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Specialized Proresolving Mediators Enhance Human B Cell Differentiation to Antibody-Secreting Cells

Sesquile Ramon,* Fei Gao,‡ Charles N. Serhan,† and Richard P. Phipps*‡

The resolution of inflammation is an active and dynamic process critical in maintaining homeostasis. Newly identified lipid mediators have been recognized as key players during the resolution phase. These specialized proresolving mediators (SPM) constitute separate families that include lipoxins, resolvins, protectins, and maresins, each derived from essential polyunsaturated fatty acids. New results demonstrate that SPM regulate aspects of the immune response, including reduction of neutrophil infiltration, decreased T cell cytokine production, and stimulation of macrophage phagocytic activity. The actions of SPM on B lymphocytes remain unknown. Our study shows that the novel SPM 17-hydroxydocosahexaenoic acid (17-HDHA), resolvin D1, and protectin D1 are present in the spleen. Interestingly, 17-HDHA and resolvin D1, but not protectin D1, strongly increase activated human B cell IgM and IgG production. Furthermore, increased Ab production by 17-HDHA is due to augmented B cell differentiation toward a CD27⁺CD38⁺ Ab-secreting cell phenotype. The 17-HDHA did not affect proliferation and was nontoxic to cells. Increase B cell IgM and IgG production. Furthermore, increased Ab production by 17-HDHA is due to augmented B cell differentiation toward a CD27⁺CD38⁺ Ab-secreting cell phenotype. The 17-HDHA did not affect proliferation and was nontoxic to cells. Increase B cell differentiation and Ab production supports the involvement of SPM during the late stages of inflammation and pathogen clearance. The present study provides new evidence for SPM activity in the humoral response. These new findings highlight the potential applications of SPM as endogenous and nontoxic adjuvants, and as anti-inflammatory therapeutic molecules. The Journal of Immunology, 2012, 189: 000–000.

The resolution of the inflammatory response is critical to maintain homeostasis and prevent disease. Once thought of as a passive process, the resolution phase of inflammation is a multifaceted and dynamic process. Newly-identified, endogenous lipid mediators are now recognized as important players in damping inflammation. These SPM are synthesized through lipoxygenases or acetylated cyclooxygenase-2–mediated pathways. SPM constitute separate families, including lipoxins, resolvins, protectins, and maresins.

SPM play important roles during inflammation, including the inhibition of neutrophil infiltration, reduction of T cell cytokine production, and increased recruitment of monocytes with enhanced phagocytic activity. In addition, exogenous treatment with proresolving lipid mediators has been shown to alleviate symptoms in animal models of inflammatory diseases, such as colitis, periodontitis, and asthma, as well as in autoimmune disorders like arthritis.

Interestingly, SPM and key intermediates have been identified in serum and in important immunological sites, including tonsils and the bone marrow, where high numbers of B cells are present. Nevertheless, little is known about SPM role on lymphocyte function, particularly B cells, and their effect on the adaptive immune response. In this study, we asked whether SPM, particularly those found in the spleen, influence B cell function. Our initial analysis focused on several key SPM, none of which as far as we know have been evaluated for activity on human B cells. Because B cells can respond to other lipid mediators such as PGs, we asked whether certain SPM could beneficially stimulate Ab production and B cell function.

Materials and Methods

Liquid chromatography-tandem mass spectrometry–based metabolo-lipidomics

FVB/NJ mouse spleens were suspended in 1.0 ml cold methanol and gently ground, followed by protein precipitation for 12 h. Samples were next extracted by C-18 solid-phase extraction column, and methyl formate fractions were taken for liquid chromatography-tandem mass spectrometry (LC-MS/MS)–based lipidomics. LC-MS/MS was performed with an Agilent 1100 HPLC (Agilent Technologies, Santa Clara, CA) equipped with an Agilent Eclipse Plus C-18 column (4.6 mm × 50 mm × 1.8 μm) paired with an ABI Sciex Instruments 5500 QTRAP linear ion-trap triple-quadrupole mass spectrometer (Applied Biosystems, Foster City, CA). Instrument control and data acquisition were performed using AnalystTM 1.5 software (Applied Biosystems). The mobile phase consisted of methanol/water/acetic acid (55/45/0.01, v/v/v) after 10 min. 1000/0/0.01 (v/v/v) after 18 min, and 55/45/0.01 (v/v/v) after 1 min to wash and equilibrate the column. Mass spectrometry analyses were carried out in negative ion mode using multiple reaction monitoring of established specific transitions for 17-hydroxydocosahexaenoic acid (17-HDHA; m/z 343 > 245), protectin D1 (10R,17S-dihydroxy-
droxy-docosa-4Z,7Z,11E,13E,15Z,19Z-hexaenoic acid, PD1; m/z 359 > 153), and resolin D1 (75.8R,17S-trihydroxy-4Z,9E,11E,13Z,15E,19Z-docosahexaenoic acid, RdV1; m/z 375 > 215). Identification was matching retention time and diagnostic ions to synthetic standards (14).

B lymphocyte isolation

Human B cells were isolated from peripheral blood of healthy subjects under the ethical permission provided by the Research Subjects Review Board at the University of Rochester. B cells were isolated, as described (15, 16). Briefly, the buffy coat was separated and diluted in 1 × PBS. PBMCs were isolated using Ficoll-Paque (Amersham Biosciences, Piscataway, NJ) gradient centrifugation. B cells were then purified from the leukocyte layer using CD19 Dynabeads (Invitrogen, Carlsbad, CA). CD19 Dynabead-cell rosettes were disrupted using CD19 Detachable (Invitrogen). Cells obtained by this method of isolation was >98% CD19

Reagents and culture conditions

Purified CD19+ human B cells isolated from PBMCs were cultured in RPMI 1640 (Life Technologies/Invitrogen, Carlsbad, CA) supplemented with 5% FBS, 2 mM L-glutamine, 5 × 10−4 M 2-ME, 10 mM HEPES, and 50 µg/mL gentamicin. Cpg oligodeoxynucleotides (ODN) 2395 5′-TCGTCGTTTTCGGCGCGCCG-3′ was purchased from the Coley Pharmaceutical Group (Wellesley, MA) and used to stimulate B cells at a concentration of 1 µg/mL. BCR stimulation was performed using rabbit anti-human IgM Ab (Jackson ImmunoResearch Laboratories, West Grove, PA) at 2 µg/mL. Protectin D1 was prepared as in (17). Resolin D1 and 17 (R)-HDoHE (Cayman Chemical, Ann Arbor, MI) were suspended in ethanol and supplemented in culture at nanomolar concentrations. Vehicle control or SPM were added 30 min prior to B cell activation with Cpg ODN 2395 plus anti-IgM Ab, as well as for the duration of the treatment. Vehicle controls were defined as 1× PBS with 0.03% ethanol by volume, equivalent to the highest concentration of SPM used.

Proliferation assay

Purified B cells were cultured in round-bottom 96-well plates (1 × 105 cells/mL, 200 µL/well). Cells were cultured in triplicate, stimulated with Cpg ODN 2395 plus anti-IgM, and incubated with 17-HDHA [17 (R)-HDoHE]. A total of 1 µCi [3H]thymidine was added per well 12 h prior to harvest. Samples were taken at days 1, 3, 4, and 5. [3H]Thymidine incorporation was measured by scintillation spectroscopy using a Topcount Luminometer (PerkinElmer Life Sciences, Boston, MA).

ELISAs

Purified CD19+ human B cells (1 × 106 cells/mL, 200 µl/well) were cultured in triplicate in 96-well round-bottom plates for 7 d. Supernatant IgM and IgG concentrations were measured using ELISA kits, as specified by the manufacturer (Bethyl Laboratories, Montgomery, TX). Cytokines were measured from purified B cells (3 × 106 cells/mL). At days 2 and 7 after activation, IL-6 (BD Pharmingen, San Diego, CA), IL-10, and TNF-α (BioLegend, San Diego, CA) levels were measured in the supernatants.

IgM- and IgG-specific ELISPOT assay

Purified CD19+ human B cells (1 × 106 cells/mL, 200 µl/well) were cultured for 5 d. ELISPOT plates (Millipore, Billerica, MA) were coated with goat anti-human IgM and IgG Abs (BioSource, Carlsbad, CA), as recommended by the manufacturer. B cells were incubated in the ELISPOT plates for 5 h at 37°C. Ab-secreting cells were detected with alkaline phosphatase-conjugated goat anti-human IgG (Jackson ImmunoResearch Laboratories) or anti-human IgM Ab (BioSource). Plates were developed using Vector AP substrate kit III (Vector Laboratories, Burlingame, CA), and spots were counted using a CTL plate reader and immunospot software (Cellular Technologies, Shaker Heights, OH).

Real-time PCR

Following 48 and 72 h of culture of human B cells (3 × 106 cells/mL, 500 µl/well), total RNA was isolated using Qiagen RNAeasy mini kit (Valencia, CA). Superscript III and random primers (Invitrogen) were used to reverse transcribe isolated RNA to cDNA. Steady-state levels of Blimp-1, Xbp-1, AID, and 7S RNA were assessed by real-time PCR. Primers used for Blimp-1 were sense, 5′-GTGTCACAGGCGGATAC-3′ and antisense, 5′-TGGTTAGACAGGTTGAGTG-3′; Xbp-1 sense, 5′-TGCGGATTACCTTTCCTGAGG-3′ and antisense, 5′-AAGGATTACCTTTACAGG-3′; Pad5 sense, 5′-TGTGCTC-ATCAGGATTGTTACG-3′ and antisense, 5′-TAGGACCGGTGTTACGTG-C-3′; AID sense, 5′-CGTGACAGGCTGACATCC-3′ and antisense, 5′-TGCGTCTCTACAGAATGAG-3′; and 7S sense, 5′-ACCCAGATTGTGCTTAAGGA-3′ and antisense, 5′-CAGGGGATTGTTTGGACTC-3′; iQ SYBR Green Supermix (Bio-Rad, Hercules, CA) was used to quantify amplified products, and results were analyzed with the Bio-Rad iCycler software. Blimp-1, Xbp-1 and Pax5, and AID mRNA steady-state levels were normalized to 7S expression. Changes in mRNA expression were determined by comparing mRNA steady-state levels from vehicle-treated peripheral B cells with SPM-treated B cells.

Western blotting

Purified human B cells were lysed in radioimmunoprecipitation assay buffer (150 mM NaCl, 1% Nonidet P-40, 0.5% Na deoxycholate, 50 mM Tris-base, 0.1% SDS [pH 8.0]) to obtain whole cell lysate. Protein concentration was determined using Bio-Rad Dc protein assay kit (Bio-Rad). Gradient SDS-PAGE gels (Fierce/Thermo Fisher Scientific, Rockford, IL) were loaded with 4 µg protein and transferred to polyvinylidene difluoride membranes (Millipore). Western blots were probed with mouse anti-human Blimp-1, rabbit anti-human Xbp-1 (Novus, Littleton, CO), rabbit anti-human Pax5 (Millipore), mouse anti-human AID (Cell Signaling Technology, Beverly, MA), and mouse anti-human actin control (Calbiochem/EMD Chemicals, Gibbstown, NJ). HRP-conjugated goat anti-mouse or goat anti-rabbit Abs (Jackson ImmunoResearch Laboratories) were used to detect specific probes Abs. Western blots were visualized by autoradiography after incubation with ECL (PerkinElmer Life Sciences).

Flow cytometry analysis

B cell viability was assessed by 7-aminominoimidocin D (7-AAD) staining using Cell Viability Solution (BD Biosciences). Cells were surface stained for CD19-V450 or -FITC (BD Biosciences), CD27 PE-Cy7 (BD Biosciences), CD38 PerCP-Cy5.5 (BD Biosciences), IgD-PE (BD Biosciences), and IgG-allophycocyanin (BD Biosciences). Fluorescence minus one controls were included in each staining protocol. Cells were analyzed on a 12-color LSR II flow cytometer (BD Biosciences). Data were analyzed using FlowJo software (Tree Star, Ashland, OR).

Statistics

Each experiment was repeated using cells from five different donors unless otherwise specified. Data are expressed as mean ± SEM. Significance was determined by one-way ANOVA with a Tukey’s posttest, or a two-tailed unpaired Student t test where applicable. A two-way ANOVA with a Bonferroni posttest was used where two or more variables were included. The p values < 0.05 were considered statistically significant.

Results

SMP are present in mouse spleens

Lipid-derived molecules such as PGs play important roles in B cell function (18, 19). Our laboratory has previously shown that PGE2 is not only produced by B cells, but it is also important for Ab production (15, 16, 19). In light of the critical role novel SPM play during inflammation, we first investigated the presence of SPM in the spleen, where a high number of B cells reside. Using LC-MS/MS–based metabolo-lipidomics analysis, we identified DHA-derived 17-HDHA, RvD1, and protectin D1 (PD1) to be present in the spleen (Fig. 1). Interestingly, other resolvins of the D series were not detected (resolvin D2 and resolvin D5). The presence of SPM in the spleen further suggests a role for SPM and the regulation of the immune response.

SMP stimulate human B cell Ab production

The identified 17-HDHA is a known biomarker for resolvins of the D series, as well as protectin biosynthesis (17, 20). We therefore asked whether DHA-derived SPM influenced human B cell functions, particularly Ab production. Peripheral blood human B cells were purified from healthy donors by CD19-positive selection. B cells were pretreated with either 17-HDHA, RvD1, or protectin D1 (PD1) to be present in the spleen (Fig. 1). Interestingly, other resolvins of the D series were not detected (resolvin D2 and resolvin D5). The presence of SPM in the spleen further suggests a role for SPM and the regulation of the immune response.
Ab production, particularly IgM. Furthermore, DHA-derived 17-HDHA had a particularly strong effect at increasing both IgM and IgG production.

Docosanoid 17-HDHA increases B cell Ab production, but does not activate naive B cells

To determine whether the effects of 17-HDHA were due to increased activation of naive B cells, we treated freshly isolated human CD19+ B cells for 7 d with 17-HDHA alone and compared its effect to the known stimulants CpG ODN 2395 (1 μg/ml) plus anti-IgM (2 μg/ml) activation. At day 7 of culture, supernatants were collected and (A) IgM and (B) IgG production was measured by ELISA. Vehicle control or SPM was added to culture every day. Data were analyzed by one-way ANOVA with a Tukey’s post test, *p ≤ 0.05, **p ≤ 0.01, and represented as ±SEM.

FIGURE 2. Certain SPM stimulate B cell Ab production. Human CD19+ B cells were isolated by positive selection from whole units of blood from healthy subjects. Purified B cells (1 × 10^6 cells/ml) were treated for 30 min with the respective SPM (100 nM) or vehicle prior to CpG ODN 2395 (1 μg/ml) plus anti-IgM (2 μg/ml) activation. At day 7 of culture, supernatants were collected and (A) IgM and (B) IgG production was measured by ELISA. Vehicle control or SPM was added to culture every day. Data were analyzed by one-way ANOVA with a Tukey’s post test, *p ≤ 0.05, **p ≤ 0.01, and represented as ±SEM.

FIGURE 3. IgM and IgG production is enhanced by 17-HDHA. Purified B cells (1 × 10^6 cells/ml) were cultured for 7 d, and Ab levels were measured by ELISA. (A) Nonstimulated B cells were treated with 100 nM 17-HDHA, vehicle, or CpG ODN 2395 (1 μg/ml) plus anti-IgM (2 μg/ml) as a positive control. (B) Purified B cells were treated for 30 min with 17-HDHA or vehicle prior to CpG ODN 2395 (1 μg/ml) plus anti-IgM (2 μg/ml) activation. Data were analyzed by one-way ANOVA with a Tukey’s posttest, **p ≤ 0.01, and represented as ±SEM.
The 17-HDHA decreases cytokine production from activated peripheral B cells

DHA-derived SPM such as protectin D1 inhibit TNF-α and IFN-γ production by T cells (23, 24). In addition, the eicosanoid lipoxin A_4 decreases IL-12 production by dendritic cells (25, 26). Therefore, we asked whether 17-HDHA influenced B cell cytokine production. Cytokine production was measured in purified human peripheral blood B cell cultures. Purified B cells were exposed to 17-HDHA, followed by activation with CpG plus αIgM. IL-6, IL-10, and TNF-α concentrations were measured by ELISA (Fig. 4). These results showed dampened production of the proinflammatory cytokine IL-6 by 17-HDHA. Downregulation of IL-6 was detected as early as day 2 after B cell activation (Fig. 4A). Surprisingly, IL-10 production was also found to be decreased by day 7 after treatment in culture (Fig. 4B). Earlier time points of IL-10 production showed a decreasing trend, but did not achieve statistical significance (data not shown). Lastly, there were no significant changes in TNF-α production (Fig. 4C), a unique finding for B cells, as previous reports have shown SPM-mediated decreased TNF-α production in T cells (6).

Increased Ab production is not due to changes in B cell viability nor proliferation

We contemplated that the changes in Ab and cytokine levels could be attributed to changes in either cell death or proliferation. Therefore, we measured B cell viability by 7-AAD exclusion. CD19^+ purified B cells were cultured for 7 d, and viability was measured at the end of the treatment. Dying, dead, and live populations were gated and compared between 17-HDHA–treated and nontreated groups, with no significant changes found (Fig. 5A, 5B). Proliferation was also considered as a possible factor influencing Ab and cytokine secretion. To monitor cell proliferation, the tritiated thymidine DNA incorporation assay was performed in a time course (Fig. 5C). No apparent differences were found in CpG plus anti-IgM–activated cells that had been exposed to 17-HDHA. We concluded that the endogenous 17-HDHA is not toxic to B cells nor does it have proliferative effects.

The 17-HDHA increases the number of Ab-producing cells

The 17-HDHA–mediated IgM- and IgG-increased production could be due to higher Ab-production levels per cell or due to a larger number of cells secreting Ig. To address this question, we performed ELISPOT analysis. Purified B cells were treated with 17-HDHA or control vehicle and activated with CpG plus anti-IgM. Our results showed that 17-HDHA increased the number of cells secreting IgM and IgG in a dose-dependent manner (Fig. 6A, 6B). These results suggest that the increase in Ab production is due to an increase in the number of Ab-secreting B cells.

Ab-secreting B cell differentiation is enhanced by 17-HDHA

To further explore whether 17-HDHA promotes cell differentiation, we analyzed characteristic B cell surface markers that change during differentiation. Purified B cells were activated and treated with 17-HDHA. Following 7 d of culture, surface marker staining and flow cytometry were performed. Cell viability was measured by 7-AAD exclusion. Plasma cell precursors are defined as CD27^CD38^ cells (27, 28); therefore, B cell surface markers CD19, CD27, CD38, and IgD were used.

CD19 staining was performed at the beginning of culture to confirm B cell purity of >98% (data not shown). Using flow cytometry analysis, Ab-secreting cells were defined as IgD^-CD27^CD38^ and pregated on the live-lymphocyte gate (27, 28). The 17-HDHA increased the number of double-positive CD27^CD38^ cells in culture (Fig. 6C, 6D). We conclude that 17-HDHA enhanced the number of Ab-secreting cells, which in turn was responsible for the increased amounts of IgM and IgG present in culture.

The 17-HDHA increases expression of plasma cell differentiation factors

B cell maturation and differentiation is a carefully regulated process. Blimp-1 and Xbp-1 are important transcription factors required for B cell differentiation toward plasma cells. Blimp-1 represses the transcription factor Pax-5, which is a negative regulator of Xbp-1. Expression of Xbp-1 is critical for Ab-secreting cell formation (29–31). In addition, Pax-5, which is important in early B cell lineage stages, is downregulated as B cells differentiate toward memory or plasma cells (32, 33).

We analyzed the expression levels of Blimp-1 and Xbp-1 in 17-HDHA–treated B cells. mRNA steady-state levels measured by real-time PCR showed that both differentiation factors were upregulated in 17-HDHA–treated B cells (Fig. 7A, 7B). In addition, Pax-5 mRNA levels were strongly downregulated as early as day 1 in culture (Fig. 7C). Furthermore, we measured protein expression of Blimp-1, Xbp-1, and Pax-5 by Western blot (Fig. 7E, 7F). The 17-HDHA increased Blimp-1 and Xbp-1 while decreasing Pax-5 protein levels. These results show that 17-HDHA promoted differentiation of activated B cells at the molecular level.

Ag-experienced B cells undergo isotype switching and somatic hypermutations, thus increasing specificity of the Ab-mediated immune response. The transcription factor AID is involved in both of these processes (34). Considering the observed increased Ab

**FIGURE 4.** The 17-HDHA decreases IL-6 and IL-10 production, but not TNF-α. Positively selected CD19^+ human B cells (3 x 10^6 cells/ml) were treated with 17-HDHA or vehicle 30 min prior to B cell stimulation with CpG ODN 2395 (1 μg/ml) plus anti-IgM (2 μg/ml). (A) IL-6 concentrations measured by ELISA at day 2. (B) IL-10 concentrations measured by ELISA at day 7. (C) TNF-α concentrations measured by ELISA at day 2. Data were analyzed by one-way ANOVA with a Tukey’s posttest, *p ≤ 0.05, **p ≤ 0.01, and represented as ±SEM.
production by 17-HDHA–treated B cells, AID mRNA steady-state and protein levels were measured. Our results showed that AID was upregulated in 17-HDHA–treated cells (Fig. 7D, 7E). Overall, these results support our hypothesis that the SPM 17-HDHA drives human B cell differentiation toward an Ab-secreting cell.

Discussion

Docosanoids have many effects on cells of the immune system, including T cells, neutrophils, and macrophages (6–8). In an effort to better understand SPM biological functions on the immune system, we asked whether SPM are present in important lymphatic organs such as the spleen. In this study, we have confirmed the presence of DHA-derived SPM in mouse spleens, specifically 17-HDHA, RvD1, and PD1.

The SPM 17-HDHA is a characteristic marker of the resolvin synthesis pathway as it is a precursor to D-series resolvins and protectins (20). Therefore, we asked what their effects are, if any, on human B cells. In this study, we report that SPM from the...
Docosanoids family, RvD1 and 17-HDHA, but not PD1, increase B cell Ab production and cell differentiation. Consequently, we studied 17-HDHA’s effects on human B cells. We found that 17-HDHA alone does not activate B cells to produce Abs. However, in CpG plus αIgM-activated B cells, 17-HDHA increased Ab production without affecting cell death or proliferation.

Based on increases in Ab production, we hypothesized that 17-HDHA influenced B cell differentiation. In this study, we demonstrated that 17-HDHA increased the number of cells producing IgM and IgG, as well as the Ab-secreting phenotype characterized by CD27⁺CD38⁺ expression (27, 35). Such a phenotypic change was further confirmed at the molecular level with increased expression of Blimp-1 and Xbp-1, as well as the downregulation of Pax-5, which are key B cell differentiation factors.

An enhanced, but controlled Ab response promotes faster and more efficient Ag recognition and clearance, thus promoting the resolution of inflammation. Therefore, 17-HDHA might play an important role in B cell maturation and the adaptive immune response. Not all peripheral B cells will become Ab-secreting cells. Additional B cell subsets could be differentially responsive to certain SPM. The question of which B cell subset is differentiating toward an Ab-secreting cell will need to be addressed in the future.

Interestingly, 17-HDHA decreased B cell cytokine production, particularly IL-6 and IL-10, which have proinflammatory and anti-inflammatory effects, respectively. Unlike previous reports on mouse and human T cells, human B cell TNF-α production was not affected by 17-HDHA (6). Decreases in both proinflammatory and anti-inflammatory cytokine production further suggest a preferential differentiation toward an Ab-secreting cell.

A question that has not yet been addressed is whether 17-HDHA solely promotes B cell differentiation, or whether it also enhances affinity maturation or isotype switching. Our findings show 17-HDHA increases AID expression; however, we have not discerned whether this is a result of the increased number of plasma cells in culture, or whether it reflects an enhanced somatic hypermutation activity within the plasma cell population. Future studies should address the effects of 17-HDHA and other SPM during an Ag-specific Ab response and their role on the long-term immunity.

In this study, we have confirmed the presence of endogenous 17-HDHA, RvD1, and PD1 in mouse spleens. The specific cells re-
sponsors for SPM biosynthesis have not yet been determined. Nevertheless, SPM such as 17-HDHA and RvD1, but not PD1, have a strong effect on B cells and the humoral response. Therefore, it is possible that SPM present in lymphoid organs, such as the spleen and bone marrow, play an important role during the humoral response. Further investigation of SPM activity in the B cell microenvironment will provide further understanding of the role of SPM during the primary and secondary immune response.

The fact that 17-HDHA and other SPM increase B cell Ab production and promote B cell differentiation to an Ab-secreting cell raises interesting possibilities. For example, 17-HDHA and other SPM might prove useful as nontoxic and nonimmunogenic adjuvants. Furthermore, their ability to stimulate B cells in the nanomolar range further enhances their attractiveness as B cell activators. The current study provides the first set of evidence on SPM functions as immune regulators of B cells and their involvement in the humoral immune response. Considering the importance of polyunsaturated fatty acids in our diet and their known anti-inflammatory properties, SPM have great potential to be used as immune regulators, particularly during infection or vaccination.

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Disclosures
C.N.S. is an inventor on patents (resolvins) assigned to Brigham and Women’s Hospital and licensed to Resolvyx Pharmaceuticals. C.N.S. is a scientific founder of Resolvyx Pharmaceuticals and owns equity in the company. C.N.S.’s interests were reviewed and are managed by Brigham and Women’s Hospital and Partners HealthCare in accordance with their conflict of interest policies. All remaining authors have no financial conflicts of interest.

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