52}. CD11b-positive cells among LP cells were purified by magnetic-activated cell sorting using CD11b MicroBeads (130-049-601; Miltenyi Biotec, Bergisch Gladbach, Germany) following the manufacturer’s instructions.

BMDMs were prepared as described\textsuperscript{53,54}. Briefly, bone marrow cells were incubated in Dulbecco’s modified Eagle’s medium (DMEM; Sigma-Aldrich, St. Louis, MO) supplemented with 20% foetal bovine serum (FBS; HyClone, Logan, UT) and 5% antibiotic-antimycotic (Amphotericin B, Penicillin, and Streptomycin; Thermo Fisher Scientific, Waltham, MA) at 37 °C for 4 h. The cells in supernatants were then collected and cultured in 10 mL of DMEM containing 15% L929 cell-conditioned medium, 20% FBS, and 5% antibiotic-antimycotic for 10 days, with a medium change every 2–3 days. BMDMs were cultured in culture dishes or 6-well plates in the absence of FBS. On the following day, the cells were stimulated with 1 μg/mL LPS (Escherichia coli 0111:B4,
L4391; Sigma-Aldrich) for up to 4 h. 16,16-dimethyl PGE₂ (dmPGE₂; Cayman Chemical, Ann Arbor, MI) and indomethacin were added to culture before LPS treatment.

RNA extraction and quantitative reverse-transcription polymerase chain reaction (RT-qPCR). Total RNA was extracted from tissues and cells using an ISOGEN II kit (Nippon Gene, Tokyo, Japan) following the manufacturer's protocol. A High-Capacity RNA-to-cDNA Kit (Thermo Fisher Scientific) was used per the manufacturer's protocol to convert the total RNA to complementary DNA. qPCRs were run in duplicate using SYBR Select Master Mix (Thermo Fisher Scientific) in an Applied Biosystems 7500 Fast Real-Time PCR system, and data were analysed with the in-built software (Thermo Fisher Scientific). Thermal cycling conditions were as follows: 50 °C for 2 min and 95 °C for 2 min, 40 cycles of denaturation at 95 °C for 3 s and annealing at 60 °C for 30 s. The primers used are listed in Supplementary Table S1. Primers were designed by using Primer-BLAST in the NCBI genome browser. Gene expression was normalized to the expression level of Hprt mRNA.

**Western blot analysis.** Tissues and isolated cells were homogenized in RIPA lysis buffer (Thermo Fisher Scientific) containing Complete Protease Inhibitor Cocktail (Roche Diagnostics, Mannheim, Germany). Protein content was measured with a Pierce BCA Protein Assay kit (Thermo Fisher Scientific), and proteins were resuspended in Sample Buffer Solution with 3-Mercapto-1,2-Propanediol (4 ×) (Fujifilm) and heated to 98 °C for 5 min. The proteins were resolved on polyacrylamide gels and transferred to polyvinylidene difluoride membranes. The membranes were incubated with goat anti-IL-1β (1:500; Val118Ser269; R&D Systems, Minneapolis, MN), rabbit anti-caspase-1 (1:200; 14F68; Santa Cruz Biotechnology, Santa Cruz, CA), mouse anti-NLRP3 (1:1,000; Cryo-2; AdipoGen Life Sciences), and mouse anti-β-ACTIN (1:10,000; AC-15; Sigma-Aldrich) at 4 °C overnight. Proteins were detected using appropriate secondary antibodies conjugated with horseradish peroxidase and enhanced chemiluminescence (Amersham ECL Prime Western Blotting Detection Reagent; GE Healthcare Life Sciences, Buckinghamshire, UK).

**Colon tissue explant cultures.** Colon sections (1 cm) from individual mice were washed with PBS to remove faecal contents and were then cultured in 24-well plates in 500 μL of RPMI 1640 (Nacalai Tesque) containing 2% FBS and antibiotics at 37 °C and in the presence of 5% CO₂. After a 24-h incubation, the culture medium was collected and centrifuged. The supernatants were transferred to new tubes for enzyme-linked immunosorbent assay (ELISA) of PGE₂.

**ELISA.** Colon tissues and BMDMs were homogenized in 0.1 M phosphate containing 1 mM EDTA and 10 μM indomethacin. The amounts of PGE₂ in supernatants and tissue or cell lysates were determined by ELISA (PGE₂ ELISA Kit; Cayman Chemical), according to the manufacturer's protocol.

**Microarray analysis.** Total RNA extracted from colon tissues was purified using an RNAqueous-Micro Kit (Ambion, Austin, TX) for microarray analysis. The quality and quantity of purified RNA were assessed using an Agilent Technologies 2100 Bioanalyzer and NanoDrop spectrophotometer, respectively, and the RNAs were applied to a SurePrint G3 Mouse Gene Expression 8 × 60 K (Agilent, Inc., Santa Clara, CA). Raw data were extracted using Agilent Feature Extraction Software (v11.0.1.1). The raw data for each gene were automatically summarized in the Agilent feature extraction protocol to generate a raw data text file, providing expression data for each gene probed on the array. Data for array probes that had a Flag A were filtered out. Selected gProcessedSignal values were log-transformed and normalized by the quantile method. Hierarchical cluster analysis was performed using complete linkage and Euclidean distance as a measure of similarity. For GSEA, the GSEA v3.0 software (Broad institute, Massachusetts Institute of Technology and Regens of the University of California) was performed with gene set database c5.all.v6.2.symbols.gmt. The number of random sample permutations was set at 1000.

**Statistical analysis.** The data are presented as means ± standard errors of the mean (SEMs) or medians plus interquartile ranges (IQRs). Significance of differences among groups was determined by Student’s t-test, Welch’s t-test, or Mann–Whitney U test. P < 0.05 was considered significant.

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Author contributions
S.H. contributed to the study conception and design. Material preparation, investigation, data collection and analysis were performed by R.N., Y. Nakamura, S.H., H.O., Y. Nishida, T.N. The draft of the manuscript was written by R.N. and S.I., T.N. Substantial contributions to reviewing and editing this manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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