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Protectin D1_{n-3} DPA and resolvin D5_{n-3} DPA are effectors of intestinal protection

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The resolution of inflammation is an active process orchestrated by specialized proresolving lipid mediators (SPM) that limit the host response within the affected tissue; failure of effective resolution may lead to tissue injury. Because persistence of inflammatory signals is a main feature of chronic inflammatory conditions, including inflammatory bowel diseases (IBDs), herein we investigate expression and functions of SPM in intestinal inflammation. Targeted liquid chromatography-tandem mass spectrometry-based metabolomics was used to identify SPMs from n-3 polyunsaturated fatty acids in human IBD colon biopsies, quantifying a significant up-regulation of the resolvin and protectin pathway compared with normal gut tissue. Systemic treatment with protectin (PD)1_{n-3} DPA or resolvin (Rv)D5_{n-3} DPA protected against colitis and intestinal ischemia/reperfusion-induced inflammation in mice. Inhibition of 15-lipoxygenase activity reduced PD1_{n-3} DPA and augmented intestinal inflammation in experimental colitis. Intravital microscopy of mouse mesenteric venules demonstrated that PD1_{n-3} DPA and RvD5_{n-3} DPA decreased the extent of leukocyte adhesion and emigration following ischemia-reperfusion. These data were translated by assessing human neutrophil-endothelial interactions under flow: PD1_{n-3} DPA and RvD5_{n-3} DPA reduced cell adhesion onto TNF- α -activated human endothelial monolayers. In conclusion, we propose that innovative therapies based on n-3 DPA-derived mediators could be developed to enable antiinflammatory and tissue protective effects in inflammatory pathologies of the gut.

inflammation resolution | specialized proresolving mediators | omega-3 fatty acids | intravital microscopy | neutrophils

Inflammatory bowel diseases (IBDs), the main forms being Crohn's disease and ulcerative colitis, are chronic inflammatory conditions of unknown etiology that primarily affect the gastrointestinal tract (1). IBD affects ~1.5 million Americans, >2 million people in Europe, and several hundred thousands more worldwide (2). Irrespective of its etiopathogenesis, the chronic nature of IBDs is caused by inflammatory signals (i.e., cytokines, proteases) leading to overt pathology (1). Healthy intestinal mucosa rely on a complex equilibrium: when this is disrupted a robust and persistent inflammatory response develops characterized by mucosal injury, increased epithelial permeability, invasion of bacteria into lamina propria, and marked neutrophil recruitment (1). This paradigm has driven research in the field informing current treatments, including biologics like anti-TNF- α . However, these treatments are immunosuppressive (hence plagued by side-effects), expensive, and ineffective in a good proportion of patients (3). The increasing incidence of IBD and the inadequacy of current treatments makes imperative to develop new therapeutic approaches.

The ultimate remit of the inflammatory response is to protect the host from exogenous or endogenous dangers: following

removal of the noxious stimuli, the inflammatory process is finely programmed to self-resolve to avoid harmful injury (4, 5). The last step of this physiological multicomponent process is tissue repair, with return to tissue functionality, instructing the adaptive immune response and altogether regaining homeostasis (6, 7). However, if not modulated inflammatory mechanisms induce self-harm, leading to tissue injury, as in chronic diseases including IBDs (8, 9). This beneficial profile of the inflammatory response is ensured with the turning on of resolution programs and the biosynthesis of proresolving mediators that counter-regulate the action of inflammation-initiating mediators (10). Within the effectors of resolution, an important role is emerging for specialized proresolving lipid mediators (SPMs): these are families of mediators formed via the stereospecific conversion of essential polyunsaturated fatty acids (PUFAs) by enzymes, including the lipoxygenases (10). During inflammation, increased local vascular permeability leads to edema, which supplies PUFAs from blood to the site of injury, together with elevated numbers of immune cells that carry the biosynthetic enzymes required for the local SPM production (11). These mediators, in turn, interact with specific receptors to temper leukocyte reactivity,

Significance

We provide evidence for a functional role of bioactive lipid mediators of the docosapentaenoic acid (DPA) metabolome in intestinal inflammation. Supported by changes in DPA-derived mediators in colon biopsies from inflammatory bowel diseases, we studied the pharmacological properties of two mediators. Exogenous administration of protectin (PD)1_{n-3} DPA or resolvin (Rv)D5_{n-3} DPA in mice reduced dextran sulfate sodium-induced colitis through a mechanism partly linked to decreased leukocyte-endothelial interaction and reduced granulocyte trafficking, as assessed by intravital microscopy. The translational impact of these data was determined by the ability of PD1_{n-3} DPA and RvD5_{n-3} DPA to reduce human neutrophil adhesion onto TNF- α -activated human endothelial monolayers. We propose that n-3 DPA-derived mediators could represent the basis for innovative therapeutic strategies in settings of intestinal inflammation.

Author contributions: T.G., J.D., C.N.S., and M.P. designed research; T.G., R.A.C., and D.F.C. performed research; M.A., D.B., L.A., C.D., and T.V.H. contributed new reagents/analytic tools; T.G., J.D., R.A.C., and D.F.C. analyzed data; and T.G., J.D., N.V., C.N.S., and M.P. wrote the paper.

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published criteria, matching retention times and at least six characteristic ions in the MS/MS spectrum, and included n-3 DPA resolvins (RvD1_{n-3} DPA, RvD2_{n-3} DPA, RvD5_{n-3} DPA), protectins (PD1_{n-3} DPA, 10S,17S-diHDDPA), and maresins (MaR1_{n-3} DPA) (Fig. 1 *B* and *C* and Table S2). Quantification using multiple reaction monitoring demonstrated that RvD5_{n-3} DPA, PD1_{n-3} DPA, and the protectin pathway marker 10S,17S-diHDDPA were augmented in tissue biopsies from IBD patients compared with those from control (Fig. 1 *D–F*). Taken together, these findings identify SPM, including n-3 DPA-derived SPM in human colonic tissue. In addition, these results indicate an up-regulation of the resolvins and protectin pathways from the n-3 DPA metabolome in IBD.

We next tested if inhibition of endogenous SPM production would impact disease activity. Using targeted LC-MS/MS-based LM metabololipidomics, we profiled the AA, EPA, DHA, and n-3 DPA bioactive metabolomes in colon tissues from naive mice, mice receiving dextran sulfate sodium (DSS), with or without a 15-LOX inhibitor treatment. In colon tissues from naive mice, we identified mediators from all four bioactive metabolomes, including the n-3 DPA-derived PD1_{n-3} DPA (Fig. 2 *A* and *B* and Table S3). Administration of DSS leads to up-regulation of several protective pathways, including both DHA and n-3 DPA-derived protectins, as well as the E-series resolvins and the DHA-derived RvD5, suggesting the activation of host-protective mechanisms during ongoing inflammation. Administration of a 15-LOX inhibitor reduced tissue concentrations of PD1_{n-3} DPA and increased levels of several proinflammatory eicosanoids, including PGD₂ and PGE₂ (Table S3). Modulation of SPM profiles was associated with a decreased colon length, increased macroscopic tissue damage, and neutrophil infiltration (Fig. 2 *B–D*). These results suggest that inhibition of endogenous PD1_{n-3} DPA leads to a failure to counter-regulate colonic inflammation, leading to increased tissue damage.

PD1_{n-3} DPA and RvD5_{n-3} DPA Protect Mice Against DSS-Induced Colitis.

Given that the enzymatic inhibition of n-3 DPA-derived SPMs is deleterious in colitis and that levels of these mediators are regulated in human colon biopsies from IBD patients (Figs. 1 and 2 and Tables S2 and S3), we tested the pharmacological actions of PD1_{n-3} DPA and RvD5_{n-3} DPA in DSS-induced colitis (Fig. 3*A*). Five days of oral DSS administration to mice induced severe colon inflammation, evident through significant colon shortening, increased wall thickness, and macroscopic damage score (Fig. 3*B*). Systemic treatment with PD1_{n-3} DPA or RvD5_{n-3} DPA (0.3 μg per mouse daily) prevented colon length reduction. Treatment with PD1_{n-3} DPA significantly reduced colon wall thickness and macroscopic colon damage (Fig. 3*B*). In DSS-induced intestinal inflammation, tissue damage is predominantly mediated by infiltrating neutrophils (15); thus, we assessed the actions of PD1_{n-3} DPA and

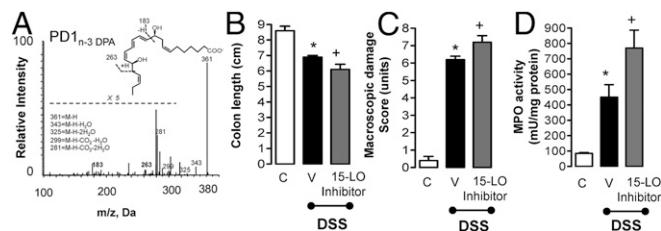


Fig. 2. 15-lipoxygenase inhibition decreased colon PD1_{n-3} DPA levels and increased tissue damage. Mice received PD 146176 (100 mg/kg orally daily). On day 8, tissues were harvested and LM were identified and quantified using LM metabololipidomics. (A) Representative MS/MS spectra used for the identification of PD1_{n-3} DPA. (B) Colon length, (C) macroscopic damage, and (D) intestinal MPO activity. Data are means ± SEM of six mice per group. **P* < 0.05 vs. control. †*P* < 0.05 vs. DSS vehicle-treated group (V), one-way ANOVA, followed by Dunnett's post hoc test.

RvD5_{n-3} DPA on this parameter by measuring tissue myeloperoxidase (MPO) activity (Fig. 3*C*). Against a marked increase in colonic MPO activity, animals receiving PD1_{n-3} DPA or RvD5_{n-3} DPA displayed reduced MPO values (Fig. 3*C*). Analyses of cytokines showed a pronounced increase in colonic TNF-α, IL-1β, and IL-6 levels—with a partial decrease in IL-10—in vehicle-treated DSS mice. PD1_{n-3} DPA, but not RvD5_{n-3} DPA, significantly reduced proinflammatory cytokines levels without correcting IL-10 (Fig. 3*D*). Both compounds restored the histological architecture of the mucosa, decreasing mucosal ulceration compared with the DSS-treated vehicle group (Fig. 3*E*).

We then assessed if PD1_{n-3} DPA was also protective when given using a therapeutic treatment. Mice were given DSS for 7 d, followed by 3 d of water. Treatment with either PD1_{n-3} DPA (0.3 μg/mouse, i.p.) or vehicle commenced at first signs of disease (day 5, beginning of weight loss): PD1_{n-3} DPA-treated group displayed significant protection as measured by reduced colon shortening, decreased macroscopic damage, MPO activity, and inflammatory cytokine levels (Fig. 3*F*).

PD1_{n-3} DPA and RvD5_{n-3} DPA Prevent Local and Systemic Inflammation Following Intestinal Ischemia/Reperfusion.

Given the association for intestinal ischemia in the pathogenesis of IBD, we tested if PD1_{n-3} DPA and RvD5_{n-3} DPA regulated host responses following gut ischemia (16). Occlusion of the mesenteric artery (ischemia) followed by reperfusion provoked a robust granulocyte infiltration in the gut with marked histological damage, as characterized by fragments of mucosa detected in the lumen, damage to the villi, and disintegration of the lamina propria (Fig. 4). Postischemic administration of PD1_{n-3} DPA (most effective at 0.1 μg per mouse) and RvD5_{n-3} DPA (0.1–1 μg per mouse) significantly reduced granulocyte infiltration (Fig. 4*A* and *B*). Administration of PD1_{n-3} DPA preserved structure and length of the villi, whereas RvD5_{n-3} DPA was less effective (Fig. 4*C*). At the dose of 0.1 μg, PD1_{n-3} DPA reduced secondary granulocyte recruitment into the lungs and plasma IL-1β levels (Fig. 4*D* and *E*).

To establish the impact of endogenous n-3 DPA-derived mediators in gastrointestinal protection, we assessed the profiles in animals with reduced expression of fatty acid elongase 2 (ELOVL2) (17), the enzyme that converts n-3 DPA to DHA (Fig. S1*A*). Administration of siRNA to mice reduced ELOVL2 expression in peripheral tissues (Fig. S1*B*). Mice were then supplemented daily with EPA and subjected to ischemia/reperfusion (I/R) injury. LM profiling identified that plasma levels of n-3 DPA-derived SPM, including RvD5_{n-3} DPA and PD1_{n-3} DPA, were significantly increased (Fig. S1 *C–E* and Table S4). These increases were associated with reduced systemic levels of LTB₄ and 12S-HHT when compared with mice given the mock plasmid alone (Fig. S1*F*). Of note, MPO levels in ELOVL2 knockdown, but not mock siRNA-treated animals supplemented with EPA, were significantly reduced compared with levels found in control mice (Fig. S1*G*).

PD1_{n-3} DPA and RvD5_{n-3} DPA Regulate Neutrophil–Endothelial Interactions.

Next, we focused on the process of leukocyte trafficking by using a model of zymosan peritonitis, followed by direct observation of postcapillary venules by intravital microscopy. Administration of PD1_{n-3} DPA and RvD5_{n-3} DPA to mice treated with zymosan markedly reduced leukocyte numbers in the exudates, compared with the vehicle-treated mice. Low doses of RvD5_{n-3} DPA decreased neutrophil counts by >40% (Fig. S2). PD1_{n-3} DPA was more effective at the lower dose of 0.1 μg.

I/R of the mesenteric microcirculation followed by intravital microscopy allowed monitoring of the processes of leukocyte rolling, adhesion, and emigration (18). The dose of 0.1 μg was selected and administered before reperfusion. Treatment with PD1_{n-3} DPA or RvD5_{n-3} DPA decreased the number of adherent leukocytes onto the postcapillary endothelium (Fig. 5*A*) but did not affect the intermittent contacts typical of the rolling process

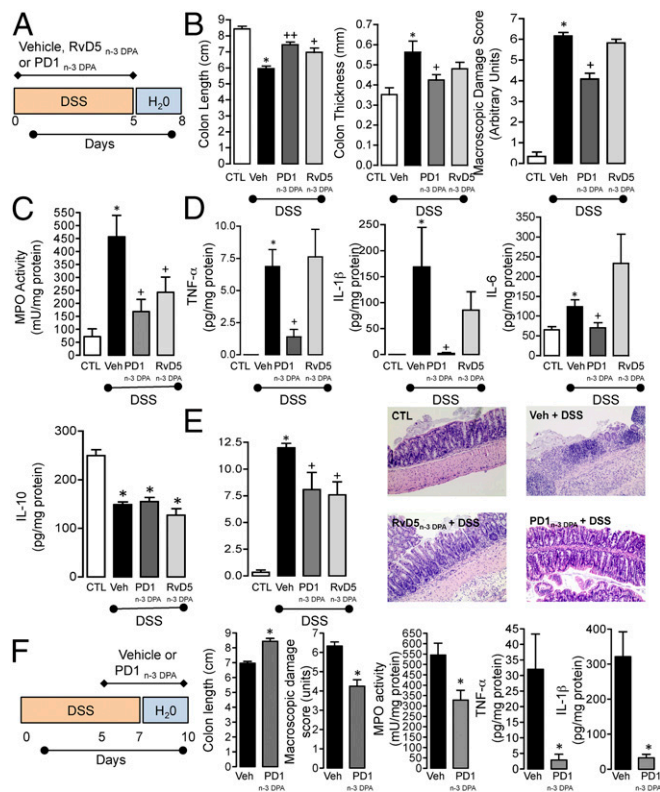


Fig. 3. PD1_{n-3} DPA and RvD5_{n-3} DPA protect mice against DSS-induced colitis. (A) Mice had access to drinking water with or without 2.5% DSS for 5 d, then switched to normal water for an additional 3 d before colon collection for analyses (day 8). Animals were treated intraperitoneally with vehicle (100 μ L PBS 0.01% EtOH) or with 0.3 μ g PD1_{n-3} DPA or RvD5_{n-3} DPA daily from days 0–5. Control mice (CTL) received only drinking water. Day 8 analyses: (B) colon length, wall thickness, and macroscopic damage; (C) intestinal MPO activity; (D) cytokines (TNF- α , IL-1 β , IL-6, and IL-10) levels as determined in colon homogenates using ELISA. (E) Histological score with representative images of colon sections. (Magnification: 50 \times) (F) In another set of experiments, mice received DSS as above, but 0.3 μ g PD1_{n-3} DPA was administered daily from day 5. Day 10 analyses included colon length, macroscopic damage score, MPO activity, and tissue cytokine levels. In all cases, data are means \pm SEM of six mice per group. * P < 0.05 versus CTL. + P < 0.05, ++ P < 0.01 vs. DSS vehicle-treated group, one-way ANOVA, followed by Dunnett's post hoc test.

(95 \pm 13% of the response in vehicle-treated animals; not significant). A significant reduction in the extent of leukocytes emigrated into the subendothelial tissue was also quantified (Fig. 5B). Representative images are in Fig. 5C.

Preincubation of human neutrophils with PD1_{n-3} DPA or RvD5_{n-3} DPA drastically reduced cell capture (Fig. 5D) compounded by an effect on cell adhesion and transmigration onto TNF- α -activated endothelial monolayers (Fig. S3), with activity between 10 pM and 100 nM. No effect was observed on the number of human cell rolling on the endothelium (Fig. S3). These results made us dwell on human neutrophil functions. Incubation of whole blood with TNF- α up-regulated cell surface expression of total CD11b. Addition of PD1_{n-3} DPA or RvD5_{n-3} DPA before TNF- α led to a 32 \pm 4% and 28 \pm 4% of reduction for total CD11b immunostaining (n = 4 donors, P < 0.05) (representative histograms in Fig. 5E). There was some degree of selectivity in these responses because the two mediators did not modulate basal CD62L expression nor TNF- α -induced CD62L shedding (102 \pm 6% with respect to appropriate control; n = 4 donors, not significant). This finding is in contrast to what observed with PAF stimulation, with an effect of PD1_{n-3} DPA or RvD5_{n-3} DPA on CD62L shedding (~50 \pm 6%

reduction of PAF response for either mediator, n = 4 donors, P < 0.05) (representative histogram in Fig. 5E).

Discussion

In the present study, we report the tissue-protective actions of two n-3 DPA-derived mediators, denoted as PD1_{n-3} DPA and RvD5_{n-3} DPA. These mediators: (i) reduced inflammation following DSS-induced colitis; (ii) prevented local and systemic inflammation following intestinal I/R; and (iii) regulated neutrophil–endothelial interactions, which may occur downstream modulation of adhesion molecule activation. Of translational relevance, we could identify n-3 DPA SPM in human colon biopsies. These results suggest a relevant profile for these SMPs as an innovative therapeutic approach to control intestinal inflammation like in IBD.

Two decades of research now support the concept that resolution of inflammation is an active process brought about by specific mediators and receptors (12). We studied herein resolution, or its lack, in the persistent and unresolved inflammation typical of IBD (1). Of relevance, the gut is in a state of underlying inflammation and it is plausible that endogenous checkpoints are properly operative to avoid progression to chronic conditions (19). Moreover, the relapsing and remitting nature of IBD together with the spontaneous resolution that sometimes is observed (20), suggest existence of contrasting endogenous processes enacted by proinflammatory and proresolving mediators during the pathogenesis of the disease. SPM are produced by leukocytes, including neutrophils and macrophages, via transcellular biosynthesis and in a cell-autonomous manner (21, 22). Other cell types, including epithelial cells (23) and platelets (21), may also contribute to the SPM production because they carry some of the initiating enzymes in the biosynthesis of these molecules. Many of these cell types, including macrophages and epithelial cells, are present in human naive (healthy) colon biopsies and their numbers are increased in IBD (24). In the present study we found an increase in both proinflammatory mediators, and a decrease in the proresolving n-3

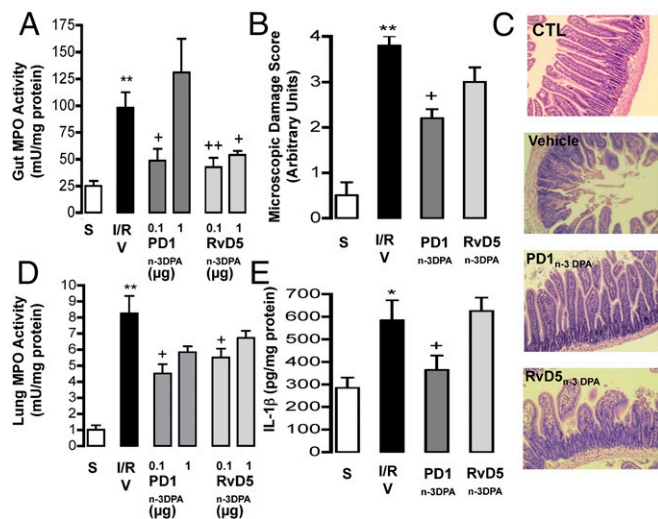


Fig. 4. Exogenous administration of PD1_{n-3} DPA or RvD5_{n-3} DPA prevents local and systemic I/R injury. Mice were subjected to intestinal ischemia (30 min) followed by 5-h reperfusion (I/R) or sham operation (S). Animals were given vehicle (100 μ L), PD1_{n-3} DPA, or RvD5_{n-3} DPA (0.1 μ g intravenously, unless otherwise specified) before reperfusion. After 5-h reperfusion, multiple markers were quantified: (A) Gut MPO activity; (B) intestinal histological score on colon sections stained with H&E; (C) representative images of colon sections (magnification: 50 \times); (D) lung MPO activity; (E) plasma IL-1 β levels. Data are mean \pm SEM of 6–8 mice per group. * P < 0.05, ** P < 0.01 vs. sham; + P < 0.05, ++ P < 0.01 vs. vehicle group, one-way ANOVA, followed by Dunnett's post hoc test.

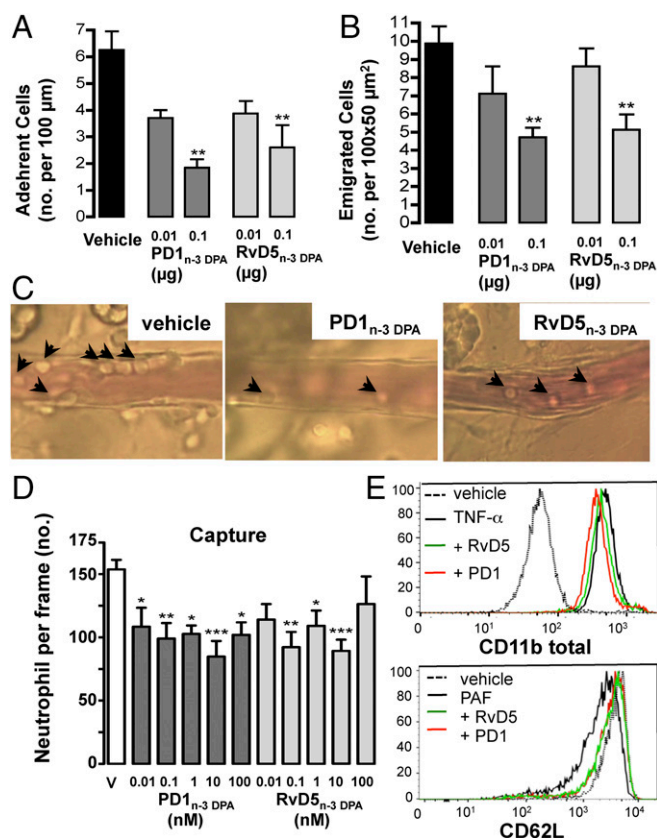


Fig. 5. PD1_{n-3} DPA and RvD5_{n-3} DPA reduce neutrophil-endothelial interactions. Mice were subjected to intestinal ischemia (30 min) followed by reperfusion (90 min) (I/R). Animals were treated intravenously before reperfusion with 0.01 or 0.1 μg PD1_{n-3} DPA, RvD5_{n-3} DPA, or vehicle (100 μL PBS 0.01% EtOH). Postcapillary venules were imaged and recorded for offline quantitation of white blood cell interaction with the endothelium. (A) Number of adherent cells to the postcapillary venule endothelium and (B) emigrated cells in the subendothelial space. (C) Representative light-microscopy images are shown for vehicle, PD1_{n-3} DPA, and RvD5_{n-3} DPA (0.1 μg per mice). Black arrows identify leukocytes adherent to postcapillary venules. (Magnification: 400×.) Data are mean ± SEM of six mice per group. ***P* < 0.01 vs. respective vehicle, one-way ANOVA followed by Dunnett's post hoc test. (D) Human neutrophils were incubated with vehicle, PD1_{n-3} DPA, or RvD5_{n-3} DPA (0.01–100 nM) prior perfusion over TNF-α-stimulated endothelial cell monolayers for 8 min, and the extent of cell capture was quantified. Results are mean ± SEM of six distinct cell donors, **P* < 0.05, ***P* < 0.01, ****P* < 0.001 vs. vehicle, one-way ANOVA, followed by Dunnett's post hoc test. (E) Whole blood from healthy volunteers was incubated with vehicle (PBS 0.1% EtOH), PD1_{n-3} DPA, or RvD5_{n-3} DPA (100 nM; indicated as PD1 or RvD5) for 15 min at 37 °C, and then stimulated with TNF-α or PAF. Histograms of CD11b and CD62L expression on human neutrophils are shown (representative from four distinct blood donors).

PUFA mediators, including PD1_{n-3} DPA. Of note, levels of these mediators were within their bioactive concentration (femtomolar to nanomolar) (13, 14), a feature aligned with recent findings where SPM at bioactive concentrations were identified in human tissues including spleen, lymph nodes, plasma (25), and also milk (26). The protective role of endogenous SPM was tested using a 15-lipoxygenase inhibitor that decreased the formation of PD1_{n-3} DPA and that was associated with an increase in the production of proinflammatory molecules and colon inflammation.

SPM derived from n-3 PUFA-like DHA and EPA have been linked to protective actions in intestinal inflammation (27). Administration of resolvin E1, resolvin D2, 17(*R*)-hydroxy DHA, aspirin-triggered resolvin D1, and Maresin 1 resulted in an improved disease activity score, reduced colonic damage, and lower polymorphonuclear infiltration in several models of colitis (27). In

the present study we report that administration of PD1_{n-3} DPA or RvD5_{n-3} DPA was effective in preventing the hallmark of intestinal inflammation after DSS administration to mice, including an effect on colon length, microscopic damage score, and granulocyte recruitment. These effects could be secondary to the regulation of proinflammatory cytokine production, as in the case of PD1_{n-3} DPA. The clinical efficacy of anti-TNF therapy in IBD (3) supports this mechanism. Of interest, both PD1_{n-3} DPA and RvD5_{n-3} DPA did not regulate colonic levels of IL-10, setting these mediators apart from LXA₄ (28) or other proresolving mediators, like Annexin-A1 (29, 30) and α-melanocyte stimulating hormone (31). To substantiate the physio-pathological impact of this new pathway, it was important to observe that inhibition of the enzyme responsible for conversion of DPA to DHA led to increased tissue levels of n-3 DPA-derived SPM, an effect linked to a reduction in systemic eicosanoid levels during I/R injury, supporting the host protective actions of these molecules.

IBD is characterized by marked infiltration of inflammatory cells and elevated concentration of inflammatory mediators, both within the gut and in the circulation (32). DSS-induced colitis is a useful model to study the early initiating event in IBD, such as the neutrophil recruitment to the intestinal wall and the enhanced production of proinflammatory cytokines that typifies the inflammatory cascade (33, 34). Among the properties of n-3 PUFA-derived mediators (10, 13), PD1_{n-3} DPA or RvD5_{n-3} DPA displayed potent bio-actions that are relevant to the pathophysiology of IBD, including their ability to regulate neutrophil recruitment (*vide infra*). Each of these mediators also displayed characteristic regulation of inflammatory cytokines whereby PD1_{n-3} DPA regulated TNF-α, IL-1β, and IL-6, whereas RvD5_{n-3} DPA only partially reduced IL-1β levels. Thus, these findings suggest that these two mediators may activate distinct protective responses potentially through engagement of different receptors.

At 0.1 μg (equivalent to ~90 nmol), PD1_{n-3} DPA and RvD5_{n-3} DPA significantly reduced neutrophil recruitment in zymosan peritonitis and intestinal ischemia reperfusion injury in mice. Because the latter model results in secondary organ injury (35), we could complement the pharmacological properties of these two compounds by establishing their ability to confer both intestinal tissue protection and reduce excessive granulocyte recruitment into the lungs, together with decreased level of plasma IL-1β.

The series of molecular and cellular events that lead to neutrophil recruitment are well characterized (36). Using intravital microscopy of mesenteric venules, we investigated the actions of these n-3 DPA mediators on specific processes within the leukocyte recruitment cascade *in vivo*, finding that both RvD5_{n-3} DPA and PD1_{n-3} DPA specifically regulated cell adhesion while leaving cell rolling unaffected. This observation was also replicated with human primary cells, using the flow-chamber system. The potent actions of PD1_{n-3} DPA and RvD5_{n-3} DPA on TNF-α-induced neutrophil recruitment under shear conditions indicate that conversion of n-3 DPA into proresolving mediators can counterbalance the actions of proinflammatory mediators. Single-cell analyses with human neutrophils identified at least one mechanism by which these mediators could regulate inflammatory responses. Both molecules modulated adhesion molecule expression on the neutrophil cell surface, with the intriguing observation that the specific target (CD11b or CD62L) might be stimulus-dependent.

In conclusion, the present study offers evidence that endogenous LMs derived from the n-3 DPA biosynthetic pathway exert beneficial actions on leukocyte reactivity and cytokine production, regulating the outcome of intestinal inflammation. Thus, PD1_{n-3} DPA and RvD5_{n-3} DPA can be used as guidance for the development of innovative therapeutic strategies to control the imbalanced inflammatory status typical of IBD and other inflammatory pathologies.

Materials and Methods

An extended version of materials and methods is presented in *SI Materials and Methods*. All procedures were performed under the ethical approval both for animal and human cell work. For studies involving mice, all procedures were performed under the United Kingdom Animals (Scientific Procedures) Act, 1986. Human cells were prepared according to an approved protocol (East London & the City Local Research Ethics Committee; no. 06/Q605/40; P/00/029 ELCHA). The Ethics Committee approved the human research protocol ([ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT01990716) to collect biopsies and clinical information. Written informed consent was received from participants before inclusion in the study.

Sample Extraction and LM Metabololipidomics. Human and mouse gastrointestinal tissues extraction and LM metabololipidomics was conducted as described previously (25).

Induction and Assessment of DSS-Induced Colitis. Mice were provided access ad libitum to water containing 2.5% DSS over a 5-d period. Every other day, the DSS solution was replenished. On day 5, DSS was replaced with normal drinking; on day 8 the animals were killed. Control mice received only drinking water. From days 1–5, RvD5_{n-3} DPA_n, PD1_{n-3} DPA_n, or vehicle (0.3 μg/100 μL in saline 0.01% EtOH) were administered intraperitoneally daily. In other cases, the 15-lipoxygenase inhibitor PD 146176 (Cayman Chemical) was used at 100 mg/kg.

For the therapeutic protocol, mice were provided access to water containing 2.5% DSS over a 7-d period. On day 7, DSS was replaced with normal drinking water for 3 d. PD1_{n-3} DPA_n or vehicle (0.3 μg/100 μL in saline 0.01% EtOH) were administered intraperitoneally daily (from day 5 to day 10). At the end of day 8 or 10, the colon was removed, measured, and examined for the consistency of the stool found within, as well as the gross macroscopic appearance and length, as described previously (37, 38). Samples harvested for histology were analyzed by light microscopy and scored (37, 38). MPO

activity was performed as previously described (39). Intestinal levels of cytokines were assayed using ELISA kit. Colonic samples were harvested for metabololipidomics analysis, as previously described (25).

Intestinal I/R Injury. In anesthetized mice, the superior mesenteric artery (SMA) was occluded for 50 min; on reperfusion, PD1_{n-3} DPA_n, RvD5_{n-3} DPA_n, or vehicle was given, and tissues collected at 5 h. MPO activity was measured as an index of neutrophil infiltration (39). Tissue sections (5 μm) were stained with H&E. Microscopic histological damage score was evaluated blindly (40). Cytokines were assayed by ELISA. Specimens of the jejunum-ileum were also collected for intestinal lipid extraction and LC/MS/MS measurements. In other experiments, mice were transfected with either 40 μg mock or ELOVL2 siRNA and given EPA (every other day for 6 d) or vehicle. ELOV2 expression in the liver was determined after 6 d by Western blot analysis. ELOVL2 siRNA or control animals were subjected to 50-min intestinal ischemia followed by a 5-h reperfusion, as described above.

Intravital Microscopy of the Mouse Mesenteric Microcirculation. I/R was performed with 30-min ischemia and 90-min reperfusion; intravital microscopy of the mesentery was conducted as described previously (18). One to three postcapillary venules were analyzed for the extent of cell rolling, adhesion, and emigration.

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