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David Meriwether, … , Alan M. Fogelman, Srinivasa T. Reddy


Cyclooxygenase 2 (*Cox2*) total knockout and myeloid knockout (MKO) mice develop Crohn’s-like intestinal inflammation when fed cholate-containing high-fat diet (CCHF). We demonstrated that CCHF impaired intestinal barrier function and increased translocation of endotoxin, initiating TLR/MyD88-dependent inflammation in *Cox2*-KO but not WT mice. *Cox2*-MKO increased proinflammatory mediators in LPS-activated macrophages, and in the intestinal tissue and plasma upon CCHF challenge. *Cox2*-MKO also reduced inflammation resolving lipoxin A4 (LXA4) in intestinal tissue, whereas administration of an LXA4 analog rescued disease in *Cox2*-MKO mice fed CCHF. The apolipoprotein A-I (APOA1) mimetic 4F mitigated disease in both the *Cox2*-MKO/CCHF and piroxicam-accelerated *Il10*–/– models of inflammatory bowel disease (IBD) and reduced elevated levels of proinflammatory mediators in tissue and plasma. APOA1 mimetic Tg6F therapy was also effective in reducing intestinal inflammation in the *Cox2*-MKO/CCHF model. We further demonstrated that APOA1 mimetic peptides (a) inhibited LPS and oxidized 1-palmitoyl-2-arachidonoyl-sn-phosphatidylcholine–dependent (oxPAPC-dependent) proinflammatory responses in human macrophages and intestinal epithelium, and (b) directly cleared proinflammatory lipids from mouse intestinal tissue and plasma. Our results support a causal role for proinflammatory and inflammation-resolving lipids in IBD pathology and a translational potential for APOA1 mimetic peptides for the treatment of IBD.

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Cyclooxygenase 2 (Cox2) total knockout and myeloid knockout (MKO) mice develop Crohn’s-like intestinal inflammation when fed cholate-containing high-fat diet (CCHF). We demonstrated that CCHF impaired intestinal barrier function and increased translocation of endotoxin, initiating TLR/MyD88-dependent inflammation in Cox2-KO but not WT mice. Cox2-MKO increased proinflammatory mediators in LPS-activated macrophages, and in the intestinal tissue and plasma upon CCHF challenge. Cox2-MKO also reduced inflammation resolving lipoxin A4 (LXA4) in intestinal tissue, whereas administration of an LXA4 analog rescued disease in Cox2-MKO mice fed CCHF. The apolipoprotein A-I (APOA1) mimetic 4F mitigated disease in both the Cox2-MKO/CCHF and piroxicam-accelerated Il10–/–-MKO/CCHF model. We further demonstrated that APOA1 mimetic peptides (a) inhibited LPS and oxidized 1-palmitoyl-2-arachidonoyl-sn-phosphatidylcholine–dependent (oxPAPC-dependent) proinflammatory responses in human macrophages and intestinal epithelium, and (b) directly cleared proinflammatory lipids from mouse intestinal tissue and plasma. Our results support a causal role for proinflammatory and inflammation-resolving lipids in IBD pathology and a translational potential for APOA1 mimetic peptides for the treatment of IBD.

Introduction
Inflammatory bowel disease (IBD), inclusive of Crohn’s disease (CD) and ulcerative colitis (UC), is a chronic and relapsing inflammatory disorder of the gastrointestinal tract. IBD consists of a dysregulated mucosal immune response to gut microbiota, brought about by host and/or environmental triggers in genetically susceptible individuals (1). However, there exists no single pathogenic mechanism for IBD. Several genetic susceptibility loci have been identified for IBD (2). The candidate genes broadly implicate several distinct functional categories, including epithelial barrier function, innate immunity, T cell signaling, and autophagy; but even within a category, the underlying pathways are largely distinct (2, 3). The range of environmental factors associated with the development of IBD is also complex (4). In the absence of a single pathogenic mechanism, investigation into the etiology and pathogenesis of IBD requires careful elucidation of the disparate causal contributions of the many susceptibility-linked genes and disease-associated environmental factors.

IBD exhibits high morbidity, but there are currently few effective pharmacological interventions. For example, between 70% and 90% of all patients with CD eventually undergo surgery within their lifetime, and approximately 40% will require repeated surgery (5). Anti-TNF therapies including both infliximab and adalimumab offer a treatment option for IBD that is refractory to conventional therapies, including corticosteroids and immunomodulators (6). However, treatment failures with anti-TNF therapies are common (7). There exists a pressing need for new IBD therapies.

Cyclooxygenase 2 (COX2) is the inducible form of cyclooxygenase and is responsible for the rate-limiting step in the conversion of arachidonic acid to prostanooids (prostacyclins, prostaglandins, and thromboxanes). Prostanoids have been well-characterized as proinflammatory mediators, and their biosynthesis is blocked by nonsteroidal antiinflammatory drugs, including selective COX2 inhibitors. However, COX2 can play a role in the resolution of inflammation, partially through its induction of the proresolving eicosanoid lipoxin A4 (LXA4) (8, 9).

Multiple genetic susceptibility studies have linked COX2 to an altered risk of developing IBD (2, 10, 11), but the exact nature of the link is not clear due to conflicting associations when utilizing...
data from COX2 polymorphisms. Nevertheless, it has been shown that COX2 and COX2-dependent prostaglandin E2 (PGE2) help maintain adaptive immune tolerance to dietary antigens in the proximal small intestine (12, 13). Consistent with the observations of Newberry et al., we previously reported that both Cox2 total knockout (Cox2-TKO) and Cox2 myeloid-specific knockout (Cox2-MKO) mice or COX2-inhibited (COX2i) mice develop severe CD-like inflammation in their ileo-ceco-colic junctions when challenged with a cholate-containing or cholate-containing high fat diet (CCHF) (14, 15). Our prior findings suggested that COX2 plays an anti-inflammatory role at the ileo-ceco-colic junction in mice. In the present study, we investigated the molecular mechanisms by which COX2 deficiency can contribute to the development of IBD.

Apolipoprotein A-I (APOA1) mimetic peptides, including both 4F and 6F, are short (18 amino acids) sequences that recapitulate the secondary structure and partial function of APOA1, the main structural protein of high density lipoprotein (HDL) (16). 4F and transgenic 6F (Tg6F; extract from tomatoes expressing the 6F peptide) are protective in numerous animal models of inflammatory diseases, including atherosclerosis (16). We previously reported that circulating 4F selectively targets the small intestine, where it is transported into the intestinal lumen, reabsorbed by the intestinal mucosa, and mediates the trans-intestinal efflux of cholesterol (17). We also demonstrated that both orally and subcutaneously administered 4F reduce the levels of proinflammatory fatty acid metabolites in enterocytes of low-density lipoprotein receptor-null (LDLR-null) mice on Western diet (18). We thus sought further to determine whether orally administered 4F and Tg6F could inhibit the development of intestinal inflammation in animal models of IBD including the Cox2-KO/CCHF model.

Our results indicate that (a) myeloid COX2 generates an anti-inflammatory/proresolving lipid and proinflammatory check on TLR-dependent mural inflammation that can drive intestinal inflammation in IBD when compromised; (b) proinflammatory lipids play a causal role in IBD pathology, while antiinflammatory/proresolving lipids play a protective role; and (c) APOA1 mimetic peptides hold promise as therapies for IBD, in part through their capacities to inhibit both LPS and oxidized 1-palmitoyl-2-arachidonoyl-sn-phosphatidylcholine (oxPAPC) signaling and to promote luminal clearance of mural proinflammatory lipids.

Results

Dysregulated response to TLR-dependent signaling drives intestinal inflammation in the Cox2-KO/CCHF models of IBD. Consistent with our prior reports (15), Cox2-TKO and COX2i but not control mice developed severe chronic inflammation in their ileo-ceco-colic junctions when fed CCHF for 2 weeks (Figure 1A). Disease was characterized by inflammatory lesions in both the submucosa and muscularis propria, sporadic foci of mucosal ulceration, and thickening of the intestinal wall.

Bile acids pose a risk of cellular toxicity (19), while disrupted barrier function (20) or epithelial damage itself (21) can cause intestinal inflammation. We thus sought to determine if altered barrier function or epithelial damage preceded the development of inflammation in the COX2 models. C57BL/6J (BL6) mice were fed CCHF or CCHF with the COX2 inhibitor celecoxib (CX). We assessed intestinal epithelial damage (22), changes in whole intestinal barrier permeability (23), translocation of endotoxin into portal vein serum, and ileo-ceco-colic inflammation over 2 weeks (Figure 1B). CCHF significantly increased whole intestinal permeability equally in both CX-treated and control mice, as assessed by urinary excretion of sucralose (Figure 1B). We also observed that CCHF comparably increased intestinal permeability in wild-type (WT) and Cox2-TKO mice (Supplemental Figure 1), indicating that off-target effects of celecoxib had not confounded our prior result (24).

The increase in permeability was accompanied by a significant increase in endotoxin translocated into portal vein serum in CX-treated and control mice (Figure 1B). The increases in both permeability and endotoxin preceded histological evidence of inflammation in the ileo-ceco-colic junctions of CX-treated mice (Figure 1B). Nonetheless, despite similar increases in both permeability and endotoxin levels, CX-treated but not control mice developed severe intestinal inflammation. Epithelial ulceration was observed in CX-treated mice at day 14 only (Figure 1B), after the initiation of inflammation in these mice. Since ulceration followed the development of inflammation, ulceration itself was likely a consequence rather than a cause of the inflammation.

We pursued the hypothesis that CCHF initiates intestinal inflammation by causing loss of barrier function and translocation of pathogen-associated molecular patterns (PAMPs), including lipopolysaccharide (LPS). We thus determined the dependence of the intestinal inflammation on gut microbiota. We pretreated Cox2-TKO and BL6 mice for 1 week with antibiotics (AB) to eliminate intestinal bacteria (25). We challenged the mice with CCHF or CCHF + CX for 2 weeks, while continuing AB. AB treatment abrogated inflammation in the ileo-ceco-colic junctions of Cox2-TKO and CX-treated mice, as assessed by gross pathology (Figure 1C) and histological disease score (Supplemental Figure 2). (For this and subsequent histological measures of disease, we employed a 0–12 point scoring system that incorporated assessments of both inflammation and epithelial damage/ altered mucosal architecture; see Supplemental Methods.) To exclude the possibility that AB treatment inhibited inflammation by blocking the bacterial conversion of cholate to the more cytotoxic deoxycholate (26), we determined the effects of cholate and deoxycholate on barrier permeability in AB-treated TKO mice, which treatment prevented the conversion of cholate to deoxycholate. Cholate and deoxycholate significantly increased barrier permeability, but we observed no difference between their effects (Supplemental Figure 3).

LPS induces the production of proinflammatory mediators by stimulating the LBP/CD14/MD-2/TLR4 cell surface formation. This complex then signals through the adaptor protein MyD88 to trigger the nuclear translocation of NFXB and the transcription of proinflammatory target genes (27). We inhibited MyD88 with the small molecule T6167923 (28) in Cox2-TKO and COX2i mice fed CCHF. As with AB treatment, MyD88 inhibition abrogated gross pathological (Figure 1D) and histological (Supplemental Figure 4) evidence of ileo-ceco-colic inflammation.

If LPS/PAMP translocation and TLR/MyD88 signaling initiate disease in our models, there must exist a differential response to bacteria-dependent TLR ligands in COX2-deficient versus control mice. Cox2 myeloid conditional knockout (MKO) mice (Cox2fl/fl LysMcre) developed ileo-ceco-colic inflammation comparable to
that of Cox2-TKO and COX2i mice, upon 7- or 10-week CCHF (Supplemental Figure 5). Elevated levels of neutrophils and macrophages but not CD4+ or CD8+ T cells characterized the inflammatory lesions (Supplemental Figure 6) (15). CCHF significantly increased intestinal barrier permeability over the course of 0–7 days independently of CX (upper left panel). Endotoxin in portal vein serum significantly increased in both groups by 7 days (lower left panel). Inflammation significantly increased in CX-treated mice by 11 days (upper right panel), whereas epithelial damage did not significantly increase until day 14 (lower right panel) (histological assessments, 0–4 point scale). (C) Mice were pretreated with vancomycin, ampicillin, neomycin, and metronidazole for 7 days and continued on antibiotics (AB) for 14 days of CCHF or CCHF + CX (n = 4/group). AB abrogated intestinal inflammation in mice treated with CDX2-TKO (upper panel) and mice treated with C57BL6 + CX (BL6 + CX) (lower panel), as assessed by ileo-ceco-colic thickness. (D) Cox2-TKO and WT mice were treated 3 times per week i.p. with the MyD88 inhibitor T6167923 (0.25 mg/injection) over the course of 2 weeks on CCHF (n = 5/group). MyD88 inhibition (MyD88i) significantly inhibited intestinal inflammation in Cox2-TKO mice. (E) Cox2-MKO significantly enhanced RNA expression of Il1b (upper panel) and Tnf (lower panel) in LPS-activated BMDMs compared with FLOX controls (n = 3/group) (fold change vs. FLOX, 0 hours) (statistics shown only for FLOX vs. MKO). Two-way ANOVA and Tukey’s multiple comparisons test with adjusted P values were used for statistical analyses.

Figure 1. Dysregulated response to TLR-dependent signaling drives intestinal inflammation in the COX2-KO/CCHF models of IBD. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. (A) Mice treated with Cox2-TKO (upper panel) and BL6 + celecoxib (CX) (lower panel) develop transmural and sporadically ulcerating inflammation in their ileo-ceco-colic junctions when fed CCHF for 2 weeks (representative images). Scale bars: 250 μm. (B) C57BL/6J mice fed CCHF diet with or without CX were assessed at multiple time points (n = 4 mice/group/time point). CCHF significantly increased whole intestinal barrier permeability over the course of 0–7 days independently of CX (upper left panel). Endotoxin in portal vein serum significantly increased in both groups by 7 days (lower left panel). Inflammation significantly increased in CX-treated mice by 11 days (upper right panel), whereas epithelial damage did not significantly increase until day 14 (lower right panel) (histological assessments, 0–4 point scale). (C) Mice were pretreated with vancomycin, ampicillin, neomycin, and metronidazole for 7 days and continued on antibiotics (AB) for 14 days of CCHF or CCHF + CX (n = 4/group). AB abrogated intestinal inflammation in mice treated with CDX2-TKO (upper panel) and mice treated with C57BL6 + CX (BL6 + CX) (lower panel), as assessed by ileo-ceco-colic thickness. (D) Cox2-TKO and WT mice were treated 3 times per week i.p. with the MyD88 inhibitor T6167923 (0.25 mg/injection) over the course of 2 weeks on CCHF (n = 5/group). MyD88 inhibition (MyD88i) significantly inhibited intestinal inflammation in Cox2-TKO mice. (E) Cox2-MKO significantly enhanced RNA expression of Il1b (upper panel) and Tnf (lower panel) in LPS-activated BMDMs compared with FLOX controls (n = 3/group) (fold change vs. FLOX, 0 hours) (statistics shown only for FLOX vs. MKO). Two-way ANOVA and Tukey’s multiple comparisons test with adjusted P values were used for statistical analyses.

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BMDMs (Figure 1E). Disregulation of TLR/MyD88-dependent proinflammatory signaling within the macrophage compartment could thus partially explain the differential response to CCHF between control and Cox2-deficient mice. Cox2-MKO mice fed CCHF exhibit elevated levels of proinflammatory lipid mediators in intestine and plasma, while the loss of inflammation-resolving LXA4 in intestine appears partially causal for disease. Cox2-MKO enhanced the acute inflammatory response and inhibited its resolution in the carrageenan hind paw model (Supplemental Figure 10). COX2 can modulate the production of proresolving lipid mediators including LXA4 (8), an eicosanoid that promotes resolution in part by stimulating efferocytosis (31). Macrophage COX2 can directly mediate intracellular production of LXA4 through LPS-dependent generation of 15HETE (32). Loss of macrophage COX2 can also shunt arachidonic acid (AA) through the 5-lipoxygenase (5LOX) and 12- or 15-lipoxygenase (12/15LOX) pathways, leading to the production of proinflammatory eicosanoids (32). We thus hypothesized that Cox2-MKO mice fed CCHF would exhibit higher proinflammatory and lower proresolving lipid mediator levels compared with FLOX controls.

We developed a multiple reaction monitoring LC-MS/MS method to measure a panel of proinflammatory, antiinflammatory, and proresolving lipid mediators (see Supplemental Methods for details). The method quantitatively evaluates most of the bioactive signaling metabolites of AA, DHA, and EPA in the COX and LOX pathways, together with pathway markers and stable end products (Table 1 and Supplemental Table 1). We validated the method for intestine, plasma, and cell culture lysate and media (Supplemental Tables 2–5 and Supplemental Figure 11).

We fed Cox2-MKO and FLOX mice either chow or CCHF for 8.5 weeks then determined the levels of the above mediators in the ileo-ceco-colic junction and plasma (Figure 2, A and B, and Supplemental Tables 6 and 7). See Supplemental Tables 8–10 for application of Benjamini-Hochberg procedure (33) to all lipidomic data sets for the control of false discovery rate (FDR) at level α = 0.05. On CCHF, Cox2-MKO mice had significantly increased intestinal levels of several proinflammatory signals compared with FLOX controls (Figure 2C), including the proinflammatory LOX mediators 12HETE, 15HETE, and 13HODE, and the proinflammatory COX-dependent PGJ2 degradation product 6ketoPGF1α. Cox2-MKO fed CCHF had significantly increased plasma levels of the proinflammatory LOX signals 12HETE, 5HETE, and 15HETE, and the proinflammatory COX signal PGF2α together with its degradation product 13,14-dihydro-15-PGF2α, and TXB2 (Figure 2D). In tissue and plasma, Cox2-MKO fed CCHF also had a significantly increased total level of all LOX and COX proinflammatory mediators. In plasma, PGE2 was also increased (Figure 2E).

We investigated whether these elevated proinflammatory signals in plasma might constitute biomarkers for IBD. In an independent experiment, we determined the levels of proinflammatory LOX mediators in plasma of Cox2-MKO and FLOX mice fed chow, and those fed CCHF for 3 or 8.5 weeks (Supplemental Figure 12). Proinflammatory LOX signals, including 12HETE and 5HETE, were increased total level of all LOX and COX proinflammatory mediators in mice) pathways, leading to the production of proinflammatory eicosanoids (32). We thus hypothesized that Cox2-MKO mice fed CCHF would exhibit higher proinflammatory and lower proresolving lipid mediator levels compared with FLOX controls.

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Cox2-MKO also significantly reduced proresolving LOX signals in the ileo-ceco junctions of CCHF-fed mice (Figure 2F). Both LXA4 and the total of 5LOX specialized proresolving mediators

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Table 1. Bioactive lipid inflammatory mediators measured by LC-MS/MS either directly or via stable products

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-INF COX&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Δ12-PGJ2</td>
<td>PPARgamma agonist; PGJ2/J2 and 15d-PGJ2 marker</td>
</tr>
<tr>
<td>15d-PGJ2</td>
<td>Negative regulator of NFκB, PPARgamma agonist</td>
</tr>
<tr>
<td>Pro-INF/Pro-RES COX&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>PGE2</td>
<td>Vascular leakage (pro-INF); class-switching (pro-RES)</td>
</tr>
<tr>
<td>PGD2</td>
<td>PMN chemotaxis through DP1 DP2 (pro-INF); class-switching (pro-RES)</td>
</tr>
<tr>
<td>Pro-INF COX&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>[PGJ2]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Vasodilatation</td>
</tr>
<tr>
<td>PGF2α</td>
<td>Pro-INF in rheumatoid arthritis, atherosclerosis</td>
</tr>
<tr>
<td>[TXA2]&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Microvascular constriction, PMN adherence</td>
</tr>
<tr>
<td>TXB2</td>
<td>PMN chemotaxis and adherence</td>
</tr>
<tr>
<td>1HETE</td>
<td>Leukocyte regulation; COX activity marker</td>
</tr>
<tr>
<td>PGE3</td>
<td>Induce COX2 and IL-6 in macrophage</td>
</tr>
<tr>
<td>TXB3</td>
<td>Induce COX2 and IL-6 in macrophage</td>
</tr>
<tr>
<td>Pro-INF LOX&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>LTB4</td>
<td>Vascular leakage; leukocyte chemotaxis and adhesion</td>
</tr>
<tr>
<td>LTC4</td>
<td>Vascular permeability</td>
</tr>
<tr>
<td>5HETE</td>
<td>PMN chemotaxis and degranulation</td>
</tr>
<tr>
<td>12HETE</td>
<td>Neutrophil chemotaxis and adhesion</td>
</tr>
<tr>
<td>15HETE</td>
<td>Activator of leukotriene biosynthesis; lipoxin pathway marker</td>
</tr>
<tr>
<td>5oxoETE</td>
<td>PMN chemotaxis and degranulation</td>
</tr>
<tr>
<td>13HODE</td>
<td>PMN chemotaxis</td>
</tr>
<tr>
<td>9HODE</td>
<td>PMN chemotaxis</td>
</tr>
<tr>
<td>Pro-RES LOX&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>LXA4</td>
<td>Promote M1 to M2; NFκB negative regulator; inhibit neutrophil chemotaxis and adhesion</td>
</tr>
<tr>
<td>LXB4</td>
<td>Promote M1 to M2; NFκB negative regulator; inhibit neutrophil chemotaxis and adhesion</td>
</tr>
<tr>
<td>MαR-1</td>
<td>Tissue regeneration; M1 to M2; inhibit neutrophil migration; increase efferocytosis</td>
</tr>
<tr>
<td>RvD1</td>
<td>Limit neutrophil infiltration</td>
</tr>
<tr>
<td>RvD2</td>
<td>Enhance survival in sepsis; protect against colitis</td>
</tr>
<tr>
<td>RvD3</td>
<td>Limit leukocyte migration, enhance macrophage efferocytosis</td>
</tr>
<tr>
<td>RvD4</td>
<td>Limit leukocyte migration, enhance macrophage efferocytosis</td>
</tr>
<tr>
<td>RvD5</td>
<td>Enhance bacterial containment and phagocytosis</td>
</tr>
<tr>
<td>PDx</td>
<td>Inhibit neutrophil infiltration and platelet aggregation</td>
</tr>
<tr>
<td>RvE1</td>
<td>Reduce PMN infiltration; promote resolution in colitis model</td>
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<sup>a</sup>Antiinflammatory cyclooxygenase products. <sup>b</sup>Proinflammatory/ proresolving cyclooxygenase products. <sup>c</sup>Proinflammatory cyclooxygenase products. <sup>d</sup>Analytes in brackets are measured indirectly via stable metabolic or degradation products. <sup>e</sup>Proinflammatory lipoxygenase products. <sup>f</sup>Proresolving lipoxygenase products.
We thus hypothesized that the significant reduction \( (P < 0.05) \) of LXA4 in the ileo-ceco-colic junctions of Cox2-MKO + CCHF mice was partially causal for the chronic inflammation in these mice. We administered a stable analog of LXA4 (BML111) to Cox2-MKO mice across the course of an 8.5-week CCHF (37). BML111 treatment significantly improved several measures of intestinal inflammation, including gross pathology and histological disease score (Figure 2G). (See Supplemental Figure 14 for additional measures and representative images.)

We had observed the copresence of neutrophils and macrophages in the inflammatory lesions of Cox2-MKO mice fed CCHF (Supplemental Figure 6). This copresence suggested that the resolution of inflammation, which involves the efferocytosis of neutrophils by macrophages, failed (35). LXA4 can block neutrophil migration and stimulate efferocytosis (36). We thus hypothesized that the significant reduction \( (P < 0.05) \) of LXA4 in the ileo-ceco-colic junctions of Cox2-MKO + CCHF mice was partially causal for the chronic inflammation in these mice. We administered a stable analog of LXA4 (BML111) to Cox2-MKO mice across the course of an 8.5-week CCHF (37). BML111 treatment significantly improved several measures of intestinal inflammation, including gross pathology and histological disease score (Figure 2G). (See Supplemental Figure 14 for additional measures and representative images.)
The APOA1 mimetic peptides 4F and Tg6F inhibit the development of intestinal inflammation in the Cox2-MKO/CCHF model of IBD. Gene expression of APOA1 is reduced in the ilea of patients with ileal or colonic CD (38). Colonic APOA1 is also protective in mouse models of UC (39). We determined both APOA1 protein and gene expression in the proximal colons and terminal ilea of MKO and FLOX mice fed CCHF or chow for 8.5 weeks (Supplemental Figure 15). Gene expression was significantly reduced in the proximal colons of Cox2-MKO mice fed CCHF.

4F protected against ileo-ceco-colic inflammation in Cox2-MKO mice at both time points. 4F treatment blocked the increase in ileo-ceco-colic thickness (Figure 3A), significantly improved disease score (Figure 3B), and blocked the increase in muscularis thickness (Figure 3C). 4F treatment significantly inhibited the influx of F4/80+ macrophages and Ly6G+ neutrophils into the ileo-ceco-colic junctions (Figure 3D). 4F-treated mice also expressed significantly less Tnf but more Il10 in their ileo-ceco-colic junctions (Figure 3F).

To determine whether the protective effect of 4F in this model extended to other APOA1 mimetic peptides, we cotreated Cox2-MKO mice fed CCHF with 0.06% Tg6F (wt/wt) for 10 weeks (40). To assess whether Tg6F could inhibit the progression of existing disease, we delayed the start of treatment with Tg6F in another group until the mice had been fed CCHF for 5 weeks. Both early
and late onset Tg6F therapy rescued gross pathology and improved histology (Figure 3, G and H and Supplemental Figure 16). These results suggest that APOA1 mimetic peptides as a class may be protective against intestinal inflammation in the Cox2-KO/CCHF model of IBD even after the disease has been well established.

4F treatment improves the balance of proinflammatory and inflammation-resolving lipid mediators in Cox2-MKO mice fed CCHF, while suppressing the proinflammatory phenotype of LPS-treated macrophages. We determined the effect of oral D-4F therapy on the levels of lipid inflammatory mediators in the ileo-ceco-colic junctions and plasmas of Cox2-MKO mice fed CCHF for 8.5 weeks (Figure 4, A and B; Supplemental Table 6 and Supplemental Table 7). 4F treatment rescued the intestinal levels of total proinflammatory LOX products as well as 12HETE and 13HODE (Figure 4C). In plasma, 4F significantly reduced the level of every proinflammatory LOX mediator elevated by Cox2-MKO together with most additional mediators in this class (Figure 4D). 4F also significantly reduced in plasma most proinflammatory COX mediators as well as PGE2 (Figure 4E).

We determined the effect of 4F on plasma paraoxonase-1 (PON1) activity as a measure of HDL and on plasma total cholesterol (TC) (Supplemental Figure 17) (41). 4F significantly reduced TC, consistent with our prior reports that 4F can enhance the trans-intestinal efflux of cholesterol (17). 4F also significantly improved PON1 activity. We last determined the effect of 4F on broad classes of plasma lipids in Cox2-MKO mice fed CCHF. We employed ion mobility MS/MS shotgun lipidomics (42) through the SCIEX Lipidyzer platform to determine the concentrations of 1103 lipid species from 13 lipid classes. We observed significant reductions in total triacylglycerols, diacylglycerols, cholesterol esters, and lyso-phosphatidylethanolamines (Supplemental Figure 18).

Last, we investigated the protective mechanism of 4F by asking whether 4F could inhibit the proinflammatory effects of LPS on mouse macrophages. We cotreated LPS-activated BMDMs with 4F (15 μg/mL) and assessed the expression of NFκB proinflammatory target genes. 4F significantly inhibited expression of Il1b (Figure 5A) and Tnf (Figure 5B) in Cox2-MKO and FLOX BMDMs for 24 hours. We also assessed the effect of 4F on lipid inflammatory mediators in BMDM cell lysates. 4F significantly reduced the levels of LPS-induced COX metabolites including PGE2 (Figure 5C) and PGD2 (Figure 5D). Comparing the mono-hydroxylated COX and LOX AA-metabolites 11HETE and 15HETE, respectively, we observed that 4F treatment significantly lowered the levels of 11HETE but not 15HETE (Figure 5E). This differential effect on structurally comparable lipids suggests that 4F did not lower the levels of COX.
D = approximately 1 nM and K₄F bound to both with high affinity (binding affinity between 4F and both LPS and lipid A (Figure 6C). We employed surface plasmon resonance analysis to determine the affinity by preventing lipid A from activating LBP/CD14/MD-2/TLR4. We hypothesized that 4F inhibits the LPS-dependent activation of the NFκB pathway by binding to the lipid A moiety of LPS, thereby preventing lipid A from activating LBP/CD14/MD-2/TLR4. We employed surface plasmon resonance analysis to determine the binding affinity between 4F and both LPS and lipid A (Figure 6C). 4F bound to both with high affinity (Kᵦ = approximately 1 nM and 17 nM, respectively), supporting our hypothesis.

We next investigated the effect of 4F on the LPS-dependent proinflammatory response of human intestinal epithelium ex vivo. We isolated small intestinal crypts from human surgical samples and cultured them in Matrigel (see Supplemental Methods). Under these conditions, the crypts expand into small intestinal epithelial structures known as enteroids (Figure 6D) that recapitulate intestinal epithelial physiology (44). We examined the way that small intestinal epithelium might amplify the proinflammatory response of macrophages. We collected conditioned media (CM) from THP-1 macrophages that had been treated with LPS or LPS + 4F for either 4 or 12 hours. We exposed the human enteroids to CM for 12 hours and determined proinflammatory gene expression by qPCR. CM from LPS-activated macrophages significantly induced gene expression of TLR4, COX2, IL1B, IL6, and TNF, with 12-hour CM significantly increasing expression over 4-hour CM (Figure 6, E–I). By contrast, CM from macrophages treated with LPS + 4F did not increase expression of these genes. The magnitude of the proinflammatory response of the enteroids to CM from LPS-activated macrophages highlights the potential amplifying effect of proinflammatory stimuli in the lamina propria by intestinal epithelium. The degree of the inhibition of this epithelial response by 4F suggests a protective mechanism by which 4F not only inhibits the primary proinflammatory response of resident macrophages but also interrupts the positive feedback loops that propagate and amplify this response within the intestinal mucosa.

OxPAPC is elevated in the plasma and cecum of Cox2-MKO + CCHF mice, while 4F reduces oxPAPC in vivo and inhibits the oxPAPC-dependent proinflammatory response of human macrophages. Lipid peroxidation markers are elevated in plasma (45) and affected intestinal tissue (46) of patients with CD, and lipid peroxides may be pathogenic for CD (47). POVPC, PGPC, and KDiAPC are all phospholipids in which the polyunsaturated arachidonoyl group at the sn2 position of PAPC oxidatively truncates following a peroxidation step at one of its double bonds (48). Because PAPC is a common and representative phospholipid, these oxPAPC species are considered biomarkers of lipid peroxidation (49).

We determined the levels of POVPC, PGPC, and KDiAPC (Supplemental Methods) in the ileo-ceco-colic junction (Figure 7A).
therapy, oral D-4F had significantly reduced oxPAPC in the cecum and plasma (Figure 7C and D) of both groups. Oxidized PAPC species are themselves biologically active and can be proinflammatory (48, 50). We considered the hypotheses that oxPAPC helps amplify intestinal inflammation, while 4F inhibits this effect. POVPc significantly increased IL1B gene expression in THP-1 macrophages by 3 hours (Figure 7E). 4F cotreatment blocked this induction, while 3F(14) — an 18 amino acid peptide that lacks the affinity of 4F for oxidized lipids (43) — did not.

4F can directly clear lipid proinflammatory mediators from intestinal tissue and plasma. 4F reduced the levels of proinflammatory LOX but not COX mediators in Cox2-MKO mice fed CCHF (Figure 8A and Figure 4C). This selective reduction of disease-
elevated mediators weighs against the possibility that 4F only indirectly lowers these mediators by otherwise inhibiting disease. We hypothesized that 4F could directly clear proinflammatory LOX mediators from intestinal tissue. We previously employed Ussing chambers to study the effect of 4F on trans-intestinal cholesterol efflux (TICE) ex vivo (17). We now mounted in Ussing chambers small intestinal explants from Cox2-KO mice fed CCHF for 8.5 weeks. Mucosal/luminal side D-4F significantly increased the clearance into the mucosal media of most proinflammatory LOX mediators (Figure 8B). 

We previously reported that proinflammatory LOX mediators associate with lipoproteins, including HDL (51, 52). We also demonstrated that 4F enhances TICE, while showing ex vivo that luminal 4F can increase the trans-intestinal efflux of lipoprotein-associated cholesterol (17). We now asked whether luminal 4F could also affect the trans-intestinal transport of lipoprotein-associated proinflammatory mediators. We combined deuterated 15HETE, 12HETE, inflammatory LOX mediators (Figure 8B). 

Oral D-4F (500 μg/mL drinking) improved several markers of disease in PAC Ilio⁻/⁻ mice. PX significantly decreased colon length while 4F therapy rescued this effect (Figure 9A). Colitis severity was determined on a 4-point scale by assessing the thickening of the mucosa, vascularity, granularity, and feces consistency (53). 4F therapy significantly improved histological disease score, as assessed by the degree of lesions, hyperplasia, ulceration, and percentage of area involved (53) (Figure 9B). 

The PAC Ilio⁻/⁻ model depends on the presence of gut microbiota to initiate disease (53). Given the off-target epithelial toxicity of PX, it has been conjectured but not demonstrated that PX accelerates disease by impairing barrier function (54). We thus assessed the effect of PX on whole intestinal permeability in Ilio⁻/⁻ mice over the course of 9 days of PX challenge. We observed a biphasic response with an early increase in permeability (day 0–3) followed by a late increase (day 7–9) that coincides with increased disease (Figure 9C, left). We also observed increased endotoxin in portal vein serum at day 9 (Figure 9C, right).

**Figure 7. Oxidized PAPC is elevated in the plasma and ceca of Cox2-MKO and CCHF-fed mice, whereas 4F both reduces oxidized PAPC in vivo and inhibits oxidized PAPC-dependent proinflammatory response in human macrophages. **

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Levels of oxPAPC products (POVPC, PGPC, and KodiaPC) were determined in the ceca (n = 10) (A) and plasmas (n = 10) (B) of Cox2-MKO (MKO) and FLOX mice fed CCHF or Chow for 8.5 weeks. Independently, the effect of 4F on oxPAPC in the ceca (C) and plasma (D) of MKO and FLOX mice fed CCHF for 3 weeks was determined. Last, human THP-1 macrophages were treated with 1 μM POVPC for 3 hours with and without the apoA-I mimetic peptides 4F and 3F(14); and expression of IL-1β was determined by qPCR (n = 3–6) (fold change vs. NT) (E)

After 8.5 weeks, MKO + CCHF diet significantly increased oxidized oxPAPC species in both ceca and plasma. 4F significantly lowered levels of oxPAPC in vivo, and 4F but not 3F(14) significantly inhibited the proinflammatory effect of POVPC on macrophages. For each analyte in A–D, 2-way ANOVA with Tukey’s multiple comparisons test and adjusted P values was used. A 1-way ANOVA with Tukey’s multiple comparisons test and adjusted P values was used for E.

4F treatment inhibits development of colitis in the piroxicam-accelerated Ilio⁻/⁻ model. 4F binds to cholesterol with high affinity (K_D = approximately 11 nM) (43). We considered the possibility that 4F is protective in the Cox2-KO/CCHF models by binding and inactivating dietary cholate. We determined the binding affinity between D-4F and cholate, observing only low-affinity binding (Supplemental Figure 23). 4F also did not inhibit the early effect of CCHF on barrier permeability (Supplemental Figure 24), further indicating that 4F does not inactivate CCHF.

We nonetheless assessed the efficacy of 4F therapy in a second mouse model of IBD, the piroxicam accelerated (PAC) Iilo⁻/⁻ model (53). Iilo⁻/⁻ mice that are fed the NSAID piroxicam (PX) for 9 days develop colitis that is characterized by hyperplasia and focal transmural inflammation reminiscent of human CD. In the PAC Iilo⁻/⁻ model, disease is assessed at day 14 (5 days after PX) by determining colon length, colitis severity, and histopathological score (53).

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4F inhibited the effect of LPS on II10−/− peritoneal macrophages treated with PX (Figure 9D). We also determined the levels of lipid inflammatory mediators in colon from II10−/− and PAC III10−/− mice with or without 4F. Multiple proinflammatory mediators were increased in the colon of PAC III10−/− mice (Figure 9E and Supplemental Table 11). 4F therapy rescued the increase of all proinflammatory LOX products, without inhibiting the significant increase in the sole elevated COX product TXB2 (Figure 9F).

Finally, we determined the effect of 4F on plasma lipid inflammatory mediators in these mice (Supplemental Table 12). 4F therapy significantly reduced the levels of most proinflammatory LOX and COX mediators. The broad nature of the effects of 4F on plasma lipids suggests a broad clearance mechanism capable of affecting all plasma-associated mediators together.

Discussion
We investigated the pathogenic mechanisms of the Cox2-KO/CCHF models of IBD together with the protective mechanisms of APOA1 mimetic peptides (Figure 10, A and B). CCHF increased barrier permeability and initiated TLR/MyD88-dependent intestinal inflammation, while the loss of myeloid COX2 enhanced the proinflammatory response of LPS-activated macrophages, elevated the levels of lipid proinflammatory mediators in intestinal tissue and plasma, and reduced the level of proresolving LXA4 in intestinal tissue. An LXA4 analog (BML111) rescued disease in the Cox2-MKO/CCHF model. 4F therapy inhibited the proinflammatory effects of LPS and oxPAPC on macrophages (and intestinal epithelium), and it reversed the disease-dependent increase of lipid proinflammatory mediators while enhancing luminal clearance of mural proinflammatory lipids. These observations support the correlate hypotheses that dysregulated levels of inflammatory mediators are pathogenic for disease in the Cox2-MKO model, and that rescue of these levels is protective.

Disease in the Cox2-MKO/CCHF model overlaps with human CD in several respects. The inflammation is localized to the ileo-colic junction and is transmural in nature (Figure 1A). Apoα expression is inhibited in this region (Supplemental Figure 14), as occurs in human CD (38). Lipid peroxidation markers are elevated in plasma and inflamed intestine (Figure 6), as in human CD (55, 56). However, unlike human CD (38), expression of Duox2 was not increased in the terminal ileum of diseased mice (Supplemental Figure 22). Furthermore, the reduction of Apoα expression in the colon was offset by lack of change in the ileal and plasma compartments.

Increased intestinal permeability has been proposed as a primary etiologic factor in IBD (57). However, the inflammatory process itself can impair barrier function (58). There remains debate whether changes in barrier permeability are early events in pathogenesis or merely secondary phenomena (59). Our results indicate that alteration in barrier function is an important initiating event in both the Cox2-KO/CCHF and PAC III10−/− models (Figure 1 and Figure 8C). Nonetheless, we did observe delayed increases in barrier permeability in both the Cox2-MKO/CCHF and PAC III10−/− models, indicative of an inflammation-dependent secondary effect (Supplemental Figure 7 and Figure 9C).

Loss of barrier function alone was not sufficient to cause intestinal inflammation in Cox2-MKO mice fed CCHF. Barrier
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**Cox2-MKO enhanced the proinflammatory phenotype of LPS-activated BMDMs (Figure 1E) (compare our results with those in refs. 60, 61). Cox2-MKO also prevented production of PGJ2 and 15d-PGJ2 in these macrophages (Supplemental Figure 9). The cyclopentenone prostaglandins PGJ2 and 15d-PGJ2 can act as negative regulators of NFκB (36). It is possible that a loss of negative feedback control of NFκB partially explains the proinflammatory effects of Cox2-MKO in LPS-activated macrophages. Because additional PAMPs including lipoteichoic acid (LTA) and peptidoglycan (PG)

Permeability increased along the whole intestine (Figure 1B and Supplemental Figure 1), but inflammation was localized to the ileo-ceco-colic junction. We originally considered the possibility that disease depended on the conversion of cholate to deoxycholate within this compartment, but we observed no difference between the functional effects of these bile salts (Supplemental Figure 3). However, disease in this model did depend on TLR/MyD88-specific ligands (Figure 1), whose concentrations are greatest in the ceca of mice.

Figure 9. 4F treatment inhibits the development of colitis in the piroxicam-accelerated Il10−/− model of IBD. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. (A–B) Il10−/− mice were treated with and without piroxicam (PX) and 4F (n = 4–7/group). (A) 4F partially rescued the effect of PX on colon length and colitis score. (B) 4F also improved disease histopathology: representative H&E images (scale bars, 200 μm) and H&E disease score are shown. Green bars indicate thickness of mucosa in Il10−/− + PX. Red arrows indicate inflammatory foci. (C) PX increased both whole intestinal barrier permeability of Il10−/− mice in a biphasic manner, as determined by LC-MS/MS of urinary excretion of sucralose (left), as well as the translocation of endotoxin into portal vein serum at day 9 (right). (D) 4F inhibited the production of prostanoids including PGE2 in LPS-activated Il10−/− BMDMs with or without PX (n = 3/group). (E–F) The lipid inflammatory mediator profile in the colons of the mice shown in A and B was determined by LC-MS/MS (see Supplemental Table 8) (n = 4–8 mice/group). (E) Heatmap of changes. (F) All significant differences are represented. 4F significantly inhibited the disease-dependent increase of proinflammatory LOX mediators. Tt, total. Statistical analyses were as follows: 1-way (A, B), repeated measures 1-way (C, left), or 3-way (D) ANOVA with Tukey’s multiple comparisons test and adjusted P values; Student’s t test (C, right). For E–F, we used the Benjamini-Hochberg procedure applied to 1-way ANOVA for each lipidic analyte with FDR at level α = 0.05, followed by Tukey’s multiple comparisons test and adjusted P values.
also can signal through NFκB (27), we speculate that Cox2-MKO also enhances the proinflammatory effects of these additional PAMPs.

The proinflammatory LOX and COX mediators 15HETE, 12HETE, 5HETE, 11HETE, PGE2, and TXB2 are elevated in the colons of human patients with UC (62). We observed comparable increases in proinflammatory LOX and COX mediators in the intestinal tissues of both the Cox2-MKO and PAC Il10 –/– models (Figure 2 and Figure 9). The overlap between the proinflammatory eicosanoid signatures of these mouse models with that of human UC suggests that the common elevations may constitute a more general signature for IBD. Intestinal inflammation also correlated with elevated plasma lipid proinflammatory mediators including both LOX metabolites and oxPAPC (Figure 2, Figure 7, and Figure 9; Supplemental Figure 12 and Supplemental Figure 21). These plasma-associated inflammatory mediators may thus be of interest as possible IBD biomarkers.

Proinflammatory lipid mediators may be causative for disease in both IBD models. 4F therapy rescued the increase of proinflammatory LOX mediators in the intestinal tissue without affecting the levels of proinflammatory COX mediators (Figure 4C, Figure 8A, and Figure 9F). 4F can also directly clear proinflammatory LOX mediators from intestinal tissue (Figure 8). Together, these observations support a causal role for the LOX mediators. Of note, 12HETE enhances vascular permeability and neutrophil recruitment (63), and elevated 12HETE has been associated with human IBD (62). Furthermore, disease correlated with elevated oxPAPC in intestine, and oxPAPC induced a proinflammatory response in macrophages.

Plasma clearance (proximal SI)

Cecum with disease:
- Pro-INF LOX: 12HETE, 15HETE, total
- Pro-INF COX: PGF2α, TXB2, total
- PGE2
- oxPAPC

Figure 10. Proposed pathogenic mechanisms of Cox2-MKO and CCHF-dependent intestinal inflammation together with the protective mechanisms of APOA1 mimetic peptides. (A) Cholate increases barrier permeability, initiating both bacteria and MyD88-dependent inflammation in Cox2-KO mice. Cox2-KO macrophages in the lamina propria exhibit an increased proinflammatory response to PAMP/LPS activation in response to PAMP/LPS activation, perhaps through loss of negative feedback control of NFκB. In turn, intestinal epithelium amplifies the proinflammatory stimuli of macrophages, resulting in a dysregulated proinflammatory positive feedback loop. Elevated oxPAPC levels further amplify the proinflammatory response. Loss of LXA4 production in Cox2-KO macrophages impairs the resolution of inflammation. The APOA1 mimetic peptide 4F interrupts the positive feedback loops by inhibiting the LPS-dependent activation of macrophages, blocking proinflammatory signal amplification by intestinal epithelium, and reducing the levels of oxPAPC while directly blocking their effects on macrophages. (B) Upon CCHF challenge, Cox2-MKO elevates lipid proinflammatory mediators in intestinal tissue and plasma while reducing proresolving mediators in tissue. 4F reduces the levels of proinflammatory LOX mediators in the cecum (right), possibly through direct clearance. 4F also reduces the levels of proinflammatory lipid mediators in plasma (left), possibly through enhancement of the TILT of lipoprotein-associated lipid inflammatory mediators into the lumen of the proximal small intestine (SI), in a manner comparable to the effect of 4F on the TICE of free cholesterol (FC) (17).
tions, including blocking neutrophil migration and stimulating efferocytosis (36). LXA4 formation is lower in colonic mucosal biopsies from patients with active UC versus healthy controls (64), while stable analogs of LXA4 were protective in dextran sodium sulfate (DSS) and trinitrobenzene sulfonic acid models of UC (65, 66). The stable LXA4 analog BML111 inhibited disease in Cox2-MKO mice fed CCHF (Figure 2G), supporting the hypothesis that COX2-dependent loss of LXA4 is partially causative of disease in this model. By contrast, LTB4 is a potent proinflammatory mediator involved in neutrophil recruitment (67) whose production can be enhanced by loss of COX2 (9). Altered balance of SLOX-associated resolving mediators to LTB4 has been associated with plaque instability in atherosclerosis (68) and could further explain the chronic inflammation of this model.

We concluded that myeloid COX2 constitutes a check on TLR-dependent intestinal inflammation in the ileo-colic junction. Our account thus extends earlier work that also demonstrated a role for COX2 in intestinal immune tolerance. Newberry et al. showed that COX2 and PGE2 help maintain adaptive immune tolerance to dietary antigens in the proximal small bowel (12), which function they further attributed to constitutive Cox2 expression and PGE2 production by stromal cells in the lamina propria (13). Despite the general overlap, however, our current account differs from this prior work in at least one respect. Contrary to Newberry et al., we did not observe an expansion of the T lymphocyte population in the inflammatory lesions of the Cox2-MKO/CCHF model (Supplemental Figure 5). Rather, the immunosuppressive effect of myeloid COX2 in our model appears to bear primarily on innate immunity. However, Newberry et al. identified stromal-derived PGE2 as the essential mediator of COX2-dependent adaptive immune suppression. By contrast, myeloid COX2-KO did not result in a decrease in PGE2 within the ileo-ceco-colic junctions of Cox2-MKO mice fed CCHF (Figure 2E), indicating complimentary production of PGE2 by other cell types. Our results thus appear consistent with the earlier work of Newberry et al. on COX2 and intestinal immune tolerance.

The APOA1 mimetic peptides 4F and Tg6F inhibited intestinal inflammation in our mouse models. In the Cox2-MKO/CCHF model, 4F directly suppressed LPS-dependent signaling and appeared to interrupt many of the amplifying proinflammatory feedback loops present in disease. In particular, 4F blocked the proinflammatory effect of LPS-activated macrophages on intestinal epithelium, lowered oxPAPC while inhibiting its proinflammatory effects, and cleared mural proinflammatory lipids. These results thus extend earlier reports on the efficacy of mimetic peptides against DSS-induced colitis (39, 69). APOA1 mimetic peptides offer several advantages over existing IBD therapies. These drugs are not immune suppressive, and unlike current biologics, they can be taken orally and are not antigenic (70–72).

4F therapy reduced the plasma levels of lipid proinflammatory mediators (Figure 4, Figure 7, and Figure 8). We demonstrated ex vivo that lipid inflammatory mediators can be transported across intestinal explants from lipoproteins into mucosal media, indicating that there may exist trans-intestinal lipid transport (TILT) of lipid species over and above cholesterol. 4F can enhance this trans-intestinal transport (Figure 8D). It is thus possible that TILT mediates the effect of 4F on plasma lipid inflammatory mediators in vivo. We previously demonstrated that 4F can enhance TICE (17), and that 4F lowered total cholesterol in the plasmas of Cox2-MKO/CCHF mice (Supplemental Figure 17). 4F may mediate the reduction of plasma cholesterol and plasma prolipid inflammatory mediators through comparable trans-intestinal mechanisms (Figure 10). It is also possible that 4F may act like APOA1 to increase macrophage expression of the antioxidant APOE (73), which effect could further account for the antioxidant properties of 4F.

The current study contains several limitations. First, inflammation is a dynamic process but we sampled lipid inflammatory mediators at only a few time points. Second, we do not fully elucidate the mechanisms by which loss of myeloid COX2 results in the dysregulated lipid mediator levels that we observe. Third, we do not fully establish the causal significance of most of the dysregulated lipid inflammatory mediators.

Nonetheless, the present study identifies a role for myeloid COX2 in limiting TLR-dependent intestinal inflammation. It highlights the potential importance of proinflammatory and pro-resolving lipids as modulators of IBD. Finally, we offer further evidence of the possible translational value of APOA1 mimetics as therapy for IBD, while adding to our understanding of their protective mechanisms.

**Methods**

**Mice.** Cox2-MKO (Cox2f/f LysMcCre/+), Cox2 FLOX (Cox2f/f), Cox2-TKO (Cox2f/f), Cox2f/f Mrp8Cre/+ and Il10−/− mice were all generated, bred, and maintained as described (15, 30, 53, 74). C57BL/6J mice were from The Jackson Laboratory. Equal numbers of male and female mice were used in all in vivo studies.

**APOA1 mimetic peptides.** D-4F and 3F(14) peptides were synthesized as previously described (17, 75). For in vivo studies, D-4F was supplied as 500 μg/mL in drinking water, and the mice were allowed to drink ad libitum. For cell culture studies, D-4F or 3F(14) were added at 15 μg/mL to the cell culture media. Tg6F extracts were prepared as described previously (40, 76). For studies involving Tg6F, the final diets contained 0.06% of Tg6F extract by weight (40).

**Study approval.** All human tissues used in this study were obtained from de-identified and discarded surgical specimens following clinical surgical pathology evaluation. The UCLA Institutional Review Board approved procurement and use of surgical samples. The requirement for informed consent for tissues obtained from the UCLA Translational Pathology Core Laboratory (IRB 11-002504) was waived. The Animal Research Committee at UCLA approved the animal protocols used in this study and the methods were carried out in accordance with the approved guidelines.

**Statistics.** For lipidomic analyses (Figure 2, Figure 4, and Figure 9), FDR corrections were performed by application of the Benjamini-Hochberg procedure to P values determined for each analyte by 1-way ANOVA, with FDR controlled at level α = 0.05. For each analyte within a data set deemed a discovery/significant, post hoc analyses consisted of Tukey’s multiple comparisons test. For all other multiple comparisons, 1-, 2-, or 3-way ANOVA was followed by Tukey’s or Dunnnett’s multiple comparisons test. For comparisons involving 1 independent variable with 2 groups, we performed Student’s t tests, and for studies consisting of more than 1 Student’s t test, we performed Holm-Sidak’s correction for multiple tests. In all cases of
multiple comparisons/tests, we adjusted \( P \) values in accord with the appropriate correction. Statistical significance was set at \( P \) less than 0.05 with respect to final (adjusted) \( P \) values. Variation is reported as SEM. Additional information about Methods and Materials can be found in the Supplemental Materials.

Author contributions

DM designed studies, conducted experiments, analyzed data, and wrote the manuscript. DS conducted the Ussing chamber experiments. CV, AD, and HT helped conduct the animal studies and maintain the animal colonies. VG performed macrophage studies. ND did Western blots and lipid extractions. RSSV and JW helped perform the human enteroid studies. EO and JP helped develop the LC-MS/MS lipid analysis methods. ML provided the Ussing chambers and training for their use. MNP and GMA synthesized the APOAi mimetic peptides D-4F and 3F. HRH generated and provided the Cox2-TKO and neutrophil-specific knockout mice and reviewed the experimental protocols and results. MGM and AMF provided guidance, feedback, supervision, and financial support. STR provided guidance and feedback regarding the overall study and individual experiments, edited the manuscript, and provided laboratory and financial support.

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