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Crucial Role of Macrophage Selenoproteins in Experimental Colitis

Naveen Kaushal,* Avinash K. Kudva,* Andrew D. Patterson,* Christopher Chiaro,* Mary J. Kennett,* Dhimant Desai,† Shantu Amin,‡ Bradley A. Carlson,‡ Margherita T. Cantorna,* and K. Sandeep Prabhu*

Inflammation is a hallmark of inflammatory bowel disease (IBD) that involves macrophages. Given the inverse link between selenium (Se) status and IBD-induced inflammation, our objective was to demonstrate that selenoproteins in macrophages were essential to suppress proinflammatory mediators, in part, by the modulation of arachidonic acid metabolism. Acute colitis was induced using 4% dextran sodium sulfate in wild-type mice maintained on Se-deficient (<0.01 ppm Se), Se-adequate (0.08 ppm; sodium selenite), and two supraphysiological levels in the form of Se-supplemented (0.4 ppm; sodium selenite) and high Se (1.0 ppm; sodium selenite) diets. Selenocysteinyl transfer RNA knockout mice (Trsp<sup>−/−</sup>Lys<sup>F<sup>+</sup></sup>) were used to examine the role of selenoproteins in macrophages on disease progression and severity using histopathological evaluation, expression of proinflammatory and anti-inflammatory genes, and modulation of PG metabolites in urine and plasma. Whereas Se-deficient and Se-adequate mice showed increased colitis and exhibited poor survival, Se supplementation at 0.4 and 1.0 ppm increased survival of mice and decreased colitis-associated inflammation with an upregulation of expression of proinflammatory and anti-inflammatory genes. Metabolomic profiling of urine suggested increased oxidation of PGE<sub>2</sub> at supraphysiological levels of Se that also correlated well with Se-dependent upregulation of 15-hydroxy-PG dehydrogenase (15-PGDH) in macrophages. Pharmacological inhibition of 15-PGDH, lack of selenoprotein expression in macrophages, and depletion of infiltrating macrophages indicated that macrophage-specific selenoproteins and upregulation of 15-PGDH expression were key for Se-dependent anti-inflammatory and proresolving effects. Selenoproteins in macrophages protect mice from dextran sodium sulfate–colitis by enhancing 15-PGDH–dependent oxidation of PGE<sub>2</sub> to alleviate inflammation, suggesting a therapeutic role for Se in IBD. The Journal of Immunology, 2014, 193: 000–000.
type (WT) mice (12). These studies suggest a pleiotropic role for PGE₂, where the control of PGE₂ production and catabolism may have a significant bearing on the pathophysiology of UC. Little is known about the impact of selenoprotein expression on PGE₂ catabolism and subsequent effect on pathways of inflammation and resolution. In fact, the role of 15-hydroxy PG dehydrogenase (15-PGDH), an NAD⁺-dependent dehydrogenase that oxidizes many eicosanoid metabolites, including PGE₂, in IBD is well-known (14). 15-PGDH is abundantly expressed in the gut and is involved in the catabolism of PGs and other proresolving lipid mediators, including resolvin E1, which affect resolution pathways in colitis, suggesting a dual role for this enzyme (15–17). However, 15-PGDH has been reported to preferentially bind to PGE₂, leading to the production of downstream 15-keto PGE₂ metabolites (PGEMs) (18, 19). It appears that such a metabolic event in response to selenoprotein expression may serve as a key determinant in the pathology of UC to modulate resolution of inflammation to alleviate colitis.

Increasing dietary Se intake from adequate levels to supranutritional levels that are well within the nontoxic dose range could potentially facilitate the resolution of UC-associated inflammation by regulating the metabolism of key lipid metabolites, such as PGD₁. A recent study reported increased levels of PGD₁ in colitis patients in remission (20). In this study, we demonstrate the ability of Se supplementation to upregulate the expression of 15-PGDH, leading to the increased catabolism of PGE₂ that alleviates inflammation by activating pathways of resolution in the dextran sodium sulfate (DSS) model of experimental colitis. Macrophage-specific selenoprotein knockout mice (Trepling/ LysM(−/−)) that do not express the selenoproteome because of the loss of both selenocysteinyl transfer RNA alleles reproduced the effects of whole-body Se deficiency on colitis susceptibility toward an inflammatory phenotype. Thus, nutritional intervention with Se supplementation may alleviate gastrointestinal inflammation and promote resolution through the expression of macrophage selenoproteins.

Materials and Methods

Mice
WT male C57BL/6 mice (3 wk old; Taconic, Madison, WI) containing <0.01 ppm Se. To the basal diet either no Se was added (Se-deficient [Se-D]), or sodium selenite was added at adequate levels of 0.08 ppm (Se-adequate [Se-A]), or supplemented with supranutritional doses of selenite at 0.4 ppm Se (Se-supplemented [Se-S]) or 1.0 ppm Se (Se-high [Se-H]) for at least 10–12 wk. Trsp(1⁰⁰) mice were prepared as described earlier (21) and crossed with LysM(−/−) mice to produce Trsp(1⁰⁰) LysM(−/−) mice that lack the expression of selenoproteins in monocytes and macrophages (and to a minor extent in granulocytes) (22). These mice were maintained on Se-D or Se-S diets. In experiments using Trsp(1⁰⁰) LysM(−/−) mice, corresponding sex-, age-, and diet group–matched WT littermates were used for comparison. All procedures were reviewed and approved preapproved by the Institutional Animal Care and Use Committee at The Pennsylvania State University.

Induction of colitis
Mice were administered 4.0% DSS (molecular mass = 40 kDa; ICN Biomedicals, Aurora, OH) dissolved in Milli-Q water ad libitum for 5 d, after which the mice were switched to Milli-Q water for the remainder of the experiment. Animals were weighed daily and monitored clinically for rectal bleeding, diarrhea, and general signs of morbidity. Moribund mice or mice that had lost >25% of their body weight were sacrificed and listed as dead after induction of colitis. Day −1 represented 1 d before the initiation of DSS administration.

Pathological examination and scoring of colitis
The entire colon (from cecum to anus) was removed, and the length was measured and reported as colonic length as described previously (23). Distal colon was removed, fixed in 10% formalin, sectioned, and stained with H&E for histopathology (at the Pennsylvania State University Animal Diagnostic Laboratory). Histological analysis was performed blindly and the slides were scored as follows: severity of inflammation (0–4: none, slight, moderate, severe), extent of injury (0–4: none, mucosal, submucosal, and transmural), and crypt damage (0–4: none, basal 1/3 damaged, basal 2/3 damaged, only surface epithelium intact with a loss of entire crypt) (24). Sections were scored for a total of 12 points and divided by 3 main criterion scores to fit within the 0–4 scale.

Cell culture and Se treatments
Murine RAW264.7 immortalized macrophage-like cell line and primary bone marrow-derived macrophages (BMDMs) isolated from mice were cultured in DMEM media supplemented with 5% defined FBS (American Type Culture Collection) with low Se (∼6 nM by atomic absorption spectroscopy) and sterile filtered 10% (v/v) L929 murine fibroblast culture media supernatant. These Se-D cells were conditioned with LPS (100 ng/ml) and then cultured with sodium selenite (0.1–0.5 μM). To address whether bioavailable Se was critical to effect the expression of 15-PGDH, we changed the source of Se from inorganic sodium selenite to organo-Se compounds such as methylseleninic acid (MSA), L-selenomethionine (SeMet), or 1,4-phenylenebis(methylene)selenocyanate (pXSC) at the concentrations indicated. Cells were allowed to grow for at least 3 d with daily media changes before being used for experiments. None of these treatments affected cell viability or growth rates (data not shown).

Gene and protein expression analysis
Changes in various classical inflammatory markers and 15-PGDH expression were studied in the macrophages and the colonic tissues extracted from mice as described elsewhere.

Cells. Cells cultured in different Se concentrations (as described earlier) were harvested and used for isolation of RNA and protein. Expression of 15-PGDH, TNF-α, IFN-γ, and IL-1β was analyzed by quantitative real-time PCR (qPCR) using an ABI 7300 real-time system (Thermo Fisher Scientific, Waltham, MA). Data were analyzed according to the method of Livak et al. (25, 26) and results were expressed as 2^ΔΔCt, which is the expression of target gene relative to the housekeeping gene (GAPDH) that is normalized to the negative control in the Se-D group. To study protein expression, we prepared lysates using the mammalian protein extraction reagent (Thermo Fisher Scientific, Waltham, MA), and immunoblots were developed using specific primary and appropriate secondary Abs for 15-PGDH (Cayman Chemicals, Ann Arbor, MI), glutathione peroxidase 1 (GPX)-1 (Abcam, Cambridge, MA), and GAPDH (Fitzgerald Industries, Acton, MA). The bands were visualized by using an ECL assay kit.

Mice. Distal colon samples from mice were collected and washed in PBS containing penicillin (100 U/ml) and streptomycin (100 μg/ml). RNA was isolated from the colon with TRIzol reagent and used for qPCR analysis with TaqMan probes (Thermo Fisher Scientific, Waltham, MA).

Western immunoblotting. Tissue homogenates were prepared in ice-cold RIPA buffer and centrifuged at 13,000 rpm for 5 min at 4°C. Protein concentrations were determined in the supernatants using BCA method (Thermo Fisher Scientific, Waltham, MA) and subjected to Western immunoblots as described earlier.

Biochemical analyses
The activity of 15-PGDH was measured by recording the changes in absorbance due to formation of NADH from NAD⁺ by 15-PGDH (27). Myeloperoxidase activity assay in the colonic tissue was performed as described previously (28).

Metabolomics and ELISA
Urine samples were diluted 1:10 in 50% acetonitrile:water containing 5 μM chloropropamide (internal standard). The samples were vortexed and centrifuged at maximum speed for 20 min at 4°C. The supernatants were subjected to mass spectrophotometry, and the spectral data were deconvoluted using MarkerLynx software program (Waters). Characteristics such as mass-to-charge ratio, retention time, and peak area of each data point were used for analysis. Principal components analysis and supervised projection to latent structures discriminant analysis were conducted using SIMCA P12+ software (Umetrics). Candidate biomarkers were searched using METLIN (Metabolite and Tandem MS Database), database from the Scripps Center for Metabolomics. Levels of PGE₂, and its downstream metabolites (PGEMs) 13, 14-dihydro-15-keto PGE₂ and 13,14-dihydroxy-15-keto-PGA₁, were measured by respective ELISA kits (as per manufacturer’s protocol) in the plasma isolated from mice maintained on different Se diets upon treatment with DSS.
Depletion of macrophages

Macrophages were depleted using clodronate-containing liposomes (CLs; ClodronateLiposomes.org, Amsterdam, the Netherlands) as described previously (29). In brief, mice were anesthetized and 200 μl CLs was injected via retro-orbital sinus into mice on days −1, 1, 3, and 5 after DSS treatment. PBS-encapsulated liposomes were not used as a negative control, because the uptake of these liposomes by colonic macrophages was shown to result in partial reduction of macrophages (28).

Synthesis of 2-hydroxy-5-(4-ethoxycarbonylphenylazo) benzeneacetic acid (CAY10397)

Ethyl p-aminobenzoate (compound 1; 0.33 g, 2 mmol; Supplemental Fig. 1A) was dissolved with cooling to 0°C in HCl (6 N, 4 ml). A solution of sodium nitrite (0.21 g, 3 mmol) in water (2 ml) was added, and the reaction mixture was stirred at 0°C for 25 min. The diazonium salt solution was added to 2-hydroxy-benzeneacetic acid (0.33 g, 2.2 mmol) and sodium hydroxide (0.11 g, 2.8 mmol) in water (2 ml) at 4°C. The reaction mixture was adjusted to pH 9–10, and the mixture was stirred for 2 h and acidified by HCl. The precipitated product was filtered and washed with water, which on recrystallization from ethanol/water resulted in 0.38 g (57%) pure compound. Ethyl p-aminobenzoate was treated with NaNO2 under acidic conditions to form a diazonium salt (compound 2; Supplemental Fig. 1A), which was used in the next step without purification. Compound 2 was coupled with 2-hydroxy-benzeneacetic acid under basic conditions to form compound 3, which on recrystallization from aqueous ethanol resulted in pure 2-hydroxy-5-(4-ethoxycarbonylphenylazo)benzeneacetic acid (compound 3; Supplemental Fig. 1A) with a 57% yield. The structure of compound 3 was confirmed by [1H]-NMR (Supplemental Fig. 1B).

Selected physical data for compound 3: [1H]-NMR (d6-DMSO) δ 1.35 (t, 3H, CH3), 3.61 (s, 2H, CH2), 4.35 (dd, 2H, OCH2), 7.01 (d, 1H, aromatic, J = 8.5 Hz), 7.81–7.70 (m, 2H, aromatic), 7.90 (d, 2H, aromatic, J = 8.5 Hz), 8.13 (d, 2H, aromatic, J = 9.0 Hz); [13C]-NMR: δ 14.63, 35.78, 61.47, 115.80, 122.65, 123.77, 125.37, 126.17, 130.90, 131.31, 145.55, 155.29, 160.43, 165.70, 172.81. Purity of compound 3 was further confirmed by reverse-phase HPLC using a Vydac C18 Analytical column. Solvent gradient of 0–100% methanol in 30 min with a flow rate of 1 ml/min was used. The purity of compound 3 (retention time = 25 min) was >97% (data not shown).

Pharmacological inhibition of 15-PGDH activity

CAY10397, a selective inhibitor of 15-PGDH, was administered by oral gavage at a dose of 100 mg/kg to Se-S mice every alternate day starting at day −1 of DSS treatment. CAY10397 (100 mg/ml) was formulated in 20 mM glycine buffer (pH 10) containing 5% (v/v) cell culture grade DMSO (Sigma-Aldrich, St. Louis, MO). CAY10397 was synthesized as described in the supplemental methods (Supplemental Fig. 1A). [1H]-NMR spectroscopic analysis (Supplemental Fig. 1B) and enzyme inhibition of purified human recombinant 15-PGDH (Cayman Chemicals, Ann Arbor, MI) analysis indicated that the chemically synthesized compound was identical to the commercially available CAY10397 (from Cayman Chemicals). Based on the inhibition of 15-PGDH activity in kidney extracts of mice (Supplemental Fig. 2), an in vivo dose of 100 mg/kg CAY10397 was used. No toxic effects were observed in mice at this dose (data not shown).

Data analysis

All data are expressed as mean ± SEM. An unpaired, two-tailed t test was used to compare the mean for each treatment group with the mean of the control group, and one-way ANOVA (Tukey multiple comparison method) or two-way ANOVA was performed to compare various treatment groups within in vivo studies using GraphPad Prism 5.0 program (GraphPad Soft-
ware, San Diego, CA). The \( p \) values \( \leq 0.05 \) were considered statistically significant.

**Results**

**Se supplementation alleviates DSS-induced colitis**

Fecal occult blood was detected in the feces of Se-D and Se-A diet–fed mice as early as day 1 after DSS administration. Body weight changes, diarrhea, and bleeding were significantly more severe in Se-D and Se-A mice compared with mice on Se-S and Se-H diets. Se-D and Se-A had significantly lower body weights than Se-S and Se-H mice (Fig. 1A). The colons of Se-D and Se-A mice were significantly shorter and had higher blood scores compared with Se-S and Se-H mice (Fig. 1B). Colonic sections from Se-D and Se-A mice showed edema and complete destruction of the mucosal surface with the loss of the epithelium and crypt damage. In addition, there was significant neutrophil infiltration and damage to colonic mucosa of Se-D and Se-A mice, indicating higher levels of tissue inflammation and failure to resolve epithelial injury (Fig. 1C). In contrast, colonic sections from DSS-treated Se-S and Se-H mice indicated negligible signs of inflammation and mucosal damage in addition to pronounced healing of the colon (Fig. 1C). Also, the classical molecular hallmarks of colitis such as TNF-\( \alpha \), IFN-\( \gamma \), IL-1\( \beta \), and COX-2 were significantly higher in Se-D and Se-A than in Se-S and Se-H mice groups (Fig. 2A). In contrast, Arg-1, Fizz-1, and Muc-2 were increased in Se-S and Se-H groups compared with Se-D and Se-A mice (Fig. 2B). These studies suggest a critical role for Se in the control of inflammation in DSS-induced experimental colitis.

**Macrophage-specific selenoproteins are required for resolution of colitis-associated inflammation**

Because macrophages play a key role in the DSS model of experimental colitis (30), and selenoprotein expression in macrophages was earlier demonstrated to be key to mitigate inflammation (6), we used the Trsp\( ^{fl/fl} \)LysM\( ^{Cre} \) mice. DSS-treated Trsp\( ^{fl/fl} \)LysM\( ^{Cre} \) mice lost weight despite being on an Se-S diet (Fig. 3A). Se-D and Se-S Trsp\( ^{fl/fl} \)LysM\( ^{Cre} \) mice displayed shorter colons (Fig. 3B) and increased histopathological scores than the Se-S WT mice (Fig. 3C). The symptoms of DSS colitis in the Se-S Trsp\( ^{fl/fl} \)LysM\( ^{Cre} \) mice resembled those of the Se-D WT mice treated with DSS. Histological analysis of the colon of Se-S Trsp\( ^{fl/fl} \)LysM\( ^{Cre} \) mice treated with DSS confirmed severe colitis and increased infiltration of neutrophils along with edema, cryptitis, and increased tissue inflammation (Fig. 3C). WT littermates maintained on an Se-S diet showed no lesions after DSS treatment (Fig. 3C). The expression of TNF-\( \alpha \), IFN-\( \gamma \), IL-1\( \beta \), and COX-2 were upregulated in the Se-S Trsp\( ^{fl/fl} \)LysM\( ^{Cre} \) mice compared with the littermate Se-S controls (Fig. 3D). These studies suggested that selenoprotein expression in macrophages was critical for the protective effects of Se in DSS colitis.

**Se-dependent changes in the metabolome indicate oxidation of PGE\(_2\)**

A 24-h pooled urine was collected from Se-D, Se-A, and Se-S mice before treatment with DSS for metabolomic analysis. Results indicated differences in the metabolite pools in three diet groups (Supplemental Fig. 3). Of the various metabolites that were differentially regulated by increasing Se in the diet, 13,14-dihydro-15-keto PGE\(_2\), a product of PGE\(_2\) oxidation, was produced at higher levels in the urine of Se-S mice compared with other groups (data not shown). Following this lead, a targeted liquid chromatography–mass spectrometry analysis was performed on the urine and plasma of mice on day 5 after DSS treatment. Although urinary 13,14-dihydro-15-keto PGE\(_2\) was higher in the Se-S mice after DSS treatment, the differences were not statistically significant (data not shown). However, 15-keto-PGE\(_2\) in the plasma of Se-S mice after DSS treatment was significantly higher than in Se-D mice (Fig. 4A). The role of 15-PGDH in the oxidation of PGE\(_2\) to 15-keto-PGE\(_2\) that is further reduced to form 13,14-dihydro-15-keto-PGE\(_2\), which subsequently undergoes dehydration to form 13,14-dihydro-15-keto-PA2\(_{\text{a}}\), is well-known (31). Plasma levels of PGE\(_2\), 15-keto-PGE\(_2\), and 13,14-dihydro-15-keto-PGE\(_2\) were estimated by ELISA from DSS-treated mice on day 10. PGE\(_2\) levels decreased in the plasma as a function of dietary Se, whereas 15-keto metabolites of PGE\(_2\) increased as a function of dietary Se (Fig. 4B). Se-dependent increase in 15-PGDH expression of mRNA, protein, and enzymatic activity were seen in DSS-treated Se-S and Se-H mice (Fig. 4C–E). Similarly, GPX2, a Se-dependent GPX predominantly expressed in the gastrointestinal tract, was also increased in a dose-dependent manner in the diet groups with saturation in the Se-S and Se-H groups (Fig. 4D). Taken together, these results suggest that Se-dependent upregulation of 15-PGDH may play a critical role in alleviating inflammation with a subsequent increase in resolution of mucosal injury.
Macrophage expression of 15-PGDH is regulated by selenoproteins

To examine the regulation of expression of 15-PGDH by selenoproteins, we cultured BMDMs isolated from mice maintained on the Se-D diet and murine RAW264.7 macrophage-like cells in the presence of graded levels of sodium selenite (0–500 nM Se). The expression (and activity) of 15-PGDH in RAW264.7 cells and BMDMs displayed Se dose response with a saturation at ∼250 nM Se (Fig. 5A, 5B). Western immunoblot analysis of GPX-1 expression confirmed the ability of both cell types to readily incorporate Se into selenoproteins (data not shown). Interestingly, BMDMs and RAW264.7 cells treated with two organo-Se compounds, SeMet or pXSC, which do not readily release Se for incorporation into selenoproteins, failed to increase the expression of 15-PGDH (Fig. 5C, 5D). However, treatment with MSA that releases Se for incorporation into selenoproteins increased the
expression of 15-PGDH. To further provide conclusive evidence of the requirement of selenoproteins, we examined the colonic expression of 15-PGDH in DSS-treated \textit{Trsp}^{fl/fl}LysM\textit{Cre} mice. Analysis of the colonic extracts on day 10 after DSS treatment clearly showed an increased expression and activity of 15-PGDH only in Se-S WT littermates, whereas significantly reduced levels of 15-PGDH were seen in \textit{Trsp}^{fl/fl}LysM\textit{Cre} mice on Se-S diet (Fig. 5E, 5F).

Macrophage depletion in Se-S mice blocks the protective effects during DSS treatment
To examine whether macrophages were essential players in the Se-mediated protective effects in DSS-colitis, we used CLs to deplete infiltrating macrophages (29). Depletion of macrophages even under Se-S conditions exacerbated the severity of colitis compared with PBS control Se-S mice (Fig. 6A–D). CL treatment of Se-S mice resulted in symptoms of colitis such as weight loss and rectal bleeding that resembled DSS-treated Se-D mice (Fig. 6A). As a control, we examined Se-D mice treated with CL to address whether Se deficiency was a critical factor to support our results with the \textit{Trsp}^{fl/fl}LysM\textit{Cre} mice. As shown in Fig. 6A and 6B, clodronate treatment of Se-D mice also exhibited severe DSS-colitis as in Se-D mice. Clodronate treatment significantly affected the onset of the disease in the Se-D mice, but was more or less similar to the PBS-treated Se-D mice subjected to DSS treatment. Taken together with the \textit{Trsp}^{fl/fl}LysM\textit{Cre} data, these results suggest that selenoprotein status and Se-dependent macrophage function are critical in DSS-colitis susceptibility (Fig. 6D).

Pharmacological inhibition of 15-PGDH abrogates protective effects of Se
To examine the role of 15-PGDH in the Se-S mice, we used CAY10397, a selective inhibitor of 15-PGDH (32). Oral gavage of CAY10397 at 100 mg/kg significantly blocked the protective effects of Se supplementation and exacerbated symptoms of colitis (Fig. 7A, 7B, 7E). Although there was no effect on the expression of 15-PGDH mRNA per se (Fig. 7C), CAY10397 blocked the Se-mediated inhibition of colonic expression of TNF-\(\alpha\), IL-1\(\beta\), IFN-\(\gamma\), and COX-2 (Fig. 7C, 7D). Furthermore, Se-mediated increase in Muc-2 (Fig. 7D) and colonic 15-PGDH activity were also significantly reduced in Se-S mice treated with DSS and CAY10397 (Fig. 7F). Taken together, these studies suggest a critical role for Se-dependent modulation of 15-PGDH expression in alleviating inflammation and promoting resolution of the gut epithelium (Fig. 8).

Discussion
Increased levels of inflammatory mediators, such as PGE\(_2\) and cytokines, are a hallmark in IBD patients (33). Dysregulated COX-dependent metabolism of arachidonic acid has been implicated in UC (34–36). However, emerging evidence suggests that the role of the COX pathway may be more complex given both the protective and harmful effects of COX-derived eicosanoids (37, 38). Therefore, pharmacological inhibition of COXs may have unintended consequences. In fact, COX-1\(^{-/-}\) and COX-2\(^{-/-}\) mice are more susceptible to DSS colitis (39), and nonsteroidal anti-inflammatory drugs exacerbate experimental models of IBD (40). Thus, targeting PG metabolic pathways downstream of COX could lead to more effective pharmacological strategies to treat colitis.

PGs are widely reported to have a dual role in inflammation and anti-inflammation. Whereas PGD\(_2\) and its downstream metabolites, \(\Delta^{12}\)-PGJ\(_2\) and 15d-PGJ\(_2\), exert significant anti-inflammatory effects (8, 9), PGE\(_2\) has been linked with the promotion of inflammation and ulceration (11, 39). A prominent role for PGE\(_2\) in UC was suggested by studies where increased incidences of active UC (36, 41, 42) were associated with increasing PGE\(_2\) concentrations, leading to the activation of the PGE\(_2\)-TNF\(\alpha\) axis (43, 44).
However, PGE₂ is also essential in the suppression of colitis, as shown in the EP4⁺/⁻ mice, where increased sensitivity toward experimental colitis was seen (12). Thus, Se status appears to be a key regulator of the levels of PGE₂ by 15-PGDH–dependent metabolism to 15-keto-PGE₂ and 13,14-dihydro-15-keto-PGA₂ to alleviate inflammation, as well as promote resolution. In addition, Se-dependent lowering of proinflammatory PGE₂ could be further complemented by the ability of the downstream PGEMs to activate PPARγ that together effect an active resolution program in addition to their already known tumor-suppressor functions (45). These data support an inverse causal relationship between Se status and PGE₂ production, which establishes Se as a key dietary factor in colitis. Clinical studies have shown supplementation with Se in UC patients leads to symptomatic relief from active colitis (2–4). Furthermore, our studies demonstrating the need for selenoproteins to alleviate DSS-colitis are in agreement with a recent study demonstrating that decreased levels of dietary Se exacerbate DSS-colitis (5). More importantly, our studies implicate macrophage selenoproteins to be critical in resolution of injury.

**FIGURE 5.** Selenoprotein-dependent expression of 15-PGDH in vitro and in vivo. RAW264.7 macrophage-like cells and BMDMs were treated with increasing concentrations of Se (as sodium selenite, 0–500 nM) for at least 3 d and analyzed for 15-PGDH expression. (A) Western immunoblot analysis of RAW264.7 cells (left panel) and BMDMs (right panel). (B) Enzymatic activity of 15-PGDH in RAW264.7 cells (left panel) and BMDMs (right panel). *p < 0.05 compared with cells without any exogenously added Se. (C) RAW264.7 cells were cultured in Se-D media and supplemented with indicated concentrations of Se in various forms, whereas BMDMs were isolated from untreated Se-D mice and cultured ex vivo in the presence of selenite (NaSe; 100, 500 nM), MSA (100, 500 nM), SeMet (100, 500 nM), or pXSC (100, 500 nM) for at least 3 d. RAW264.7 cells (left panel) and BMDMs (right panel) and protein expression were examined by Western immunoblot of 15-PGDH expression. Representative blot of n = 3 shown. (D) Modulation of 15-PGDH expression by qPCR in RAW264.7 cells (left panel) and BMDMs (right panel) treated with earlier mentioned Se compounds. *p < 0.05 compared with untreated cells. (E–G) Colonic extracts isolated from DSS-treated Trsp⁽⁰⁰⁾LysM⁽⁰⁾ mice and WT littermate control mice (on day 10) maintained on Se-D and Se-S diets were used to examine the (E) expression of 15-PGDH mRNA by qPCR, (F) upregulation of 15-PGDH expression by Western immunoblot, and (G) modulation of enzymatic activity of 15-PGDH. qPCR and enzymatic assay data for Trsp⁽⁰⁰⁾LysM⁽⁰⁾ mice shown are mean ± SEM of n = 3 per group. *p < 0.05 Western blot data shown are representative of n = 3 independent observations; #p < 0.05 and a p < 0.05 when compared with Se-A and Se-S groups, respectively.
associated with inflammation in DSS-treated Se-D and Se-A mice compared with the Se-S and Se-H mice. These studies suggest that macrophages infiltrate in response to myriad of inflammatory signals to alleviate inflammation and aid in resolution. Along these lines, studies from our laboratory have demonstrated that Se status has a significant impact on the polarization of macrophages toward an M1 (classically activated) or M2 (alternatively activated)-like phenotype that are endowed with proinflammatory or anti-inflammatory properties, respectively (46). In agreement with these observations, colonic expression of Arg1, a prototypical M2 marker, was significantly increased along with Fizz1 in Se-S mice treated with DSS, whereas the M1 markers (Tnfα, Ifnγ, Il1β) were significantly decreased. In addition, Muc2, predominantly expressed by goblet cells that help in maintaining gut homeostasis and protection from pathogenic microbes by secreting mucin, was downregulated in Se-D mice, rendering them susceptible to colitis as reported in Muc2−/− mice (47). These results suggest that macrophages may impact other cells to alleviate inflammation and facilitate resolution responses. It remains to be seen whether the expression of specific selenoproteins in macrophages changes the microenvironment in the gut to facilitate resolution over continued inflammation.

This study extends the paradigm that cellular metabolism is sensitive to selenoprotein expression as seen in form of global changes in the metabolome. More specifically, the metabolism of PGE2 being sensitive to the expression of the selenoproteome suggests the importance of redox-modulated metabolic pathways in alleviating inflammation. These studies also suggest that maintenance of inflammatory PGs and cytokines at homeostatic levels are important in the protection against colitis. In fact, whereas low concentrations of PGE2 are essential for angiogenesis and mucous production (48), high levels of PGE2 at the site of inflammation are associated with increased colitis activity (36), therefore making PGE2 one of the major drivers of pathology in IBD (49, 50). Thus, increased oxidative catabolism of PGE2 by Se through the upregulation of 15-PGDH is a critical event in reducing excess PGE2 that effectively alleviates inflammation. Although it is possible that 15-PGDH can oxidize other eicosanoids (e.g., resolvins) that aid in anti-inflammation and resolution, 15-PGDH has been reported to preferentially oxidize PGE2, particularly in inflamed tissues (18, 19). It remains to be seen whether 15-PGDH expression is spatiotemporally regulated during inflammation to limit the metabolism of other anti-inflammatory eicosanoids to initiate pathways of resolution.

Use of various forms of Se along with macrophages that lack selenoproteins clearly demonstrates that bioavailable Se is crucial for the upregulation of proteins, such as 15-PGDH, outside the selenoproteome. Preliminary studies suggest that 15-PGDH expression is regulated by the nuclear receptor, PPARγ, in the colonic tissue, which is in agreement with that reported by Lu et al. (45). Given that PPARγ activation is seen in Se-S macrophages via the generation of endogenous ligands in the form of PGD2 metabolites (6) and the ability of PGDMs to activate PPARγ (45), it is very likely that a feed-forward loop may be initiated leading to the
upregulation of 15-PGDH and hematopoietic PGD2 synthase to effect anti-inflammatory and proresolution pathways. The ability of PPARγ to activate 15-PGDH in cancer cell types is reminiscent of an anti-inflammatory cell type that is programmed to effectively accelerate wound healing while alleviating inflammation (45). It remains to be seen whether pharmacological agonists of PPARγ also enhance PGE2 catabolism to the extent that they can be used as a therapy for colitis. Based on our studies, it appears that 15-PGDH expression and activity may be associated with alternatively activated macrophages that are endowed with anti-inflammatory and wound-healing properties (51).

In summary, our studies demonstrate the anti-inflammatory role of selenoproteins to impinge on the modulation of PG metabolic pathways to protect against experimental colitis (Fig. 8). Our data suggest that macrophages are key players that mediate the protective effects of selenoproteins where metabolic inactivation of PGE2 appears to be the major regulator of disease pathogenesis. Based on our data, it is clear that the metabolic inactivation of PGE2 increases with increased dietary Se, suggesting that higher than adequate levels of Se may be beneficial in IBD. Currently, little is known about the molecular basis of these therapies in IBD. Therefore, a better understanding of these redox-dependent processes may help develop more efficient regimens to effectively resolve inflammation and restore the gut epithelium in IBD.

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**References**


