Characterization of an arachidonic acid-deficient (Fads1 knockout) mouse model

Yang-Yi Fan,* Jennifer M. Monk,* Tim Y. Hou,*,§ Evelyn Callway,*,§ Logan Vincent,*,§ Brad Weeks,†† Peiying Yang,§§ and Robert S. Chapkin†,*,§

Program in Integrative Nutrition and Complex Diseases,* Center for Environmental and Rural Health,† Departments of Biochemistry & Biophysics,‡ and Veterinary Pathobiology,¶ Texas A&M University, College Station, TX 77843; and Department of Cancer Biology,§§ University of Texas MD Anderson Cancer Center, Houston, TX 77030

Abstract  Arachidonic acid (20:4\(^\Delta\)\(\Delta\)\(\Delta\)) AA)-derived eicosanoids regulate inflammation and promote cancer development. Previous studies have targeted prostaglandin enzymes in an attempt to modulate AA metabolism. However, due to safety concerns surrounding the use of pharmaceutical agents designed to target Ptgs2 (cyclooxygenase 2) and its downstream targets, it is important to identify new targets upstream of Ptgs2. Therefore, we determined the utility of antagonizing tissue AA levels as a novel approach to suppressing AA-derived eicosanoids. Systemic disruption of the Fads1 (\(\Delta\)5 desaturase) gene reciprocally altered the levels of dihomo-\(\gamma\)-linolenic acid (20:3\(^\Delta\)\(\Delta\)\(\Delta\)), DGLA and AA in mouse tissues, resulting in a profound increase in 1-series-derived and a concurrent decrease in 2-series-derived prostaglandins. The lack of AA-derived eicosanoids, e.g., PGE\(_2\), was associated with perturbed intestinal crypt proliferation, immune cell homeostasis, and a heightened sensitivity to acute inflammatory challenge. In addition, null mice failed to thrive, dying off by 12 weeks of age. Dietary supplementation with AA extended the longevity of null mice to levels comparable to wild-type mice. We propose that this new mouse model will expand our understanding of how AA and its metabolites mediate inflammation and promote malignant transformation, with the eventual goal of identifying new drug targets upstream of Ptgs2.—Fan, Y-Y., J. M. Monk, T. Y. Hou, E. Callway, L. Vincent, B. Weeks, P. Yang, and R. S. Chapkin. Characterization of an arachidonic acid-deficient (Fads1 knockout) mouse model. J. Lipid Res. 2012. 53: 1287–1295.

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Prostaglandins of the 2-series (e.g., PGE\(_2\)) found in high abundance in colorectal tissue, are downstream products of cyclooxygenase-2 (Ptgs2). A significant increase in Ptgs2 gene expression has been shown to promote PGE\(_2\)-dependent colon cancer development, in part by enhancing cell proliferation and repressing protective proapoptotic signaling pathways (1, 2). Recent controversies associated with the role of aspirin and other Ptgs2 inhibitors indicate that more work is needed to elucidate the effects of PGE\(_2\) in cancer initiation, progression, and metastasis (3). The preferred substrate for cyclooxygenase catalysis of either Ptgs1 or Ptgs2 is arachidonic acid (20:4\(^\Delta\)\(\Delta\)\(\Delta\), AA) (4). AA is derived metabolically from linoleic acid (18:2\(^\\Delta\)\(\Delta\)), LA), the major polyunsaturated fatty acid (PUFA) in the diet. Dietary LA is the major source of tissue dihomo-\(\gamma\)-linolenic acid (20:3\(^\Delta\)\(\Delta\)\(\Delta\)), DGLA and AA, and its metabolism is regulated by the complementary action of fatty acid desaturases (Fads1 and Fads2) (5) (see supplementary Fig. 1). In addition to LA, dietary AA can contribute to tissue AA levels. However, compared with the dietary intake of LA (10–20 g/day), AA intake (100–500 mg/day) is a very minor contributor (6).

AA-derived eicosanoids, including PGE\(_2\), function as mediators of immune inflammation. Despite the traditional belief that PGE\(_2\) acts as an immunosuppressant based on its inhibition of T-cell activation and cytokine [tumor necrosis factor (TNF)\(\alpha\), interleukin (IL)-12] production in vitro (7), PGE\(_2\) has now emerged as an immunomediator, which facilitates Th1 differentiation and Th17 cell expansion, two

Abbreviations:  AA, arachidonic acid; Ptgs2, cyclooxygenase 2; DGLA, dihomo-\(\gamma\)-linolenic acid; DSS, dextran sodium sulphate; EdU, 5-ethyl-2'-deoxyuridine; Fads1, fatty acid desaturase 1 (\(\Delta\)5 desaturase); Hct, heterozygous; IL, interleukin; LA, linoleic acid; MHC II, major histocompatibility complex class II; PGE, prostaglandin E; PI(4,5)P\(_2\), phosphatidylinositol-4,5-bisphosphate; TNFa, tumor necrosis factor \(\alpha\); Wt, wild-type.

*To whom correspondence should be addressed.

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T-cell subsets involved in adaptively mediated inflammation (8). Therefore, PGE\textsubscript{2} has the ability to influence the cytokine microenvironment, thereby skewing naïve T-cell differentiation, and ultimately function, toward inflammatory T-cell subsets. For example, it has been reported that PGE\textsubscript{2} propagates inflammatory bowel disease (IBD) by enhancing the development and function of IL-17-producing Th17 cells (9). In addition to its role in cytokine regulation, AA is a major constituent of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P\textsubscript{2}), and via the action of phospholipase C, results in the accumulation of inositol trisphosphate (IP\textsubscript{3}) and 1-stearoyl-2-AA-diacylglycerol to elicit intracellular Ca\textsuperscript{2+} mobilization (10). The hydrolysis of PI(4,5)P\textsubscript{2} plays an important role in membrane rapid cytoskeletal remodeling (11) and Ras/Erk, as well as Ca\textsuperscript{2+} signaling in lymphocytes (12).

Most studies have targeted prostaglandin biosynthetic and degradation enzymes in an attempt to suppress AA-derived eicosanoid-mediated inflammation and tumor-promoting action (13). Surprisingly, no investigators to date have attempted to target AA (substrate levels) as a way of modulating prostaglandin biosynthesis and tumor development. The controversies associated with the role of aspirin and Pgs inhibitors indicate that more work is needed to elucidate the effects of eicosanoids in colon cancer and inflammatory diseases (3). Therefore, we generated a novel genetic model, i.e., the Fads1 (Δ5 desaturase) knockout mouse, to determine the role of AA-derived 2-series eicosanoids in mucosal physiology and inflammation. This model allows for the specific investigation of AA deficiency without the underlying complications of essential fatty acid (LA and DGLA) deficiency.

**MATERIALS AND METHODS**

**Generation of Fads1 null mice**

Mutant Fads1 mice were generated using a gene-trapping technique (14). Mice (strain C57BL/6) were cloned from an ES cell line (IST11525H2; Texas Institute for Genomic Medicine, TIGM). The ES cell clone contained a retroviral insertion in the Fads1 gene identified from the TIGM gene trap database, and was microinjected into C57BL/6 host blastocysts to generate germ-line chimeras using standard procedures. The retroviral OmniBank Vector 76 (Fig. 1) contained a splice acceptor sequence (SA) followed by a 5′ selectable marker β-gal, a functional fusion between the β-galactosidase and neomycin resistance genes, for identification of successful gene trap events followed by a polyadenylation signal (pA). Insertion of the retroviral vector into the Fads1 gene led to the splicing of the endogenous upstream exons into this cassette to produce a fusion transcript that was used to generate a sequence tag (OST) of the trapped gene by 3′ RACE (15). Chimeric males were bred to C57BL/6 females for germline transmission of the mutant Fads1 allele.

**Animals and diets**

Three genotypes [wild-type (Wt), heterozygous (Het) and null (Null)] of Fads1 mice were derived from heterozygous males and females. All procedures followed the guidelines approved by Public Health Service and the Institutional Animal Care and Use Committee at Texas A&M University. All animals were fed commercial 10% safflower oil diet (D03092902R; Research Diets), free of AA. In a separate experiment, the basal 10% safflower oil diet was supplemented with ARASCO oil (containing 42% AA, w/w) to determine the effect of dietary AA on the life span of Null mice.

**Genotype/phenotype**

Mice were screened after weaning at the age of 3–4 weeks. For genotyping, tail snip DNA was extracted using a Qiagen DNeasy blood and tissue kit (cat# 69506). PCR was performed to determine the presence of the gene trap using Hot Start Taq Mastermix (#CB40303; Denville Scientific) (16). Primers (Fads1 band, 310 bp product, 5′-CGTGTTTCTCCTGGAATGCTCA-3′, 5′-CGACTCAGACTGAAAGCTGTC T-3′; Gene Trap Vector 76, 270 bp product, 5′-CTTGCAAATGCGTTACGACTA-3′, 5′-CCAATAAAAACCTGTGTTGTCG-3′). For phenotyping, total lipids were extracted from 0.5 cm mouse tail using the method of Folch et al. (17) and transesterified in 6% methanolic HCl. Fatty acid methyl esters were subsequently analyzed by capillary gas chromatography as previously described (18).

**Real-time PCR**

Fads1 and Fads2 mRNA expression levels in colon mucosa and liver were determined by real-time PCR on an ABI 7900 instrument. cDNA was synthesized from 2 μg total RNA using random hexamers and oligo(dT) primers with Superscript II RT (Invitrogen). PCR was performed (primer sequences available online) using predeveloped Taqman assays (Applied Biosystems). Expression levels were normalized to ribosomal 18S expression using assay kits from Applied Biosystems (cat# Mm00507605 for Fads1, Mm00517221 for Fads2, and Mm03928990 for 18S).

**Measurement of total phospholipid fatty acid composition**

Total lipids in colon mucosa, liver, spleenocytes, and serum were extracted by the method of Folch (17). Total phospholipids were separated by one-dimensional thin-layer chromatography on silica gel 60 G plates using chloroform/methanol/ acetic acid/water (90:8:1:0.8, v/v/v/v) as the developing solvent. Isolated total phospholipids were transesterified in 6% methanolic HCl overnight, followed by GC analysis as previously described (18).

**Eicosanoid analysis**

Eicosanoids were extracted from colon mucosa, small intestine mucosa, and lung using a previously described method (19, 20). Briefly, snap-frozen tissues were ground to a fine powder and homogenized with an Ultrasonic Processor (Misonix). An aliquot of homogenate was subjected to extraction with hexane/ethyl acetate (1:1). The upper organic layer was collected, and the organic phases from three extractions were pooled and then evaporated to dryness under a stream of nitrogen. All extraction procedures were performed at minimum light levels at 4°C. Samples were then reconstituted in 100 μl of methanol/10 mmol/l ammonium acetate buffer, pH 8.5 (50:50, v/v), before liquid chromatography/tandem mass spectroscopic analysis. Protein concentration was determined by the method of Bradford according to the manufacturer’s instructions (Pierce). Liquid chromatography/tandem mass spectroscopic analyses were performed using a QuattroUltima mass spectrometer (Waters) equipped with an Agilent 1100 binary pump high-performance liquid chromatography system (Agilent) using a modified version of the method of Yang et al. (19). Eicosanoids of interest were chromatographically separated using a Luna 3 μm phenyl-hexyl 4.6 × 100 mm analytic column (Phenomenex). The mobile phase
consisted of 10 mmol/l ammonium acetate (pH 8.5) and methanol using a linear methanol gradient consisting of 50% to 60% in 10 min, and then from 60% to 80% in 4 min. This was then increased to 100% methanol concentration over the next 6 min and kept at this condition for an additional 2 min to achieve chromatographic baseline resolution. The flow rate was 0.5 ml/min with a column temperature of 60°C. The mass spectrometer was operated in negative electrospray ionization mode with a cone voltage of 40 V. Source temperatures were 125°C with a desolvation gas temperature at 350°C. Collision-induced dissociation of the eicosanoids was performed using argon gas at a cell pressure of 1.6 × 10⁻³ Torr. Eicosanoids were detected and quantified by multiple reaction mode monitoring of the transitions m/z 351→271 for AA-derived PGE₂ and m/z 353→317 for DGLA-derived PGE₁ (21).

**Analysis of colonic cell proliferation**

In vivo colonic cell proliferation was determined by immunohistochemical detection. Mice were intraperitoneally injected with 5-ethyl-2'-deoxyuridine (EdU, 30 mg/kg body weight) 2 h prior to termination. One centimeter of distal colon was removed, fixed in 4% paraformaldehyde, followed by a series of ethanol washes, and embedded in paraffin. The incorporation of EdU into DNA of actively dividing cells was determined using a commercially available kit (Click-IT EdU Alexa Fluor 647 Imaging Kit; Invitrogen). Briefly, after deparaffinization, samples were washed in 3% BSA in PBS, treated with 0.5% Triton in PBS for 20 min, washed again with 3% BSA in PBS, then incubated with Click-IT reaction cocktail for 30 min. Coupling of EdU to the Alexa fluor substrate was then observed using fluorescence microscopy (22).

**Polyphosphoinositide (PI(4,5)P₂) analysis**

Spleens were removed aseptically, and CD4⁺ T cells were isolated by positive selection using magnetic CD4 (L3T4) microbeads according to the manufacturer’s protocol (Miltenyi Biotec). Cells (5 × 10⁵) were washed with cold 1× PBS, and then pelleted at 4,000 g for 5 min at 4°C. Supernatant was removed, and the pellet was resuspended in 800 l of 1:1 methanol:CHCl₃. The mixture was vortexed for 1 min, and then centrifuged at 7,500 g for 5 min at 4°C. The supernatant was discarded, and pellets resuspended in 400 l of 80:40:0.3 methanol:CHCl₃:HCl. The mixture was vortexed for 5 min and subsequently centrifuged at 3,000 g for 1 min at 4°C. An additional 80 l of 1 N HCl was added to the extract and vortexed for 15 s prior to centrifugation at 18,000 g for 15 s at 4°C. The organic layer was collected and dried under a stream of N₂. The lipid film was dissolved in 1× PBS supplemented with 0.0025% of protein stabilizer (Echelon Biosciences) and used for detection of PI(4,5)P₂. PI(4,5)P₂ dissolved in 1× PBS supplemented with 0.0025% protein stabilizer was added in duplicate into 96-well flat-bottom plates and incubated at room temperature for 2 h. The wells were then washed with 1× PBS three times, and then blocked in 5% BSA in PBS overnight at 4°C. The wells were again washed with 1× PBS three times, and then incubated with primary mouse anti-PI(4,5)P₂ (Abcam) in blocking solution at a dilution of 1:2500 for 1.5 h at room temperature. The wells were washed with 1× PBS and incubated with secondary goat anti-mouse IgG labeled with horseradish peroxidase (KPL, Gaithersburg, MD) in blocking solution at a dilution of 1:5,000 for 1 h and protected from light. This was followed by incubation in TMB high-sensitivity substrate solution (BioLegend) for 5 min at room temperature and protected from light. The reaction was stopped by the addition of 1 N H₂SO₄, and the absorbance was read at 450 nm. A standard curve was generated with known concentrations of PI(4,5)P₂ (Echelon Biosciences). To test the cross-reactivity of the primary antibody, 50 pmol of PI(4)P and PI(3,4,5)P₃ (Avanti) were dissolved in 1× PBS supplemented with 0.0025% protein stabilizer, added into separate wells, and subjected to the same ELISA protocol as described. No detectable signals were observed (data not shown). In addition, ELISA data were validated by mass spectrometry (23).
Characterization of immune cell populations

A single-cell suspension was produced by combining spleen and mesenteric and inguinal lymph nodes, which were pushed through a sterile stainless-steel wire screen (100 mesh) and resuspended in RPMI 1640 medium with 25 mmol/l HEPES (Irvine Scientific), supplemented with 10% fetal bovine serum (FBS, Irvine Scientific), 2 mmol/l GlutaMAX (Gibco), penicillin 100 U/ml, and streptomycin 0.1 mg/ml (Gibco); henceforth, “complete medium.” Subsequently, a mononuclear cell suspension was produced by density gradient centrifugation using Lympholyte-M (Cedarlane Laboratories). Cell numbers were determined using a hemocytometer, and viability assessed by trypan blue exclusion always exceeded 96% in each genotype. The T-cell compartment was identified by surface expression of CD3, and the antigen-presenting cell (APC) compartment was identified by surface expression of major histocompatibility complex (MHC) class II (i.e., I-A[b]). For this purpose, 10^6 viable mononuclear cells were incubated for 10 min with a FcγR-blocking monoclonal antibody (1 µg/ml) (2.4G2; BD Pharmingen) on ice and were subsequently stained with either 1 µg/ml of PE-anti-mouse I-A[b] (clone AF6-1201; BD Bioscience) or 1 µg/ml of APC-anti-mouse CD3ε (clone 145-2C11; Bio-Rad) antibodies for 30 min. Flow cytometric analysis was conducted using an Accuri C6 flow cytometer (Accuri Cytometers).

In vivo stimulation of mononuclear cells and measurement of cytokine production

Using 96-well Falcon plates (#3072; Becton-Dickinson), 5 × 10^5 viable mononuclear cells were added to each well in a final volume of 200 µl of complete RPMI. Cell cultures were either unstimulated (complete RPMI alone) or stimulated under various conditions, namely, 5 µg/ml of plate-bound anti-CD3 (clone 145-2C11; BD Bioscience) plus 20 µg/ml of soluble anti-CD28 (clone 37.51; Bio-Rad), 10 µg/ml LPS (E. coli 055:B5; Sigma) or 10 µg/ml anti-CD40 (clone 1C10; Bio-Rad). All cultures were incubated at 37°C for 24 h, and then supernatants from similarly treated culture wells were pooled together and subsequently aliquoted for storage at −80°C. Under each stimulation condition, in vitro cytokine production was measured using the Bio-Plex Pro Mouse Cytokine Group I multiplex kit (Bio-Rad) and the Bio-Plex 200 System and accompanying software package, Bio-Plex Manager 6.0 (Bio-Rad).

Colitis induction

Animals were exposed to dextran sodium sulfate (DSS, molecular weight, 36,000–50,000; MP Biomedicals) treatment as previously described (24, 25). To induce intestinal inflammation, 2.5% DSS was administered in the drinking water for 5 days, followed by 14 days of tap water.

Histological scoring

Various tissues (brain, heart, lung, liver, kidney, stomach, colon, and small intestine) were removed, fixed in 10% neutral buffered formalin and paraffin embedded. Sections were stained with hematoxylin and eosin. Histological examination was performed in a blinded manner by a board-certified pathologist (B. Weeks), and the degree of inflammation (score, 0–3) and epithelial injury (score, 0–3) in microscopic cross-sections of the tissues was graded as described previously (20).

Statistical analysis

Data were analyzed using two-way ANOVA. Differences of P < 0.05 were considered significant.

RESULTS

Targeted deletion of Fads1 in mice

Fads1 knockout mice were generated by a gene-trapping technique described in Materials and Methods. Tail DNA was genotyped by PCR. As shown in Fig. 1, Fads1 gene product (310 bp) was only detected in Wt and Het animals, whereas the gene trap product (270 bp) was amplified in both Null and Het animals. To confirm that targeted deletion resulted in the anticipated reduction in Δ5 desaturase expression, Fads1 and Fads2 mRNA expression levels in mouse colon mucosa and liver were measured by RT-qPCR. As shown in Fig. 2, Fads1 expression levels were altered as expected in haploinsufficient Het and Null mice compared with Wt siblings. In comparison, Fads2 expression levels were not different (P > 0.05) among the three genotypes.

Deletion of Fads1 reduces viability

To limit the exogenous source of AA for the Fads1 mouse, animals were fed an LA-enriched 10% safflower oil-based commercial diet (cat# D03092920R; Research Diets) devoid of AA. There was no significant effect of genotype on animal body weights (P > 0.05) (supplementary Fig. II). With respect to longevity, Null mice began to die gradually starting at 5–6 weeks of age, with no survivors past 12 weeks of age (Fig. 3). The average age at death of male Nulls was 7.6 weeks and of female Nulls, 7.5 weeks. From a gross anatomical perspective, no overt physical differences between Wt/Het and Null mice were observed. Occasionally, Null mice exhibited hip dysplasia at ~5 weeks of age, but this did not occur in all mice (~20% of the time). Often, there were no visible signs of any illness or infirmity as close as
Deletion of Fads1 alters 1- and 2-series eicosanoid levels

DGLA and AA are precursors to 1- and 2-series prostaglandins (PGE\(_1\) and PGE\(_2\)), respectively. Therefore, we further probed the effect of gene deletion-induced alterations in DGLA and AA levels with respect to the biosynthesis of 1- and 2-series prostaglandins. Prostaglandins were extracted from colonic mucosa, small intestine mucosa, and lung and measured by mass spectrometry (Fig. 5). As expected, PGE\(_2\) levels were significantly lower, whereas PGE\(_1\) levels were higher in Null versus Wt mice (P < 0.05). Het mice exhibited an intermediate PGE\(_2\)/PGE\(_1\) phenotype.

Fads1 deletion decreases colonic cell proliferation

It has been reported that AA-derived prostaglandins enhance cell proliferation in colon cancer cells (26). Because intestinal PGE\(_2\) was decreased in Fads1 null mice (described above), we examined the level of cell proliferation in mouse colon crypts. As shown in Fig. 6, deletion of the Fads1 gene did not alter the number of cells in colonic
compared basal immune cell populations in *Fads1* Wt, Het, and Null mice. Specifically, mononuclear cells were isolated, and CD3+ T-cell and MHCII-cell populations were quantified by flow cytometry. As shown in Fig. 8, the total number of mononuclear cells and percentage of CD3+ T cells were significantly (P < 0.05) reduced in Null mice. In contrast, there was no difference in the level of MHCII-expressing cell populations across the three genotypes. In complementary experiments, culture supernatants were collected from mononuclear cells and isolated from spleen, and then mesenteric and inguinal lymph nodes were incubated overnight with agents designed to stimulate APCs (LPS, αCD40) or T cells (αCD3 + αCD28). Following overnight stimulation ex vivo, mixed cultures (i.e., mononuclear cell cultures composed of both T cells and APCs) from both Het and Null mice contained lower levels of IFNγ and TNFα following T-cell activation (Fig. 9 and supplementary Table V).

**DISCUSSION**

In this study, we determined the utility of antagonizing tissue AA levels as a novel approach to suppressing AA-derived eicosanoids (e.g., PGE₂) biosynthesis. For this purpose, we generated a previously unknown genetic model, namely, the *Fads1* (Δ5 desaturase) knockout mouse. In many tissues, LA is converted to AA by an alternating sequence of Δ6 desaturation (*Fads2* gene product), chain elongation, and Δ5 desaturation (*Fads1* gene product), in which hydrogen atoms are selectively removed to create new double bonds, and then two carbon atoms are added to lengthen the fatty acid chain (29). Dietary AA

**Fig. 5.** Levels of DGLA and AA-derived prostaglandins. Prostaglandins (PGE₁ and PGE₂) extracted from (A) colon mucosa, (B) small intestine mucosa, and (C) lung were measured by LC-MS; mean ± SEM, n = 3. Values not sharing the same letter indicate a significant difference within respective prostaglandins (P < 0.05).

**Fig. 6.** Effect of *Fads1* deletion on colonic cell proliferation. Levels of cell proliferation in mouse colonic crypts were measured by the EdU Click-It assay. Data are expressed as (A) total number of cells per crypt and (B) percentage of EdU-labeled cells relative to the total number of cells per crypt; mean ± SEM, n = 3. Values not sharing the same letter indicate significant differences (P < 0.05). crypts, although the percentage of EdU-labeled cells was significantly (P < 0.05) decreased in Null compared with Wt and Het mice. Het mice exhibited an intermediate proliferative phenotype between Wt and Null mice, consistent with the levels of AA and PGE₂ (Figs. 4 and 5).

**CD4+ T cell PI(4,5)P₂ levels are reduced in *Fads1* null mice**

As membrane phospholipid PI(4,5)P₂ is a key node of second messenger metabolism in T cells (12) and its acyl backbone is predominantly composed of 1-stearoyl-2-arachidonoyl species, we examined whether the absence of AA associated with the deletion of the *Fads1* gene altered the PI(4,5)P₂ concentration in CD4+ T cells.

The basal level of PI(4,5)P₂ was significantly (P < 0.05) decreased by approximately 24% in *Fads1* Null CD4+ T cells compared with Het and Wt siblings (Fig. 7).

**Immune cell populations are altered by *Fads1* deletion**

As AA-derived eicosanoids play an important role in immune regulation (27, 28), we next investigated the immunomodulatory effects associated with a deficiency in AA-derived 2-series prostaglandins. For this purpose, we compared basal immune cell populations in *Fads1* Wt, Het, and Null mice. Specifically, mononuclear cells were isolated, and CD3+ T-cell and MHCII-cell populations were quantified by flow cytometry. As shown in Fig. 8, the total number of mononuclear cells and percentage of CD3+ T cells were significantly (P < 0.05) reduced in Null mice. In contrast, there was no difference in the level of MHCII-expressing cell populations across the three genotypes. In complementary experiments, culture supernatants were collected from mononuclear cells and isolated from spleen, and then mesenteric and inguinal lymph nodes were incubated overnight with agents designed to stimulate APCs (LPS, αCD40) or T cells (αCD3 + αCD28). Following overnight stimulation ex vivo, mixed cultures (i.e., mononuclear cell cultures composed of both T cells and APCs) from both Het and Null mice contained lower levels of IFNγ and TNFα following T-cell activation (Fig. 9 and supplementary Table V).
acid analysis also confirmed that *Fads1* null mice have negligible levels of AA (~1 mol%) compared with heterozygous (~14 mol%) and wild-type (~15 mol%) mice (Fig. 4 and supplementary Tables I–IV). Similar effects were observed in the liver and spleen, indicating a systemic depletion of AA. In contrast, *Fads1* ablation resulted in the massive enhancement of dihomo-γ-linolenic acid (20:3Δ5,8,11,14, DGLA), the 1-series prostaglandin substrate in select tissues (Fig. 4). As anticipated, the alteration in prostaglandin precursor levels was associated with a profound shift in DGLA and AA-derived eicosanoid biosynthesis (Fig. 5).

Interestingly, 1-series derived prostaglandins, e.g., PGE1, interact with discrete receptors compared with PGE2 (30, 31). This is noteworthy, because PGE1 is capable of inhibiting colon cancer cell proliferation (32, 33) as opposed to PGE2, which promotes growth (1, 34). Therefore, we are in a unique position to explore the impact of carcinogen exposure on mice that have virtually no 2-series prostaglandin substrate (20:4Δ5,8,11,14, AA).

Consistent with the loss of AA in membrane phospholipids, it is noteworthy that PIP2 levels were decreased in T cells isolated from Null mice (Fig. 7). Considering that PIP2 is made up primarily of AA-containing molecular species (35), we propose that the lack of AA substrate for the 1-acyl-glycero 3-phosphoinositol acyltransferase-dependent remodeling likely contributed to a reduction in its mass (23, 33, 36). Because PIP2 controls the activity of numerous proteins and serves as a source of second messengers (37), further work is needed to assess how AA deficiency affects individual phospholipid classes and PIP2-dependent signaling at the plasma membrane. These data will provide insight regarding how different tissues compensate in response to depletion of AA.

Disruption of the *Fads1* versus *Fads2* genes in mice manifests distinct phenotypic outcomes. For example, deletion of Δ6 desaturase resulted in dermal and intestinal ulceration (38–40). In contrast, we report that Δ5 desaturase null mice failed to thrive, gradually dying off at 5–6 weeks of age, with no survivors past 12 weeks of age (Fig. 3A). In most cases, the pathologies observed in *Fads2* null mice were not apparent until approximately 17 weeks of age for females and 11 weeks for males (38–40). *Fads1* Null mice did not live long enough to see if similar adverse events were present. A contributing factor for the survival of the *Fads2* null mice was likely the modest amounts of AA present in the “control” diets (39, 40). This would have allowed the mice to survive and develop fertility and spermatogenesis complications. In addition, differences in the genetic background of the mice may have affected the phenotype. All *Fads2* null mice were on a mixed (129S6/Svev/Tac/C57BL/6) background. *Fads1* mice were on a pure C57BL/6 background. In addition, differences between these metabolically related models may be attributed to the fact that DGLA-derived 1-series eicosanoids are able to compensate for some of the AA-derived eicosanoid functions (30). In contrast to *Fads1* Null mice, *Fads2* mice are unable to synthesize 1-series PGs due to the absence of DGLA (38). Although the precise cause of the loss of viability in *Fads1* Null mice is still under investigation, this
phenotype was fully reversible upon supplementation of AA to the diet (Fig. 3B). With regard to the underlying mechanisms driving the perturbation of immune cell populations observed in Fads1 knockout mice (Figs. 8 and 9), given the importance of PI(4,5)P2 and PGE2 in regulating immune cell function (41–43), it is possible that the suppression of these critical mediators contributed to a disruption in immune cell homeostasis.

The lack of AA-derived eicosanoid production is consistent with the observed reduction in intestinal crypt proliferation (Fig. 6) and the inability of Fads1 Null mice to tolerate an acute intestinal inflammatory challenge (Supplementary Fig. IV). Similar effects have been reported in microsomal PGE synthase Null mice and following cyclooxygenase-2 deficiency (44, 45). PGE2 in the intestine has a protective effect on the integrity of the epithelial intestinal wall, and its loss promotes polymicrobial sepsis, a frequently fatal disease (45). Clearly, until the full effects of Fads1 deletion on the entire spectrum of AA-derived eicosanoids is known, it is not possible to exclusively link the loss of tissue AA to the specific role of PGE2.

In summary, we have generated and characterized a novel AA-deficient (Fads1 knockout) mouse model. Although young-adult mice die prematurely, this phenotype can be rescued by adding AA to the diet. Hence, in future studies, it will be possible to titrate membrane levels of AA. Lipidomic analyses indicate that DGLA and AA-derived mediators are reciprocally modulated in this model. Given the central role of AA-derived eicosanoids in epithelial and immune cell biology, we are in a unique position to address our overall goal, to determine at a mechanistic level, the utility of antagonizing tissue AA levels as a novel approach to suppressing AA-dependent inflammation and cancer.

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