Novel n-3 Immunoresolvents: Structures and Actions

Jesmond Dalli, Romain A. Colas & Charles N. Serhan

Center for Experimental Therapeutics and Reperfusion Injury, Department of Anesthesiology, Perioperative and Pain Medicine, Harvard Institutes of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts. 02115.

Resolution of inflammation is now held to be an active process where autacoids promote homeostasis. Using functional-metabololipidomics and in vivo systems, herein we report that endogenous n-3 docosapentaenoic (DPA) acid is converted during inflammation-resolution in mice and by human leukocytes to novel n-3 products congeneric to D-series resolvins (Rv), protectins (PD) and maresins (MaR), termed specialized pro-resolving mediators (SPM). The new n-3 DPA structures include 7,8,17-trihydroxy-9,11,13,15E,19Z-docosapentaenoic acid (RvD1n-3 DPA), 7,14-dihydroxy-8,10,12,16Z,19Z-docosapentaenoic acid (MaR1n-3 DPA) and related bioactive products. Each n-3 DPA-SPM displayed protective actions from second organ injury and reduced systemic inflammation in ischemia-reperfusion. The n-3 DPA-SPM, including RvD1n-3 DPA and MaR1n-3 DPA, each exerted potent leukocyte directed actions in vivo. With human leukocytes each n-3 DPA-SPM reduced neutrophil chemotaxis, adhesion and enhanced macrophage phagocytosis. Together, these findings demonstrate that n-3 DPA is converted to novel immunoresolvents with actions comparable to resolvins and are likely produced in humans when n-3 DPA is elevated.

The resolution of acute inflammation is an active process temporally orchestrated by local-acting mediators that limit further neutrophil recruitment to sites of inflammation1–4. During the onset of inflammation, chemical mediators, including the arachidonic acid-derived eicosanoids (e.g. leukotriene (LT) B₄ and prostaglandin (PG) E₂), mount initiation and propagation of inflammation5,6, actions that are actively counter-regulated and orchestrated by pro-resolution agonists1,2,4. These pro-resolving autacoids also stimulate the clearance of debris, apoptotic cells and bacteria, promoting homeostasis1,2,7.

One of the key steps during resolution of inflammation is an increase in local vascular permeability leading to edema8 and the transport of n-3 essential fatty acids (EFA) from blood to the site of inflammation. n-3 EFA are linked with protective actions in a number of inflammatory conditions including rheumatoid arthritis9, neurological disorders10 and cardiovascular disease11. At the site of inflammation, n-3 EFA are converted to novel potent mediators by exudate leukocytes that promote inflammation-resolution1,2,12. The E-series resolvins, e.g. resolvin (Rv) E₁, are produced from 7Z,8Z,11Z,14Z,17Z-eicosapentaenoic acid (EPA). The D-series resolvins, which include Resolvin D₁ (7S,8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid; RvD1) and Resolvin D₂ (7S,16R,17S-trihydroxy-4Z,9E,10Z,12E,14E,19Z-docosahexaenoic acid; RvD2), the protectins13 and maresins14,15 are biosynthesized from 4Z,7Z,10Z,13Z,16Z,19Z-docosahexaenoic acid (DHA). These potent autacoids were initially identified using temporal lipidomics with self-resolving exudates13,14 and are now appreciated for their ability to stereo-selectively promote inflammation-resolution15,16,17,18. The resolvins, protectins and maresins are coined specialized pro-resolving mediators (SPM) that by definition limit further neutrophil recruitment to the site of inflammation and promote macrophage clearance of debris, apoptotic cells and bacteria19,20. In addition, the SPM exert potent actions in promoting wound repair and tissue regeneration as well as dampening inflammatory pain16,17,18. Targeted lipid mediator metabololipidomics of tissues obtained from a number of species ranging from primordial organisms such as planaria16 to humans19,20 indicates that SPM production, including RvD₁, RvE₁9,20 and maesrin 1,2,14, is evolutionarily conserved.

In mammals, alpha-linolenic acid (9Z,12Z,15Z-octadecatrienoic acid; ALA) is converted via elongation and desaturation to EPA and subsequently to DHA. An intermediate in the conversion of EPA to DHA is n-3 docosapentaenoic acid (7Z,10Z,13Z,16Z,19Z-docosapentaenoic acid; n-3 DPA)10,12,17. n-3 DPA carries 22 carbons and contains five double bonds, with the first double bond being found on carbon 7. The structural differences of n-3 DPA from EPA, DHA and n-6 docosapentaenoic acid (4Z,7Z,10Z,13Z,16Z,19Z-docosapentaenoic acid; n-6 DPA), a biochemically distinct form of DPA where the first double bond is found on carbon 4, are...
thought to confer unique biophysical properties that are of functional relevance, for example in neuronal systems22. In humans, genome-wide association studies demonstrate that elevation in circulating levels of n-3 DPA and a concomitant decrease in DHA levels are associated with single nucleotide polymorphisms in the gene encoding for the fatty acid elongase 2 (ELOVL2)23. n-3 DPA is present in a number of mammalian tissues including plasma, brain, retina and heart at levels comparable to those of EPA and DHA23. Since circulating levels of n-3 DPA in human cohorts of European, African, Hispanic and Chinese ancestry with mutations in the ELOVL2 gene are elevated23, we addressed herein whether n-3 DPA is a precursor to novel bioactive molecules. We report that endogenous n-3 DPA is converted to novel bioactive products, determined their structures and established their anti-inflammatory and pro-resolving actions.

**Results**

n-3 docosapentaenoic acid products exert potent anti-inflammatory and tissue-protective actions in vivo. Since even minor changes in the structural properties of EFA are of functional significance23, we first investigated whether n-3 DPA, products that contain one fewer double bond than those derived from DHA (Fig. 1a), produced by exudate leukocytes exerted protective actions during acute inflammation. For this purpose, we employed a model of surgery-induced second organ injury, the murine hind limb ischemia reperfusion model (Fig. 1b)2. Administration of an isolated mixture obtained via solid-phase extraction (see Methods) of the n-3 DPA products 10 min prior to onset of reperfusion led to protection from secondary organ injury as evidenced by reduction in lung tissue damage (Fig. 1c) and decrease in the number of infiltrated leukocytes into the lungs (−45%, p < 0.05). These actions were comparable to protection afforded by the DHA-derived pro-resolving mediator RvD1 (Fig. 1c, d).

Assessment of whole blood neutrophil-platelet aggregates in these mice, a marker of systemic inflammation24, following administration of n-3 DPA products or RvD1 gave a significant reduction (55–65%) in the levels of platelet-leukocyte aggregates found 2 h post reperfusion (Fig. 1e).

Using targeted lipid mediator metabololipidomics, we next assessed whether these n-3 DPA products also regulated pro-inflammatory eicosanoid biosynthesis following ischemia reperfusion. Administration of these products led to a significant reduction in plasma prostanoid levels including PGE2 (−75%) and thromboxane B2 (TxB2) (−80%); Fig. 1f). Here we also found a significant reduction in plasma LTB4 (−60%) levels along with a decrease in levels of its double di-oxygenation isomer 5S,12S-diHETE (−75%; Fig. 1g). Of note the n-3 DPA products displayed equal or higher potency at regulating plasma eicosanoid levels when compared to RvD1 (Fig. 1f, g). These results demonstrate that n-3 DPA products possess potent anti-inflammatory and tissue-protective actions, regulating leukocyte recruitment, pro-inflammatory mediator biosynthesis and systemic inflammation.

Targeted metabololipidomics of plasma following ischemia reperfusion injury. Having found that n-3 DPA products display potent actions during ischemia reperfusion, we next investigated the role of endogenous n-3 DPA derived in the control of acute inflammation. First we assessed the plasma levels of unesterified arachidonic acid (AA), eicosapentaenoic acid (EPA), n-3 docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in mice that were not subjected to an inflammatory stimulus. Plasma AA levels were 573.0 ng/ml, EPA levels were 116.2 ng/ml, n-3 DPA levels were 66.3 ng/ml and DHA levels were 146.1 ng/ml. Following ischemia reperfusion injury, circulating values for all 4 PUFA were elevated, with levels for n-3 DPA increasing −12 times to those found in uninjured mice (Fig. 2a).

We next sought evidence for the conversion of n-3 DPA to bioactive mediators during acute inflammation. Using lipid mediator metabololipidomics and monitoring the precursor ion m/z 345 in Q1 and the product ion m/z 327 in Q3, we found three major products eluting in LC-peaks (Fig. 2b). Analysis of the tandem mass spectra (MS-MS) for the products under each peak demonstrated that peak I, at retention time (Rt) = 17.1 min, corresponded to 17-HDPA (Supplementary Fig. 1a), the peak at Rt = 17.3 min (peak II) to 14-HDPA (Supplementary Fig. 1b) and peak III at Rt = 17.6 min corresponded to 7-HDPA (Supplementary Fig. 1c). Quantification of these novel products by multiple reaction monitoring (MRM) in the plasma of uninjured mice and mice subjected to ischemia reperfusion demonstrated that all three products were elevated following ischemia reperfusion injury (Fig. 1c). These results demonstrate that during acute inflammation, systemic n-3 DPA levels are elevated, and this n-3 EFA is converted to novel oxygenated products.

Chiral metabololipidomics of endogenous n-3 DPA products. In order to elucidate the biosynthetic origins of these novel n-3 DPA-derived products we designed chiral metabololipidomic profiling of the n-3 DPA products generated in vivo. Reverse-phase chiral LC-MS-MS metabololipidomics of plasma samples obtained from mice subjected to ischemia reperfusion injury achieved baseline separation of 7R/S-HDPA, 14R/S-HDPA and 17R/S-HDPA (Supplementary Fig. 2). Quantification of the two isomers for each of the products identified demonstrated that the R to S ratio for all of the monohydroxy products identified was ~20%:~80%. These results indicated that the conversion of the R3-DPA to these novel products is enzymatically regulated, since mammalian lipoxygenases are known to insert molecular oxygen in predominantly the S configuration3.

Targeted metabololipidomics during onset and resolution of acute inflammation uncovers novel n-3 docosapentaenoic acid products. Having found that n-3 DPA is converted in vivo to yield monohydroxy acids that predominantly possess the S chirality, we next investigated whether these n-3 DPA monohydroxy products were precursors and/or pathway markers for the biosynthesis of bioactive mediators. To this end, we employed targeted LM metabololipidomics with plasma from mice subjected to ischemia reperfusion injury. Multiple reaction monitoring of m/z 377 in Q1 and m/z 143 in Q3 yielded two peaks, the first at Rt = 11.6 min and the second at Rt = 12.1 min (Fig. 3a). Inspection of the MS-MS spectrum for the product eluting in peak at RT 11.6 min demonstrated that this material corresponded to RvD2n-3 DPA with the following characteristic ions assigned: m/z 307, m/z 279, m/z 249, m/z 233, and m/z 143 (c.f. Supplementary Fig. 3a, b); while assessment of the MS-MS spectrum for the products at RT = 12.1 min demonstrated that this material corresponded to RvD1n-3 DPA (Fig. 3b). Multiple reaction monitoring of m/z 361 in Q1 and m/z 249, m/z 233, and m/z 143 (Fig. 3a). Assessment of MS-MS spectra for the product with Rt = 13.6 min gave characteristic fragmentation corresponding to RvD5n-3 DPA (Fig. 3c). MS-MS fragmentation for the material at RT = 13.7 min demonstrated characteristic fragmentation corresponding to PD1n-3 DPA (Fig. 3d). The peak eluting at RT = 14.4 min was identified as PD2n-3 DPA with the following characteristic ions assigned: m/z 233, m/z 247, m/z 189 (c.f. Supplementary Fig. 4c, d). These findings demonstrate that n-3 DPA is converted to novel products that are cognate to pro-resolving mediators from DHA; therefore, we employed the nomenclature from the DHA bioactive metabolome to describe each of these new structures.

Since vascular leakage during inflammation supplies the site of inflammation with PUFA4, we next investigated tissue levels of n-3 DPA products during the onset and resolution of inflammation. For this purpose, we applied a self-limited model of inflammation where, following the administration of a pro-inflammatory stimulus into the
In mouse peritoneum, there is a rapid recruitment of neutrophils into the site that peaks at around 6 h (Tmax; Fig. 4a). This is followed by a decline in neutrophil numbers over the next 18 h. The time difference between Tmax and the point where neutrophil numbers reach 50% of maximum (T50) is defined as the resolution interval (RI)\textsuperscript{25}. Using LM metabololipidomics, we profiled the levels of LTB4 and PGE2 that were rapidly produced during the initiation phase of the inflammatory response concomitant with neutrophil infiltration into the tissue (Fig. 4b). Maximal LTB4 levels coincided with peak neutrophil infiltration, whereby LTB4 levels rapidly subsided over the next 8 h. PGE2 levels were also elevated early in the initiation phase of the response, with levels for this mediator persisting into the
Figure 2 | n-3 DPA levels increase in acute inflammation and is converted to novel products in vivo. Mice were subjected to ischemia reperfusion injury (see Methods for details) at 2 h of reperfusion, blood was collected via cardiac puncture and plasma was obtained by centrifugation, products were extracted and monohydroxy n-3 DPA levels were assessed by lipid mediator metabololipidomics. (a) Plasma polyunsaturated fatty acid levels; (b) Representative chromatographs obtained by Multiple Reaction Monitoring (MRM) of the parent ion (Q₁) m/z 345 and a diagnostic daughter ion (Q₃) m/z 327. (c) Monohydroxy-containing levels in plasma of sham mice and mice subjected to I/R. Results for a and c are mean ± SEM. n = 4. Results for b are representative of n = 4. *P < 0.05; **P < 0.01; ***P < 0.01 vs. sham mice.
resolution phase. In this experimental setting, which characterizes the initiation and resolution phases of the inflammatory response, we determined the profile of novel n-3 DPA products in order to temporally stage each of these new products within the self-limited inflammatory response. Here we found that endogenous production of RvD1n-3 DPA and PD2n-3 DPA displayed a bi-phasic profile, reaching a maximum during peak neutrophil infiltration and late into resolution (Figure 4c, d). PD1n-3 DPA, MaR2n-3 DPA and MaR3n-3 DPA levels were each found to reach a maximum at the 4h interval and gradually decreased over the next 20 h (Figure 4d, e). The peak in exudate RvD2n-3 DPA levels coincided with the onset of resolution (the point where PMN levels reach \( \frac{1}{2} \) of \( T_{\text{max}} \)). RvD5n-3 DPA levels were found to gradually increase over the course of inflammation-resolution, with a maximum being reached late in the resolution phase. The n-3 DPA product corresponding to MaR1n-3 DPA gave levels that were elevated in the peritoneum of naive mice, whereupon challenge with zymosan these levels drastically decreased. Also, MaR1n-3 DPA accumulated late during resolution (Figure 4e). These results establish the endogenous production of novel n-3 DPA resolvins, protectins and maresins during acute inflammation and stage their formation primarily within the self-limited inflammatory response.

Specialized pro-resolving mediators from n-3 DPA exert potent anti-inflammatory actions in vivo. We next tested whether these novel structures carried bioactivity. Intravenous administration of 100 ng of RvD1n-3 DPA and RvD2n-3 DPA significantly reduced neutrophil recruitment into the peritoneum (A; \( \sim 45\% \), Fig. 5a). In these experiments, we also found that the novel tri-hydroxy-containing n-3 DPA products significantly reduced exudate levels of the pro-inflammatory cytokines Interleukin (IL) 6 (\( \sim 55\% \); Fig. 5b) and monocyte chemoattractant protein (MCP)-1 (\( \sim 55\% \); Fig. 5c). Administration of the di-hydroxy-containing n-3 DPA products from both 17-hydroperoxy-DPA (HpDHA; RvD5n-3 DPA and PD1n-3 DPA; B; \( \sim 47\% \)) and 14-HpDPA (MaR1n-3 DPA and MaR2n-3 DPA; C; \( \sim 50\% \)) also significantly reduced PMN recruitment and pro-inflammatory cytokine levels in these exudates (Fig. 5a–c).

Human leukocytes produce n-3 DPA immunoresolvents. Having found that these products are produced in murine systems, we next sought evidence for their production by human leukocytes. Assessment of methyl formate fractions obtained from activated human peripheral blood neutrophils incubated with n-3 DPA by lipid mediator metabololipidomics profiling gave products that displayed chromatographic and MS-MS spectra consistent with resolvins, protectins and maresins that carry the n-3 DPA backbone (Fig. 5a). Assessment of the UV absorbance spectra and MS-MS fragments for each of the products gave fragment ions that

**Figure 3** Identification of novel endogenous n-3 DPA pro-resolving mediators. Mice were subjected to ischemia reperfusion injury (see Methods and Fig. 2 for details). Two h into reperfusion, blood was collected and lipid mediators identified by lipid mediator metabololipidomics. (a) Representative chromatographs obtained by Multiple Reaction Monitoring of the parent ion (Q1) and a diagnostic daughter ion (Q3) in the MS-MS of n3-DPA resolvins, protectins and maresins. Representative MS-MS spectra used for identification of (b) RvD1n-3 DPA, (c) RvD5n-3 DPA, and (d) PD1n-3 DPA. Results are representative of \( n = 4 \).
were consistent with RvD2n-3 DPA with m/z 377 [M-H], m/z 359 [M-H-H2O] and m/z 333 [M-H-CO2] (Fig. 5a and Supplementary Fig. 3a, b). Additional diagnostic ions were identified at m/z 247, m/z 143 and m/z 279 consistent with the presence of hydroxy groups at carbon positions 7, 16 and 17. In the tri-hydroxy chromatographic regions, we also identified RvD1n-3 DPA as demonstrated by its characteristic fragmentation pattern and UV absorbance spectra, consistent with hydroxyl groups at the carbon 7, 8 and 17 positions (Fig. 3a, b and Supplementary Fig 3c, d). Assessment of the UV absorbance and MS-MS spectra in the di-hydroxy region.

Figure 4 | Self-limited inflammation: Endogenous formation of novel immunoresolvents from n-3 DPA. Mice were treated with 0.1 mg zymosan i.p.; after the indicated time intervals peritoneal exudates were collected. (a) Exudate leukocyte counts obtained by light microscopy and flow cytometry. Exudate levels for (b) prostaglandin (PG) E2 and leukotriene (LT) B4; (c) resolvins, (d) protectins and (e) maresins were measured by lipid mediator metabololipidomics. Results are mean ± SEM. n = 4 mice per time point.
macrophages produce resolvins, protectins and maresins from endogenous n-3 DPA (n = 3). These findings demonstrate that human leukocytes can convert both exogenous as well as endogenous n-3 DPA to novel n-3 DPA immunoresolvents.

**n-3 DPA products exert anti-inflammatory and pro-resolving actions on human leukocytes.** We next investigated whether these novel n-3 DPA products retained their leukocyte directed anti-inflammatory and pro-resolving actions when incubated with human leukocytes. A key step in neutrophil recruitment to the site of inflammation is firm adhesion to the vascular endothelium\(^26\). Incubation of neutrophils with RvD1\(_{n-3}\) DPA and RvD2\(_{n-3}\) DPA, (A; ~30%), RvD5\(_{n-3}\) DPA and PD1\(_{n-3}\) DPA, (B; ~25%) or PD1\(_{n-3}\) DPA and PD2\(_{n-3}\) DPA, (C; ~22%) led to a significant reduction in neutrophil adhesion to TNF-\(\alpha\) activated endothelial cells to a similar extent as RvD2 (~30%; Fig. 7a), a potent pro-resolving mediator\(^7\).

Neutrophil-endothelial cell adhesion is mediated by adhesion molecules expressed on both the endothelial and neutrophil surface that are up-regulated during inflammation\(^26\). One of these adhesion molecules is Intercellular Adhesion Molecule 1 (ICAM-1/CD54) expressed on endothelial cells\(^8\). Incubation of each of the n-3 DPA products with endothelial cells prior to incubation with TNF-\(\alpha\) also led to a significant reduction in endothelial cell ICAM-1 expression (~25–40%; Fig. 5b).

We next questioned whether the n-3 DPA pro-resolving mediators regulated human peripheral blood leukocyte recruitment to a chemotactic stimulus. Incubation of human leukocytes with RvD2 led to a significant reduction in neutrophil (~42%) recruitment towards an IL-8 gradient. When human neutrophils were incubated with RvD1\(_{n-3}\) DPA and RvD2\(_{n-3}\) DPA (1 nM), we also found a significant reduction in neutrophil chemotaxis (~45%) towards an IL-8 gradient (Fig. 7b). Incubation of human neutrophils with 1 nM of the di-hydroxy containing products (i.e. RvD5\(_{n-3}\) DPA and PD1\(_{n-3}\) DPA; C, or MaR1\(_{n-3}\) DPA and MaR2\(_{n-3}\) DPA, D) also led to a significant reduction in neutrophil chemotaxis (~40–75%) towards IL8 (Fig. 7b).

Because macrophage clearance of cellular debris and apoptotic cells is a critical process in promoting the resolution of acute inflammation\(^9\), we next incubated human monocyte-derived macrophages with RvD2, which led to a significant increase in the uptake of opsonized zymosan particles (~50%; Fig. 7c). Addition of RvD5\(_{n-3}\) DPA and PD1\(_{n-3}\) DPA (1 nM) to macrophages each also led to a significant increase (~70%) in macrophage phagocytosis of fluorescently labeled zymosan (Fig. 7c). Macrophage incubations with MaR1\(_{n-3}\) DPA and MaR2\(_{n-3}\) DPA, (Fig. 7c) also gave significant increases in macrophage uptake of fluorescently labeled zymosan (Fig. 7c). These results demonstrate that the novel n-3 DPA mediators exert potent anti-inflammatory and pro-resolving actions with human leukocytes limiting human neutrophil recruitment and macrophage phagocytosis, key processes in promoting resolution and by definition those assigned to pro-resolving mediators\(^9\).

**Discussion**

In the present report, using lipid mediator metabololipidomics, we identified novel n-3 DPA-derived products and staged their biosynthesis during self-limited inflammation in inflammatory exudates. With murine models of acute inflammation and human leukocytes, we determined their anti-inflammatory, pro-resolving and tissue protective actions, defining these novel n-3 DPA products as immunoresolvents.

In humans, circulating n-3 DPA levels do not appear to be directly associated with dietary intake, unlike other n-3 EFA including DHA and EPA\(^23,29\), thus suggesting in humans a primary endogenous metabolic origin for n-3 DPA. Along these lines, a recent genome wide association study with more than 8500 participants from various ethnicities demonstrated that elevated plasma n-3 DPA levels are

---

**Figure 5** n-3 DPA immunoresolvents display potent anti-inflammatory actions in vivo. The indicated n-3 DPA-derived product mixtures were administered by intravenous injection 10 min prior to the intraperitoneal administration of zymosan (0.1 mg, 500 \(\mu\)l PBS) to 6–8-week-old male FvB mice. At 4 h, peritoneal exudates were collected and the (a) number of infiltrated neutrophils was assessed by light microscopy and flow cytometry. Exudate levels for the pro-inflammatory mediators (b) IL6 and (c) MCP-1 were determined by cytokine array. The ratio of RvD1\(_{n-3}\) DPA to RvD2\(_{n-3}\) DPA was ~3:1 (A), the ratio of MaR1\(_{n-3}\) DPA to MaR2\(_{n-3}\) DPA was ~4:1 (B); the ratio of RvD5\(_{n-3}\) DPA to PD1\(_{n-3}\) DPA was ~9:1 (C). Results are mean ± SEM. n = 4, * \(P < 0.05\), ** \(P < 0.01\) vs. zymosan mice.

---

**The indicated n-3 DPA-derived product mixtures were administered by intravenous injection 10 min prior to the intraperitoneal administration of zymosan (0.1 mg, 500 \(\mu\)l PBS) to 6–8-week-old male FvB mice. At 4 h, peritoneal exudates were collected and the (a) number of infiltrated neutrophils was assessed by light microscopy and flow cytometry. Exudate levels for the pro-inflammatory mediators (b) IL6 and (c) MCP-1 were determined by cytokine array. The ratio of RvD1\(_{n-3}\) DPA to RvD2\(_{n-3}\) DPA was ~3:1 (A), the ratio of MaR1\(_{n-3}\) DPA to MaR2\(_{n-3}\) DPA was ~4:1 (B); the ratio of RvD5\(_{n-3}\) DPA to PD1\(_{n-3}\) DPA was ~9:1 (C). Results are mean ± SEM. n = 4, * \(P < 0.05\), ** \(P < 0.01\) vs. zymosan mice.**
associated with single nucleotide polymorphisms in the genes encoding for the fatty acid elongase 2 (ELOVL2) and glucokinase regulatory protein (GCKR)\(^2\). This increase in n-3 DPA levels is also associated with a reduction in circulating DHA levels. Of interest, circulating n-3 DPA has been associated with protection from cardiovascular disease\(^{30-32}\). Hence, our present findings indicating that n-3 DPA is a precursor to new potent bioactive products may have wide implications in individuals carrying elevated circulating levels of n-3 DPA.

Ischemia-reperfusion injury is of considerable consequence in the pathology of many diseases including periodontal disease, arthritis and stroke as well as being of relevance during surgical procedures, in particular involving extremities, causing aberrant leukocyte activation that results in local and remote tissue and organ damage\(^{33}\). Neutrophils in these settings play pivotal roles in the perpetuation of reperfusion injury giving rise to second organ injury\(^{33}\). However, phagocytes, including neutrophils when appropriately activated, are also instrumental in orchestrating resolution processes via their temporal production of pro-resolving mediators in resolving exudates\(^{14,15,34,35}\). Hence our findings indicating that n-3 DPA products obtained from phagocytes collected from self-resolving exudates display potent protective actions from second organ injury during ischemia reperfusion have implications in a wide range of human pathologies. Indeed these products markedly reduced both local tissue damage and neutrophil infiltration into the lungs (Fig. 1), a hallmark of second organ injury, to a similar extent as the DHA-derived resolvin D1, a known potent pro-resolving mediator\(^{9}\).

The formation of platelet-leukocyte aggregates in the vasculature is also a component of many inflammatory disorders including stroke, sepsis and hypertension. Formation of these microcellular aggregates enhances the production of a number of pro-inflammatory cytokines including IL-8 and MCP-1 as well as increases the levels of platelet aggregating factor, a potent pro-inflammatory lipid mediator (for a review, see ref. 24). In this context, elevated levels of circulating platelet-leukocyte aggregates are proposed as an early marker for acute myocardial infarction and are increasingly regarded as a cardiovascular risk factor\(^{36}\). In the present studies, administration of phagocyte-derived n-3 DPA products led to a reduction in circulating platelet-neutrophil aggregates following ischemia.

Related figure image

Figure 6 | Human neutrophils produce novel n-3 DPA-derived immunoresolvents. Human neutrophils were prepared from peripheral blood (see Methods for details), suspended in DPBS (80 × 10^6/ml) and incubated with serum treated zymosan (0.1 mg) and n-3 DPA (1 µM, 30 min, 37°C, pH 7.45); incubations were stopped with ice-cold methanol and products assessed by lipid mediator metabololipidomics. (a) Representative chromatographs obtained by Multiple Reaction Monitoring of the parent ion (Q1) and a diagnostic daughter ion (Q3) in the MS/MS. Representative MS/MS spectra used for identification of (b) RvD1_{n-3 DPA} and (c) RvD5_{n-3 DPA}. Results are representative of n = 4.
reperfusion injury. In addition, we also found a substantial reduction in plasma pro-inflammatory eicosanoid levels including LTB4 and TxB2 (Fig. 1), actions that were comparable to those afforded by DHA-derived RvD1. Therefore, these results demonstrate that n-3 DPA products display potent systemic anti-inflammatory and tissue protective actions.

Phagocytes carry lipoxygenase enzymes that are involved in the biosynthesis of pro-resolving mediators. These enzymes convert their substrate in a stereospecific manner, inserting molecular oxygen predominantly in the S configuration. In the present study we found that the systemic levels of n-3 DPA and the new n-3 DPA products (i.e. 17-HDPA, 14-HDPA and 7-HDPA) were each elevated during acute inflammation in vivo (Fig. 2). In addition, chiral lipidomics of these n-3 DPA products demonstrated that the hydroxy groups in these products were predominantly in the S configuration. The ratio of RvD1n-3 DPA to RvD2n-3 DPA (A) was ~3:1; the ratio of RvD5n-3 DPA to PD1n-3 DPA (B) was ~9:1; the ratio of PD1n-3 DPA to PD2n-3 DPA (C) was ~1:5; the ratio of MaR1n-3 DPA to MaR2n-3 DPA (D) was ~4:1. Results are mean ± SEM. n = 4 independent neutrophil and macrophage preparations (*p < 0.05, **p < 0.05 vs. vehicle incubated cells). Bar = 50 μM.
In light of the results from the present studies in conjunction with earlier mechanisms proposed for the biosynthesis of the D-series resolvins, protectins\textsuperscript{15,34} and maresins\textsuperscript{35}, the pathways for the novel n-3 DPA immunoresolvent are illustrated in Fig. 8. In this proposed scheme, n-3 DPA is first converted via 17 lipoxygenation to 17-hydroperoxy-8\textsuperscript{Z},10\textsuperscript{Z},13\textsuperscript{Z},15\textsuperscript{E},19\textsuperscript{Z}-docosapentaenoic acid (17-HpDPA). This intermediate can next undergo a second lipoxygenation by 5-lipoxygenase to yield 14-HpDPA that is further converted to an epoxide intermediate and then enzymatically hydrolyzed to MaR\textsubscript{1n-3DPA} and/or MaR\textsubscript{2n-3DPA}. 14-HpDPA can also undergo a second oxygenation at the omega - 1 position to yield the MaR\textsubscript{3n-3DPA}. Note that each product is depicted in the 17S and 14\textsuperscript{S} configuration based on the results obtained from chiral lipidomics that indicated S as the predominant form of each but may also carry 17R as well as 14R chirality from lipoxygenase reactions as these lesser components (see Supplementary Fig. 2 and text for details). The complete stereochemistries of these novel mediators remain to be established and are depicted in their likely configuration based on biogenic synthesis (see Supplementary Figures 3–5 for retention times, UV and MS-MS for each of these mediators).

Figure 8 | Biosynthetic schemes proposed for novel n-3 docosapentaenoic acid products and their actions. At the site of injury, n-3 DPA is converted to (a) 17-HpDPA that undergoes further conversion by lipoxygenation to the n-3 DPA resolvins. 17-HpDPA is also a substrate for enzymatic conversion to an epoxide intermediate that is next enzymatically hydrolyzed to the n-3 DPA protectin structures. (b) n-3DPA is also converted to 14-lipoxygenation to yield 14-HpDPA that is further converted to an epoxide intermediate and then enzymatically hydrolyzed to MaR\textsubscript{1n-3DPA} and/or MaR\textsubscript{2n-3DPA}. 14-HpDPA can also undergo a second oxygenation at the omega - 1 position to yield the MaR\textsubscript{3n-3DPA}. Note that each product is depicted in the 17S and 14\textsuperscript{S} configuration based on the results obtained from chiral lipidomics that indicated S as the predominant form of each but may also carry 17R as well as 14R chirality from lipoxygenase reactions as these lesser components (see Supplementary Fig. 2 and text for details). The complete stereochemistries of these novel mediators remain to be established and are depicted in their likely configuration based on biogenic synthesis (see Supplementary Figures 3–5 for retention times, UV and MS-MS for each of these mediators).
enzymatic hydrolysis to either 10,17-di-hydroxy-7Z,11,13,15,19Z-docosapentaenoic acid (PD103,3DPA) or 16,17-di-hydroxy-7Z,10,13,14,19Z-docosapentaenoic acid (PD163,3DPA). In a parallel pathway, the arachidonate 12-lipoxygenase converts n-3 DPA to 14-hydroperoxy-7Z,10 Z,12E,16Z,19Z-docosapentaenoic acid (14-HpDPA) that is further converted to an epoxide intermediate and then enzymatically hydrolyzed to yield 7,14-di-hydroxy-8,10,12,16Z,19Z-docosapentaenoic acid (MaR143,3DPA) or 13,14-di-hydroxy-7Z,9,11,16Z,19Z-docosapentaenoic acid (MaR134,3DPA). Alternatively, this 14-HpDPA can undergo a second oxygenation at the omega-1 position to yield 14, 21-di-hydroxy-7Z,10Z,12E,16Z,19Z-docosapentaenoic acid (MaR14213,3DPA). Of note, since we identified the S isomer of both 17-HDPA and 14-HDPA, the reduction products of 17-HDPA and 14-HDPA, as the major products in inflamed tissues (Supplementary Fig. 2), it is highly likely that the stereochemistry at these positions is retained in the biosynthesis of the novel n-3 DPA-derived resolvins, protectins and maresins. Of note, it is conceivable that the R-containing diastereomers of the n-3 DPA resolvins, protectins and maresins may also be of biological relevance in inflammation-resolution, since they were also obtained via lipoxigenation reaction albeit to lesser proportions than their corresponding R-containing products (see Supplementary Fig. 2).

Excessive neutrophil activation and infiltration to the inflamed site can be detrimental since it may lead to further tissue damage and propagation of the inflammatory response. When administered in vivo, RvD1n33DPA, RvD2n33DPA, RvD5n33DPA, PD1n33DPA, MaR1n33DPA and MaR2n33DPA each reduced neutrophil infiltration in murine peritonitis (Fig. 5). These mediators also demonstrated potent cytokine counter-regulatory actions reducing exude levels of IL-6 and MCP-1 (Fig. 5) to levels comparable with the pro-resolving mediator RvD2Z. The novel n-3 DPA immunoresolvents were also found to exert potent anti-inflammatory actions with human neutrophils and endothelial cells regulating central steps in the leukocyte recruitment cascades, reducing neutrophil chemotaxis and adhesion to endothelial cells (Fig. 7) as well as expression of the adhesion molecule ICAM-1 by endothelial cells (Supplementary Fig. 6). Importantly, these mediators were found to enhance macrophage phagocytosis, a key process in accelerating the onset or resolution (Fig. 7)49,50. Thus, the actions for each of these novel n-3 DPA products are in accord with the key characteristic that define an immunoresolvent, a property shared with their DHA resolution metabolome SPM counterparts.

Since mammals lack enzymes that can insert double bonds in either the n-3 or n-6 position to polysaturated fatty acids, the precursor molecules for the production of n-3 and n-6 essential fatty acids, namely alpha linolenic acid (ALA) and linoleic acid (9Z,12Z-octadecadienoic acid), must be obtained via dietary intake51. In mammalian tissues, ALA is converted by elongation and desaturation to EPA and DHA. Linoleic acid on the other hand is converted, in a parallel pathway in humans, to arachidonic acid and subsequently to n-6 DPA52,53. Thus, while n-3 DPA and n-6 DPA each share the carbon 22 and five unsaturated double bonds, their structures are different because they are produced via separate biosynthetic pathways from chemically dissimilar precursors and each possesses distinct biophysical properties54. Earlier, oxygenated products obtained from n-6 DPA were found to reduce neutrophil recruitment in a model of dermal inflammation, enhance macrophage phagocytosis55, reduce ear swelling in a model of delayed type hypersensitivity56 and protect against intestinal damage in a mouse model of intestinal inflammation57, albeit displaying lower potency than the n-3 pro-resolving mediators as well as compared to the new n-3 DPA mediators described herein. This is in accord with the finding that even subtle differences in the stereochemistry of lipid mediators can result in dramatic changes in their potency.

In summary, herein we established the structures of new n-3 DPA-derived products, their formation from endogenous sources, staged the production of each of these novel mediators during inflammation resolution and determined their anti-inflammatory, pro-resolving and tissue protective properties. These actions are characteristic of specialized pro-resolving mediators27 and define these novel n-3 DPA products as immunoresolvents. In view of the role of lipid mediators in inflammation and its timely resolution49,50,58, the n-3 DPA metabolome documented herein may mediate some of the beneficial actions associated with probiotic dietary supplementation23. Moreover, these new n-3 immunoresolvents may also serve as a compensatory mechanism in people with elevated n-3 DPA levels and lower circulating DHA levels23 to compensate for losses in DHA SPM-regulated leukocyte-mediated tissue damage and timely resolution of acute inflammation.

Methods

Materials. Zymosan A, bovine serum albumin (BSA), Roswell Park Memorial Institute media 1640 (RPMI 1640), DPBS and Histopaque 1077-1 were purchased from Sigma-Aldrich. Rat anti-mouse Ly6G (clone 1A8; BD Biosciences); rat anti-mouse F4/80 (clone: BM8); CD11b (clone: Mac-1) and CD41 (clone: eBiomWReg30) were from eBioscience. Human recombinant granulocyte-macrophage colony stimulating factor (GM-CSF) and LC grade solvents were purchased from Fisher Scientific; Agilent Eclipse E18 (4.6 mm × 100 mm × 1.8 μm; 4.6 mm × 50 mm × 1.8 μm) column; C18 SPE columns (Biotage); Fluorescently conjugated Zymosan A (Invitrogen); LC-MS-MS quantification and deuterated internal standards (d5-SS-hydroxyicosatetraenoic acid (d5-SS-HETE), d4-LTB4, d4-lipoxygen (d4-LLOX), d3-PGE2, RvD1, RvD2, Cayman Chemicals).

Animals. All animals used in the present study were male FVB mice (Charles River Laboratories) that were 6–8 weeks old (weighing 20–25 g). They were maintained in a temperature- and light-controlled environment and had unlimited access to water and food (laboratory standard rodent diet 5001 (Lab Diet)), containing 1.5% eicosapentaenoic acid, 1.9% DHA of total fatty acids. Experiments were performed in accordance with the Harvard Medical School Standing Committee on Animals guidelines for animal care (Protocol 062570).

n-3 DPA-derived mediator preparations. Exudates were obtained from mice 12 h after zymosan administration and incubated with n-3 DPA in DPBS (1 μM, 45 min, 37°C, pH 7.45); the incubations were stopped using 2 volumes of ice-cold methanol and products extracted as outlined in the sample extraction and lipid mediator metabololipidomics section below.

145-HpDPA was prepared from n-3 DPA (∼15 µM) incubated with 5.4 U/ml isolated 12-lipoxygenase (LOX) (porcine) (0.05 M phosphate buffer, 0.02% Tween 20, pH 7.4). 145-HpDHA was isolated via RP-HPLC (1100 Series; Agilent Technologies) using a C18 column and a mobile phase consisting of methanol/water (60:20 vol/vol) at 0.5 ml/min that was ramped up to 98:2 vol/vol over for 20 min. Reduction with NaBH4 yielded 145-HDPA used for mass spectrometry standard. In determined incubations 145-HpDPA was incubated with human macrophages (40 × 105/ml) or neutrophils (80 × 105/ml) in PBS (containing calcium and magnesium) and serum treated zymosan (0.01–0.1 mg/ml); the incubations were stopped after 45 min and products extracted.

175-HpDPA was prepared from n-3 DPA (∼15 µM) incubated with 100 U/ml isolated soybean-LOX (Borate buffer, 4°C, pH 9.2). 175-HpDHA was isolated via RP-HPLC. Reduction with NaBH4 yielded 175-HDPA used for mass spectrometry standard. Biogenic synthesis of the di- and tri- oxygenation products was performed with 5-LOX enzyme (200 U/ml) incubated with 175-HpDPA. In determined incubations, 175-HpDPA was added to human macrophages (40 × 105/ml) or neutrophils (80 × 105/ml) in PBS (containing calcium and magnesium) and cells were then incubated with SIZ (0.1 mg/ml) incubations; the incubations were stopped after 45 min and products extracted.

Ischemia reperfusion injury. Mice were anesthetized by intraperitoneal injection of a mixture of xylazine (80 mg/kg) and ketamine (10 mg/kg). Hind-limb ischemia was initiated using tourniquets consisting of a rubber band placed on each hind limb as in6. Ten min prior to the initiation of reperfusion n-3 DPA products (obtained as described above) or Resolvin D1 (0.5 mg) were administered by intravenous injection and compared to vehicle alone. At the end of this reperfusion period (2 h), mice were euthanized and blood collected via cardiac puncture, lungs harvested, frozen in liquid nitrogen and incubated at −80°C. Lungs were dissected and processed for histology by the Children’s Hospital Boston Core Histology Facility. The frozen lungs were homogenized from individual mice, centrifuged and the tissue myeloperoxidase (MPO) levels were determined using a mouse MPO ELISA (R&D Systems).

To investigate platelet-leukocyte aggregates in murine whole blood after ischemia reperfusion, blood was collected 2 h post reperfusion by cardiac puncture and incubated with PE conjugated rat anti-mouse Ly6G and FITC conjugated rat anti-mouse CD41 or relevant isotype controls for 30 min at 4°C. Red blood cells were lysed.
using ice-cold red-blood cell lysus buffer (BD Biosciences) and cells fixed with 1% formalin prior to analysis by a BD Canto II. Data was analyzed using FlowJo (TreeStar Inc.).

**Sample extraction and lipid mediator metabolomics.** All samples for LC-MS/MS analysis were extracted on SPE columns as in Ref. 41. Prior to extraction, 500 pg of deuterium-labelled internal standards (Waters, Inc.). d5-LTB4, d5-LXAn and d5-PGE2 were added to facilitate quantification of sample recovery.

The LC-MS-MS system, Qtrap 5500 (ABScied), was equipped with an Agilent HP1100 binary pump and diode-array detector (DAD). An Agilent Eclipse Plus C18 column (100 mM × 4.6 mm × 1.8 µm) was used with a gradient of methanol/water/acetic acid of 60: 40: 0.01 (v/v/v) to 100: 0.01 at 0.4 ml/min flow rate. To monitor and quantify the levels of the various LM, a multiple reaction monitoring (MRM) method was developed with signature ion fragments for each molecule. Identification was conducted using published criteria with at least six diagnostic ions. Calibration curves were obtained using synthetic LM mixture (d5-SS-HETE, d5-LTB4, d5-LXAn, d5-PGJ2, d5-PGE2, d5-PGF2a, RvD1, RvD2, RvDS. Protexin (PDI), Mareins 1 (MaR1), 17-hydroxydocosahexaenoic acid (17-HDHA), 14-hydroxydocosahexaenoic acid (14-HDHA) and 7-hydroxydocosahexaenoic acid (7-HDHA) at 1, 10, 100, 275 pg. Linear calibration curves for each were obtained with r2 values in the range 0.98–0.99. Quantification was carried out based on area peak area of the Multiple Reaction Monitoring (MRM) transition and the linear calibration curve for each compound. Where calibration curves for a structurally related DHA-derived product were not available (14.21-dHDP A, 13,14-dHDP A and 16,17-dHDP A), levels were monitored using a compound with similar physical properties.

For chiral lipidomic analysis, a Chiralpak AD-RH column (100 mM × 2.1 mm × 5 µm) was used with isocratic methanol/water/acetic acid 95: 5.01: 0.01 (v/v/ml). To monitor isotopic monohydoxy docosapentaenoic acid levels, a multiple reaction monitoring (MRM) method was developed using signature ion fragments for each compound.

**Zymosan peritonitis.** Zymosan (0.1 mg) was injected intraperitoneally (i.p.) in 1 ml of sterile saline. Exudates were collected at 0, 4, 12 and 24 h post zymosan. Leukocyte numbers and differential counts in the peritoneal exudates were determined as in 46. In designated experiments, mice were administered intravenously (i.v.) vehicle (saline containing 0.1% EtOH), or the indicated mixture of n-3 DPA-derived products at 100 ng/mouse 5 min prior to i.p. zymosan administration (0.1 mg). After 4 h the exudates were collected and the number of extravasated neutrophils determined using Turko's solution and flow cytometry as above.

**Neutrophil isolation and chemotaxis.** Peripheral blood neutrophils were obtained from healthy volunteers as in Ref. 17. Briefly, neutrophils were prepared following density gradient isolation and used as described above and labeled with CFDA-SE following staining with fluorescently conjugated mouse anti-human ICAM-1 antibody (Clone HCDC54; BioLegend) using the staining protocol.

**Macrophage preparation and phagocytosis.** Macrophages were prepared and phagocytosed was assessed as in Ref. 15. Briefly, cells were incubated with vehicle (0.1% EtOH in DPBS) or the indicated mixture of n-3 DPA products for 15 min. The HUVEC were then incubated with TNF-α (10 ng/ml, 37°C) for 4 h. At the end of these incubations, ICAM-1 levels were assessed by flow cytometry following staining with fluorescently conjugated mouse anti-human ICAM-1 antibody (Clone HCDC54; BioLegend) using the staining protocol.

**Statistics.** All results are expressed as means ± SEM. Differences between groups were compared using Student’s t-test (2 groups) or 1-way ANOVA (multiple groups) followed by post hoc Bonferroni test. The criterion for statistical significance was P < 0.05.
leukocyte adhesion molecule-1 and intercellular adhesion molecule-1. 

29. Sun, Q., Ma, J., Campos, H., Rexrode, K. M., Albert, C. M. & Mozaffarian, D. et al. Blood concentrations of individual long-chain n-3 fatty acids and risk of nonfatal myocardial infarction. *Am J Clin Nutr* **88**(1), 216–223 (2008).

30. Khaw, K. T., Friesen, M. D., Riboli, E., Luben, R. & Wareham, N. Plasma phospholipid fatty acid concentration and incident coronary heart disease in men and women: the EPIC-Norfolk prospective study. *PLoS Med* **9**(7), e1001255 (2012).

31. Oda, E., Hatada, K., Katoh, K., Kodama, M., Nakamura, Y. & Aizawa, Y. A case-control pilot study on n-3 polyunsaturated fatty acid as a negative risk factor for myocardial infarction. *Int Heart J* **46**(4), 583–591 (2005).

32. Rissanen, T., Voutilainen, S., Nyyssonen, K., Lakka, T. A. & Salonen, J. T. Fish oil-derived fatty acids, docosahexaenoic acid and docosapentaenoic acid, and the risk of acute coronary events: the Kuopio ischaemic heart disease risk factor study. *Circulation* **102**(22), 2677–2679 (2000).

33. Qiu, F. H., Wada, K., Stahl, G. L. & Serhan, C. N. IMP and AMP deaminase in reperfusion injury down-regulates neutrophil recruitment. *Proc Natl Acad Sci U S A* **97**(8), 4267–4272 (2000).

34. Serhan, C. N. & Petasis, N. A. Resolvins and protectins in inflammation resolution. *Chem Rev* **111**(10), 5922–5943 (2011).

35. Serhan, C. N., Yang, R., Martinod, K., Kasuga, K., Pillai, P. S. & Porter, T. F. et al. Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J Exp Med* **206**(1), 15–23 (2009).

36. Furman, M. L., Dauerman, H. L., Goldberg, R. J., Yarzebski, J., Lessard, D. & Gore, J. M. Twenty-two year (1975 to 1997) trends in the incidence, in-hospital and long-term case fatality rates from initial Q-wave and non-Q-wave myocardial infarction: a multi-hospital, community-wide perspective. *J Am Coll Cardiol* **37**(6), 1571–1580 (2001).

37. Spite, M. & Serhan, C. N. Novel lipid mediators promote resolution of acute inflammation: impact of aspirin and statins. *Circ Res* **107**(10), 1170–1184 (2010).

38. Dangi, B., Obeng, M., Nauroth, J. M., Chung, G., Bailey-Hall, E. & Hallenberg, T. et al. Metabolism and biological production of resolvins derived from docosapentaenoic acid (DPAn-6). *Biochem Pharmacol* **79**(2), 251–260 (2010).

39. Chiu, C. Y., Gomolka, B., Dierkes, C., Huang, N. R., Schroeder, M. & Purschke, M. et al. Omega-6 docosapentaenoic acid-derived resolvins and 17-hydroxydocosahexaenoic acid modulate macrophage function and alleviate experimental colitis. *Inflamm Res* **61**(9), 967–978 (2012).

40. Barrett, E., Fitzgerald, P., Dinan, T. G., Cryan, J. F., Ross, R. P. & Quigley, E. M. et al. Bifidobacterium breve with alpha-Linolenic Acid and Linoleic Acid Alters Fatty Acid Metabolism in the Maternal Separation Model of Irritable Bowel Syndrome. *PLoS One* **7**(11), e48159 (2012).

41. Yang, R., Chiang, N., Oh, S. F. & Serhan, C. N. Metabolomics-lipidomics of eicosanoids and docosanoids generated by phagocytes. *Curr Protoc Immunol* Chapter 14, Unit 14 26 (2011).

Acknowledgements
The authors thank Mary Halm Small for expert assistance in manuscript preparation. This work was supported in part by the National Institutes of Health (grant P01GM095467).

Author contributions
J.D. designed and carried out experiments, analyzed data, and contributed to manuscript and figure preparations; R.A.C. carried out experiments, analyzed data, and contributed to manuscript and figure preparations; C.N.S. carried out overall experimental design, conceived the research plan, and contributed to manuscript and figure preparations.

Additional information
Supplementary information accompanies this paper at http://www.nature.com/scientificreports

Competing financial interests: A patent application is submitted assigned to Brigham and Women’s Hospital with C.N.S. and J.D. as inventors.

License: This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-nd/3.0/

How to cite this article: Dalli, J., Colas, R.A. & Serhan, C.N. Novel n-3 Immunoresolvents: Structures and Actions. *Sci. Rep.* **3**, 1940; DOI:10.1038/srep01940 (2013).