SOCS3 transactivation by PPARγ prevents IL-17-driven cancer growth

Berger, Hélène; Végran, Frédérique; Chikh, Madijd; Gilardi, Federica; Ladoire, Sylvain; Bugaut, Hélène; Mignot, Grégoire; Chalmin, Fanny; Bruchard, Mélanie; Derangère, Valentin; Chevriaux, Angélique; Rébé, Cédric; Ryffel, Bernhard; Pot, Caroline [**and 4 more**]

How to cite

BERGER, Hélène et al. SOCS3 transactivation by PPARγ prevents IL-17-driven cancer growth. In: Cancer research, 2013, vol. 73, n° 12, p. 3578–3590. doi: 10.1158/0008-5472.CAN-12-4018

This publication URL: https://archive-ouverte.unige.ch/unige:33010
Publication DOI: 10.1158/0008-5472.CAN-12-4018

Published OnlineFirst April 25, 2013; DOI: 10.1158/0008-5472.CAN-12-4018

Cancer Research

SOCS3 Transactivation by PPARγ Prevents IL-17–Driven Cancer Growth

Hélène Berger, Frédérique Végran, Madijd Chikh, et al.

Cancer Res 2013;73:3578-3590. Published OnlineFirst April 25, 2013.

Cancer Research

SOCS3 Transactivation by PPARγ Prevents IL-17–Driven Cancer Growth

Hélène Berger[1,2], Frédérique Végran[1,2], Madijd Chikh[1,2], Federica Gilardi[5], Sylvain Ladoire[1,2,3], Hélène Bugaut[1,2], Grégoire Mignot[1,2], Fanny Chalmin[1,2], Mélanie Bruchard[1,2], Valentin Derangère[1,2], Angélique Chevriaux[1,2,3], Cédric Rébé[1,2,3], Bernhard Ryffel[4,8], Caroline Pot[6,7], Aziz Hichami[1,2], Béatrice Desvergne[5], François Ghiringhelli[1,2,3], and Lionel Apetoh[1,2,3]

Abstract

Activation of the transcription factor PPARγ by the n-3 fatty acid docosahexaenoic acid (DHA) is implicated in controlling proinflammatory cytokine secretion, but the intracellular signaling pathways engaged by PPARγ are incompletely characterized. Here, we identify the adapter-encoding gene *SOCS3* as a critical transcriptional target of PPARγ. *SOCS3* promoter binding and gene transactivation by PPARγ was associated with a repression in differentiation of proinflammatory T-helper (T_H)17 cells. Accordingly, T_H17 cells induced *in vitro* displayed increased SOCS3 expression and diminished capacity to produce interleukin (IL)-17 following activation of PPARγ by DHA. Furthermore, naïve CD4 T cells derived from mice fed a DHA-enriched diet displayed less capability to differentiate into T_H17 cells. In two different mouse models of cancer, DHA prevented tumor outgrowth and angiogenesis in an IL-17–dependent manner. Altogether, our results uncover a novel molecular pathway by which PPARγ-induced SOCS3 expression prevents IL-17–mediated cancer growth. *Cancer Res; 73(12); 3578–90. ©2013 AACR.*

Introduction

Inflammation plays contrasting roles during cancer progression. Acute inflammation can be beneficial for cancer regression as shown by the requirement of HMGB1 and interleukin (IL)-1β for the induction of CD8 T-cell polarization and anticancer immune responses (1, 2). In contrast, proinflammatory mediators can support growth of established cancers by supporting an inflammatory milieu within the tumor microenvironment that favors tumor cell survival and neoangiogenesis (3). IL-17–producing CD4 T cells [T-helper (T_H)17 cells] have recently emerged as a key T-cell subset that produces proinflammatory mediators such as IL-17A, IL-17F, IL-21, and TNF-α and promotes autoimmunity (4). T_H17 cells can be induced from naïve T cells using stimulation with TGF-β and IL-6 and are characterized by the expression of their lineage-specific transcription factor RORγt (5). Although *Rorc* is indispensable for T_H17 cell induction, STAT3 is also an essential transcription factor for T_H17 cell differentiation as it directly regulates *il17a/f* and *Rorc* gene transcription (6, 7). IL-17 secretion from T_H17 cells has been proposed to contribute to tumor progression through the promotion of tumor neoangiogenesis (8).

The beneficial anti-inflammatory properties of dietary intake of (n-3) polyunsaturated fatty acids (PUFA) were initially illustrated by epidemiologic studies reporting a lower incidence of cardiovascular diseases in Eskimos compared with Western populations (9, 10). Administration of the n-3 PUFA docosahexaenoic acid (DHA) was later shown to dampen proinflammatory mediator secretion in patients with cancer (11). Activation of the transcription factor PPARγ has been proposed to account for the ability of DHA to prevent proinflammatory cytokine release (12).

Although DHA anticancer efficacy has been reported in preclinical mouse models of established tumors (13), whether the anticancer activity of DHA relies on its ability to modulate the production of proinflammatory cytokines remains elusive. Here, we report that activation of the PPARγ transcription factor by DHA in developing T_H17 cells induces *Socs3* expression and interferes with the STAT3 signaling pathway, thereby inhibiting T_H17 cell differentiation. In addition, DHA prevents *in vivo* tumor growth of B16F10 melanoma and 4T1 mammary adenocarcinoma tumors in an IL-17–dependent manner. Altogether, not only our study shows that DHA administration prevents tumor angiogenesis and growth in an IL-17–

Authors' Affiliations: [1]Institut National de la Santé et de la Recherche Medicale (INSERM), U866; [2]Faculté de Médecine, Université de Bourgogne; [3]Centre Georges François Leclerc, Dijon; [4]University and CNRS, UMR7355, Orléans, France; [5]Center for Integrative Genomics, University of Lausanne, Lausanne; [6]Division of Neurology, Geneva University Hospital; [7]Department of Pathology and Immunology, University of Geneva, Geneva, Switzerland; and [8]Institute of Infectious Disease and Molecular Medicine (IIDMM), University of Cape Town, Cape Town, Republic of South Africa

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

H. Berger and F. Végran share first co-authorship of this article.

F. Ghiringhelli and L. Apetoh share senior co-authorship of this article.

Corresponding Authors: François Ghiringhelli, Centre de Recherche, Institut National de la Santé et de la Recherche Medicale (INSERM) U866, Facultés de Médecine et de Pharmacie, 7 Bd Jeanne d'Arc, 21079 Dijon, France. Phone: 33-3-80-39-33-53; Fax: 33-3-80-39-34-34; E-mail: fghiringhelli@cgfl.fr; and Lionel Apetoh, lionel.apetoh@inserm.fr

doi: 10.1158/0008-5472.CAN-12-4018

dependent manner, but it also uncovers a previously unrecognized molecular pathway by which PPARγ-induced SOCS3 expression in T_H17 cells prevents IL-17 secretion.

Materials and Methods

Mouse strains

All animals were bred and maintained according to both the Federation of Laboratory Animal Science Associations (FELASA) and the Animal Experimental Ethics Committee Guidelines (University of Burgundy, Dijon, France). Animals were used between 6 and 22 weeks of age. Female C57BL/6, BALB/c and Nude mice were purchased from Centre d'élevage Janvier and from Charles River Laboratories. RORγt-eGFP reporter mice were obtained from Dr. Eberl (Institut Pasteur, Paris, France) and have been previously described (14). *IL-17a*$^{-/-}$ mice were obtained from Dr. Y. Iwakura (University of Tokyo, Tokyo, Japan; ref. 15). γδTCR-deficient mice were kindly provided by Dr. Bernhard Ryffel (University and CNRS, Orléans, France). Rag2$^{-/-}$ mice were provided by the CDTA (Cryopréservation, Distribution, Typage et Archivage animal) and distributed by EMMA (European Mouse Mutant Archive, a service funded by the EC FP7 Capacities Specific Programme). PPARβ- and PPARγ-deficient mice were provided by Pr. Béatrice Desvergne (Centre for Integrative Genomics, University of Lausanne, Lausanne, Switzerland) and have been described previously (16, 17).

Diets

To investigate the effect of dietary DHA in mice, we constituted 2 diet groups. One was fed a control diet containing sunflower oil, and the other group of mice was fed a DHA-enriched diet containing Omegavie DHA90 TG (Polaris Nutritional Lipids) for at least 3 weeks before starting experiments. Diets were prepared as described by Triboulot and colleagues (Supplementary Fig. S1; ref. 18).

To investigate the effect of dietary DHA in 2 human healthy volunteers (2 of the authors), 860 mg/day of DHA (Nutrixeal) was provided as a diet supplement for 3 weeks. Naïve CD4 T-cell differentiation into T_H17 cells was then assessed.

Fatty acid analysis

Total lipids were extracted from the diets according to the method of Bligh and Dyer (19), then transmethylated by BF3/methanol after saponification and fatty acids were analyzed by gas–liquid chromatography using C_{15}:0 as internal standard with a Becker gas chromatograph (Becker Instruments) equipped with a 50 m capillary glass column packed with carbowax 20 m (Spiral-RD) as described previously (18).

Direct quantification of nonesterified fatty acid in plasma was conducted by a one-step method of derivatization and extraction using (trimethylsilyl)diazomethane (Sigma) in the presence of C_{15}:0 as internal standard (20) with subsequent analysis by conventional gas–liquid chromatography as described earlier.

Tumor growth experiments

B16F10 melanoma and 4T1 mammary adenocarcinoma cancer cells were cultured at 37°C under 5% CO_2 in RPMI-1640 with GlutaMax-1 (Lonza) supplemented with 10% (v/v) fetal calf serum (Lonza), 1% penicillin, streptomycin, amphotericin B (Gibco), 4 mmol/L HEPES (Gibco), and 1 mmol/L sodium pyruvate (Gibco). 4T1 cells were kindly provided by Dr. Trad (Université de Bourgogne, Dijon, France) and B16F10 cells were obtained from American Type Culture Collection. All cells were routinely tested for *Mycoplasma* contamination using Mycoalert Mycoplasma Detection Kit (Lonza) and found negative. To induce tumor formation, 3×10^5 B16F10 or 10^5 4T1 cancer cells were injected subcutaneously into Nude or immunocompetent mice. *In vivo* IL-6 and IL-17 neutralization were respectively achieved by injecting 200 μg intraperitoneally (i.p.) of an anti-IL-6 (clone MP5-20F3; BioXCell) or an anti-IL-17 antibody (clone 17F3; BioXCell) on day 0, 1, 2, 3, 4, and 6 following tumor implantation. *In vivo* IL-1 blockade was carried out by injecting i.p. 30 μg of IL-1 receptor antagonist (IL-1Ra; Kineret from Biovitrum) 3 times a week. All experiments were carried out in accordance with guidelines prescribed by the Ethics Committee at the University of Burgundy.

CD31 immunostaining

Tumors were cryosliced from mouse biopsies and permeabilized with 0.1% Triton X-100. Sections were probed with a rat monoclonal against CD31 (BD Biosciences—Pharmingen) followed by a secondary antibody coupled to horseradish peroxidase (HRP) and counterstained with Harris's hematoxylin.

In vitro T-cell differentiation

Naïve CD4$^+$ T cells (CD4$^+$CD62L^{hi}CD44^{lo}) were obtained from spleens and lymph nodes of C57BL/6 wild-type (WT) or PPARβ- or PPARγ-deficient mice. CD4$^+$ T cells were purified from spleen and lymph nodes with anti-CD4 microbeads (Miltenyi Biotec), then were further sorted as naïve CD4$^+$ CD62L^{hi}CD44^{lo} T cells. Isolated naïve T cells were routinely 98% pure. Isolated naïve CD4$^+$ T cells were stimulated with plate-bound antibodies against CD3 (145-2C11, 2 μg/mL; BioXCell) and CD28 (PV-1, 2 μg/mL; BioXCell) and polarized into effector CD4$^+$ T lymphocyte subsets with cytokines in presence or not of DHA (Sigma-Aldrich). Mouse IL-4 (20 ng/mL), IL-6 (25 ng/mL), IL-12 (10 ng/mL), and TGF-β (2 ng/mL) were all purchased from MiltenyiBiotec. Anti-IL-4 (clone 11B11) and anti-IFN-γ (clone XMG1.2; 10 μg/mL) antibodies were obtained from Bio-XCell. In some experiments, the PPARγ agonist troglitazone and the antagonist GW9662 have been added at final concentrations of 0.75 and 0.1 μmol/L, respectively (Cayman Chemical). The PPARα and PPARβ antagonists (GW6471 and GSK0660; Sigma-Aldrich) have been supplemented to reach a final concentration of 0.24 and 0.16 μmol/L, respectively. Cells were classically harvested on day 3 (unless otherwise specified) for detection of cytokines by ELISA and quantitative real-time PCR (qRT-PCR) analysis.

Experiments on human CD4$^+$ T cells were carried out using peripheral blood from healthy volunteers (provided by the "Etablissement Français du Sang" Besançon, France). Written informed consent was obtained from all healthy blood donors. Naïve CD4$^+$ T cells were isolated with the human CD4$^+$ T Cell

Isolation Kit II (MiltenyiBiotec), followed by stimulation with the Expansion/Activation Kit (MiltenyiBiotec), and differentiated for 3 days into T_H0 cells without cytokines or T_H17 cells in presence of TGF-β (2 ng/mL), IL-6 (10 ng/mL), and IL-23 (10 ng/mL).

siRNA transfection

For transfection experiments, naïve $CD4^+$ T cells were transfected *in vitro* with Silencer Select Predesigned siRNA specific for murine *SOCS3* (ID: s72013; Ambion, Life Technologies) or murine *PPARγ* (ID: 160219; Ambion) or Silencer Negative Control N°1 (Ambion) with transfection reagent *Trans*IT-TKO (Mirus Bio LLC) according to the manufacturer's instructions. Twenty-four hours after transfection, $CD4^+$ T cells were restimulated with anti-CD3 and anti-CD28, differentiated in T_H0 or T_H17 conditions as described earlier and cultured for an additional 24 or 48 hours before analysis.

Measurement of cytokines

After 72-hour polarization, cell culture supernatants were assayed by ELISA for mouse IL-4 (BD Biosciences), IL-17a (Biolegend), IFN-γ (BD Biosciences), or human IL-17 (Biolegend) according to the manufacturer's protocol.

For intracellular cytokine staining, cells were cultured for 5 days and then stimulated for 4 hours at 37°C in culture medium containing phorbol 12-myristate 13-acetate (PMA; 50 ng/mL; Sigma-Aldrich), ionomycin (1 μg/mL; Sigma-Aldrich), and monensin (GolgiStop; 1 μL/mL; BD Biosciences). After staining for surface markers and 7-amino-actinomycin D (7-AAD) to exclude dead cells, cells were fixed and permeabilized according to the manufacturer's instructions (Cytofix/Cytoperm Kit; BD Biosciences), then stained for intracellular products. Monoclonal antibodies (mAb) used for flow cytometry analyses were as follows: fluorescein isothiocyanate (FITC)–conjugated anti-CD4 (GK1.5; BD Biosciences), Alexa Fluor 647–conjugated anti-IL-17 (eBio 17B7; eBiosciences), and phycoerythrin (PE)-conjugated anti-IL-4 (554435; BD Biosciences) or PE-conjugated anti-IFN-γ (554412; BD Biosciences).

All events were acquired by a BD LSR-II cytometer equipped with BD FACSDiva software (BD Biosciences) and data were analyzed using FlowJo software (Tree Star).

Immunoblot analysis

Purified naïve T cells were differentiated for 24 to 48 hours into T_H0 or T_H17 cells with or without DHA (20 μmol/L), then collected and pelleted by centrifugation (5 minutes, 1,500 × *g*). Cells were lysed in boiling buffer [1% SDS, 1 mmol/L sodium orthovanadate, and 10 mmol/L Tris (pH 7.4)] containing protease inhibitor cocktail for 20 minutes at 4°C. Cell lysates were subjected to sonication (10 seconds at 10%) and protein concentration was assessed using the Bio-Rad DC Protein Assay Kit. Proteins were then denaturated, loaded, and separated on SDS-PAGE and transferred on nitrocellulose membranes (Schleicher & Schuell). After blocking with 5% bovine serum albumin (BSA) in TBS–0.1% Tween 20 (TBST), membranes were incubated overnight with primary antibody diluted in TBST containing 1% BSA, washed and incubated for 1 hour with secondary antibody diluted in TBST–1% BSA. After additional washes, membranes were incubated with luminol reagent (Santa Cruz Biotechnology) and exposed to X-ray films. The following mouse mAbs were used: anti-STAT3 (Cell Signaling Technology), anti-SOCS3 (Novus Biologicals), and anti-β-actin (Sigma-Aldrich). The following rabbit polyclonal antibody was used: anti-phospho-STAT3-Tyr705 (Cell Signaling Technology). Secondary antibodies HRP-conjugated polyclonal goat anti-mouse (Jackson ImmunoResearch) and polyclonal rabbit anti-goat immunoglobulins (Dako) were also used.

Chromatin immunoprecipitation assay

Cells were differentiated for 24 hours into T_H17 cells with or without DHA. Chromatin immunoprecipitation (ChIP) was conducted according to the manufacturer's instructions (ChiP-IT Express Enzymatic; Active Motif). Briefly, cells were fixed in a solution containing 37% formaldehyde for 10 minutes and quenched with 0.125 mol/L glycine. Chromatin was isolated and sheared to an average length of 300 to 500 bp by Enzymatic Cocktail. Twenty-five micrograms of DNA were immunoprecipitated with a PPARγ-specific antibody (H-1000; Santa Cruz Biotechnology). After chromatin elution, cross-links were reversed by a Reverse Cross-linking Buffer and qRT-PCR was conducted. Data were normalized to *Actin-b* C_t values and expressed in fold enrichment according to immunoglobulin (Ig) values. Primers designed to assess ChIP assay are as follows: *Actb* 5′-actctttgcagccacattcc-3′ and 3′-agcgtctggttcccaatact-5′; *Socs3 1679* 5′-gtcgacattccttctcaggttt-3′ and 5′-gcacccctttcccttttctttt-3′; and *Socs3 576* 5′-cccaggtcctttgcctgatt-3′ and 5′-tgagagaggggaccaggagaaa-3′.

Quantitative real-time PCR

Total RNA from T cells was extracted with TriReagent (Ambion), reverse transcribed using M-MLV Reverse Transcriptase (Invitrogen), and was analyzed by qRT-PCR with the SYBR Green method according to the manufacturer's instructions using the 7500 Fast Real-Time PCR System (Applied Biosystems). Expression was normalized to the expression of mouse *Actb*. Primers designed to assess gene expression are as follows: *Actb* 5′-atggaggggaatacagccc-3′ and 5′-ttctttgcagctccttcgtt-3′; *Tbx21* 5′-atcctgtaatggcttgtggg-3′ and 5′-tcaaccagcaccagacagag-3′; *Gata-3* 5′-aggatgtccctgctctcctt-3′ and 5′-gcctgcggactctaccataa-3′; *Foxp3* 5′-ctcgtctgaaggcagagtca-3′ and 5′-tggcagagaggtattgaggg-3′; *Rorc mus musculus* 5′-ggtgataaccccgtagtgga-3′ and 5′-ctgcaaagaagacccacacc-3′; *Rora* 5′-cccctactgttccttcacca-3′ and 5′-tgccacatcacctctctctg-3′; *Ahr* 5′-ctccttcttgcaaatcctgc-3′ and 5′-ggccaagagcttctttgatg-3′; *il17f* 5′-ttgatgcagcctgagtgtct-3′ and aattccagaaccgctccagt-3′; *il21* 5′-aaaacaggcaaaagctgcat-3′ and 5′-tgacattgttgaacagctgaaa-3′; *il22* 5′-tcgccttgatctctccactc-3′ and gctcagctcctgtcacatca-3′; *cd4* 5′-cctgtgcaagaagcagagtg-3′ and 3′-gttctgctgattccccttcc-5′; *Tgfb1* 5′-caacccaggtccttcctaaa-3′ and 3′-ggagagccctggatacaac-5′; *Rorc homo sapiens* 5′-aagcaggagcaatggaagtg-3′ and 3′-gcaatctcatcctcggaaaa-5′; *Socs3 mus musculus* 5′-aacttgctgtgggtgaccat-3′ and 5′-aaggccggagatttcgct-3′; *Socs3 homo sapiens* 5′-tggatggagcgggaggct-3′ and 5′-acgggacatctttcaccctcaggctcct-3′; *gpr120* 5′-gatttctcctatgcggttgg-3′ and 5′-

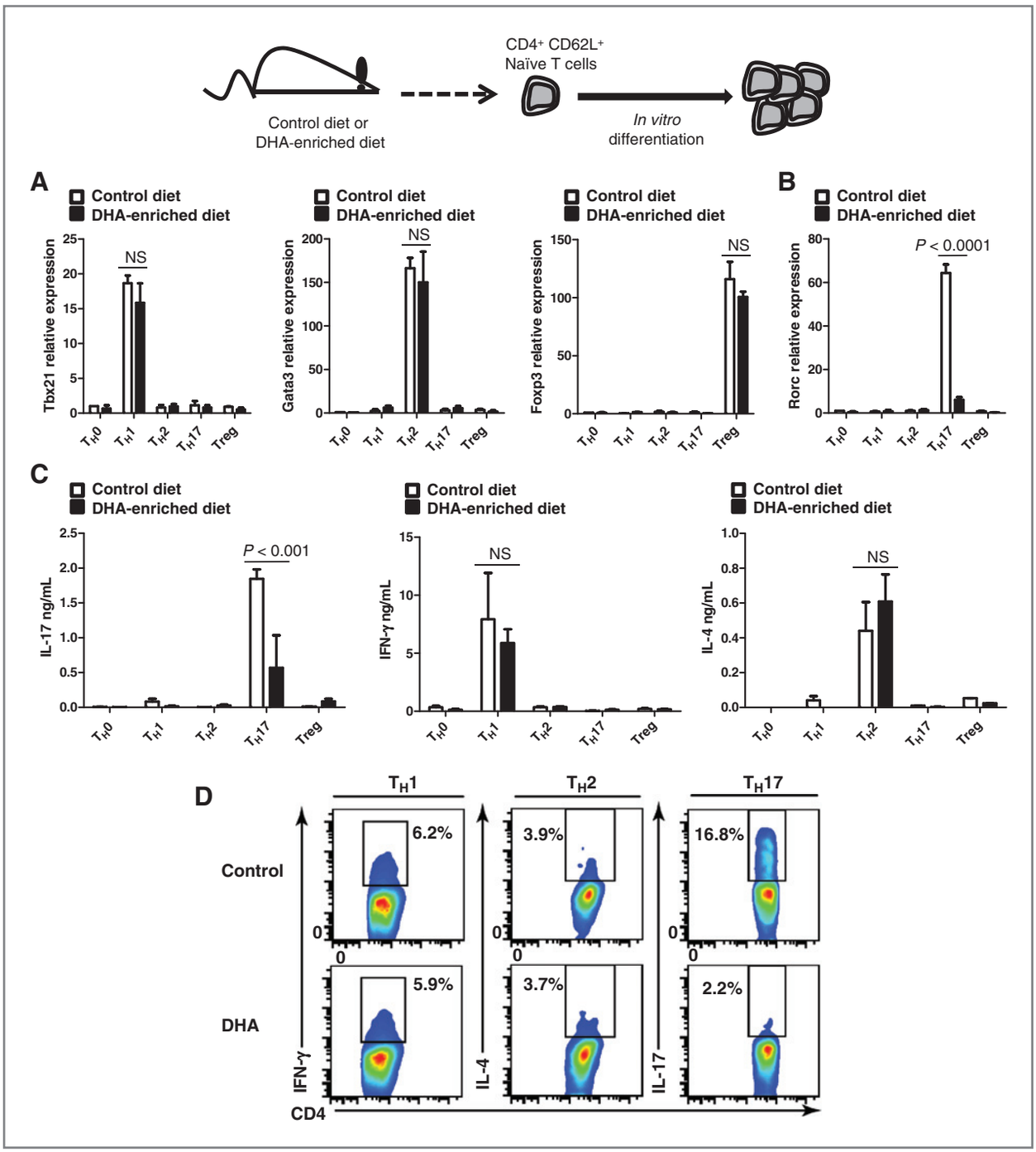
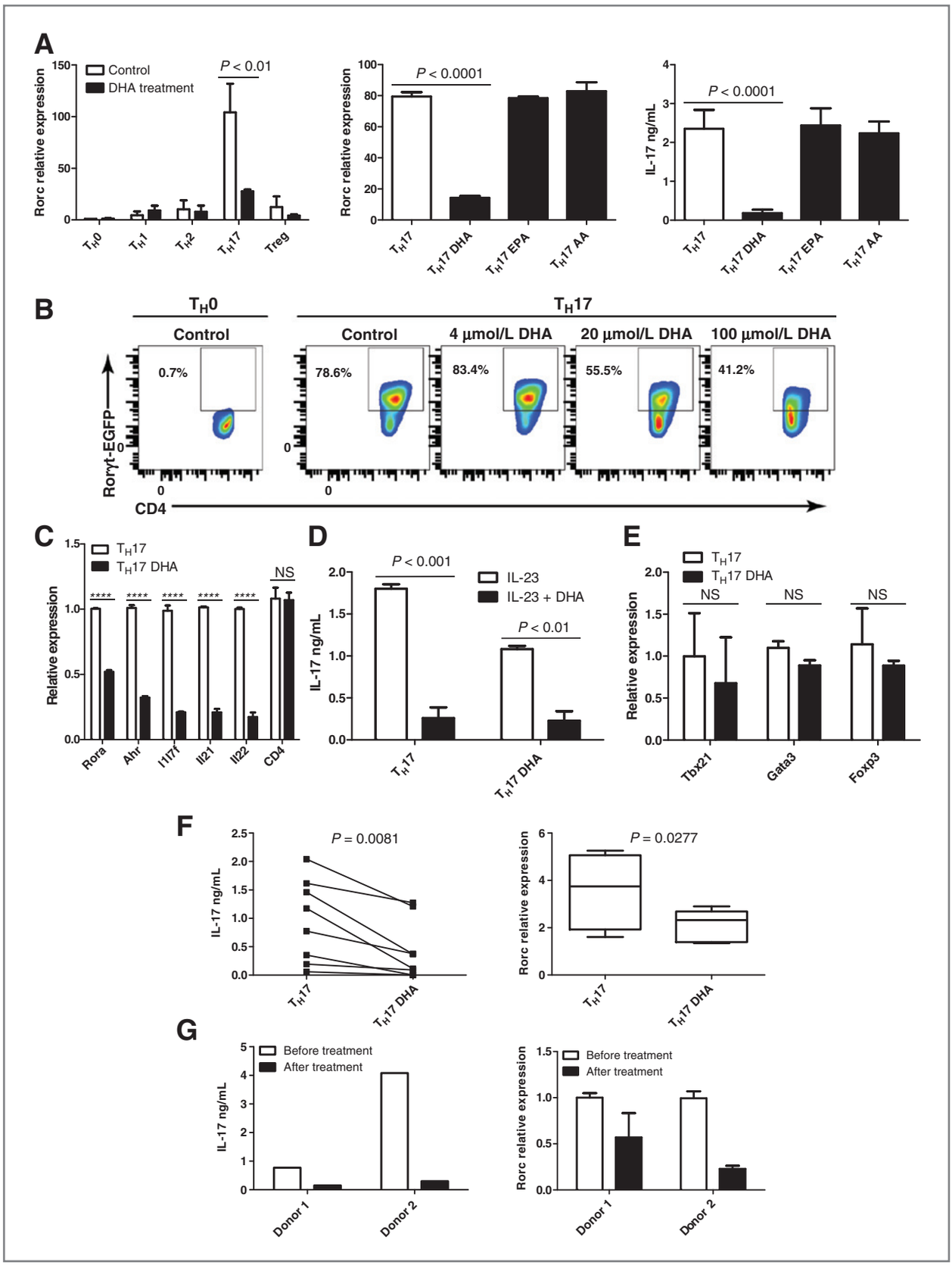


Figure 1. DHA-enriched diet reduces the capacity of naïve CD4$^+$ T cells to differentiate into T_H17 cells. Cell-sorted naïve CD4$^+$CD62L^{hi} CD44^{lo} T cells were isolated from mice under a control or a DHA-enriched diet and differentiated into T_H0, T_H1, T_H2, Treg, or T_H17 cells in the presence of anti-CD3 and anti-CD28 for 72 hours. A and B, qRT-PCR analysis of *Tbx21*, *Gata3*, *Foxp3* (A) and *Rorc* (B) mRNA expression. Expression is presented relative to *Actb* expression. C, ELISA analyses of IL-17 (left), IFN-γ (middle), and IL-4 (right) in supernatants of CD4$^+$ T cells differentiated for 3 days. D, flow cytometry analysis of intracellular staining for IFN-γ, IL-4, and IL-17, respectively, in naïve CD4$^+$ T cells polarized in T_H1, T_H2, and T_H17 conditions assessed on day 5 of culture. Numbers beside outlined areas indicate percentage cells in gate. Representative data from 1 of 3 independent experiments are shown. NS, not significant.

ccctctgcatcttgttcc-3′; *mmp9* ctgtcggctgtggttcagt-3′ and agacgacatagacggcatcc-3′; *cyp27* 5′-gggcactagccagattcaca-3′ and 5′-ctatgtgctgcacttgccc-3′, *clu* 5′-ccattgtcccagatcagca-3′ and 5′-aggaggagcgcactggag-3′; *vegfr* 5′-aagagagtctggcctgcttg-3′ and 5′-ctgctcgggtgtctgctt-3′; and *vegf* 5′-aatgctttctccgctctgaa-3′ and 5′-gcttcctacagcacagcaga-3′.


A
Control
DHA treatment
P < 0.01
Rorc relative expression
TH0
TH1
TH2
TH17
Treg
P < 0.0001
TH17
TH17 DHA
TH17 EPA
TH17 AA
IL-17 ng/mL
B
TH0
TH17
Control
4 µmol/L DHA
20 µmol/L DHA
100 µmol/L DHA
0.7%
78.6%
83.4%
55.5%
41.2%
Rorγt-EGFP
CD4
C
TH17
TH17 DHA
NS
Relative expression
Rora
Ahr
Il17f
Il21
Il22
CD4
D
P < 0.001
IL-23
IL-23 + DHA
P < 0.01
E
NS
Tbx21
Gata3
Foxp3
F
P = 0.0081
P = 0.0277
G
Before treatment
After treatment
Donor 1
Donor 2

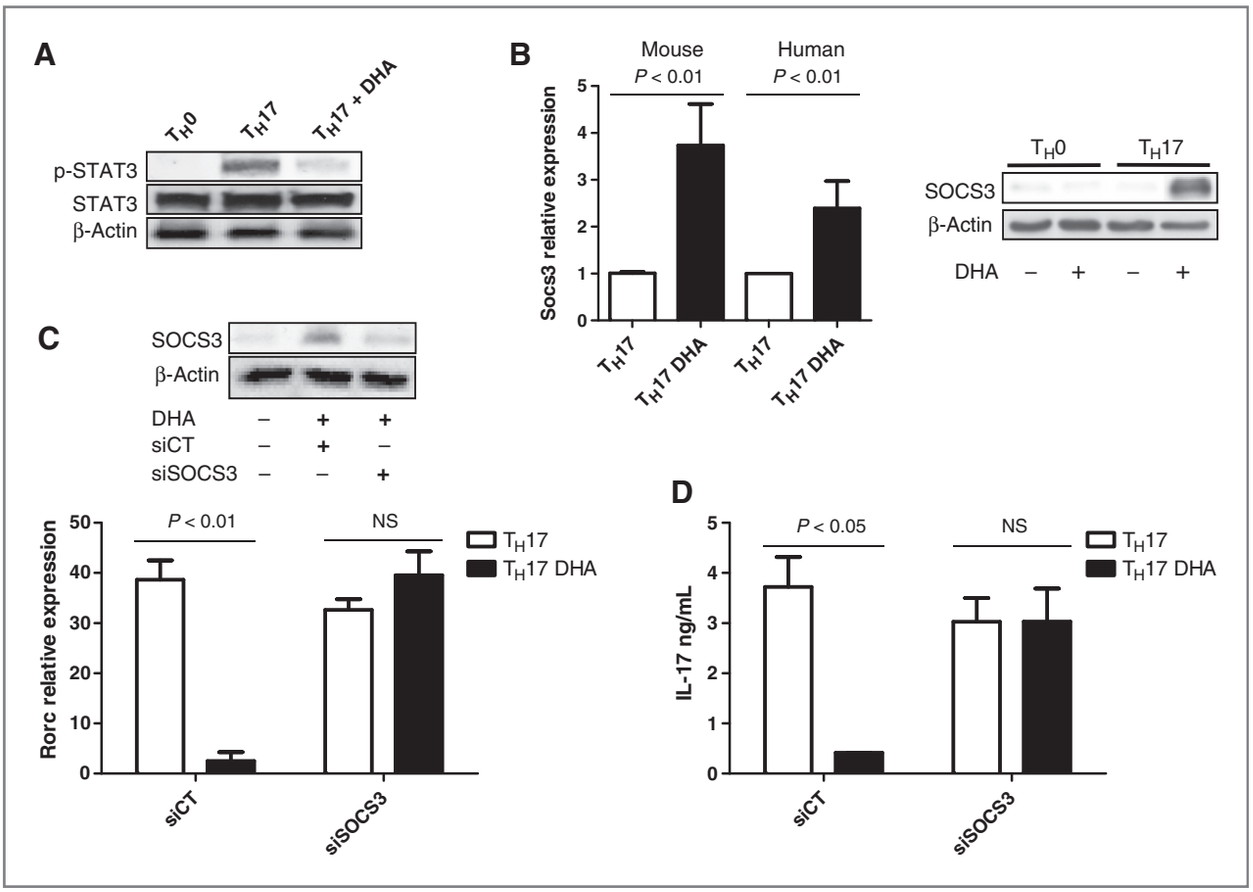


Figure 3. DHA treatment impairs STAT3 phosphorylation and upregulates SOCS3 in T_H17 cells. A, expression of mouse p-STAT3 in T_H0- and T_H17-differentiated cells for 24 hours treated or not with DHA was determined by Western blotting. B, SOCS3 mRNA expression level was assessed in mouse and human control T_H17 cells or DHA-treated counterparts after 3 hours of differentiation by qRT-PCR analysis (left) and activation of mouse protein SOCS3 was examined by immunoblotting (right) in $CD4^+$ T cells polarized under T_H0 or T_H17 conditions 24 hours long in presence or not of DHA. C, $CD4^+$ T cells were transfected with a control siRNA (siCT) or a siRNA specific for SOCS3 (siSOCS3) for 24 hours before being differentiated for further 24 hours into control or DHA T_H17-cell driving conditions, then SOCS3 expression was determined by Western blotting (top) and *Rorc* mRNA induction (bottom) monitored by qRT-PCR. D, T_H17 cells were generated and transfected as in C and IL-17 release was assayed by ELISA. Representative data from 1 of 3 experiments are shown. NS, not significant.

Statistical analyses

Statistical analysis was conducted using Prism software (Graph Pad software). For the analysis of experimental data, comparison of continuous data was achieved by the Mann–Whitney *U* test and comparison of categorical data by Fisher exact test, as appropriate. All *P* values are two-tailed. *P* values less than 0.05 were considered significant. Data are represented as mean ± SD.

Results

In vivo administration of DHA impairs mouse T_H17 cell differentiation from naïve CD4 T cells

Despite reports suggesting that DHA could dampen the production of proinflammatory cytokines from innate immune cells (21, 22), whether DHA affects naïve CD4 T-cell differentiation in a cell-intrinsic manner has not been explored. To test this, we first examined the ability of naïve $CD4^+$ $CD62L^+$ T cells

Figure 2. DHA impairs T_H17 differentiation *in vitro*. A, RNA isolated from naïve $CD4^+CD62L^{hi}$ cells differentiated into T_H0, T_H1, T_H2, Treg, or T_H17 cells with anti-CD3 and anti-CD28 antibodies in the presence or absence of DHA (20 μmol/L) was subjected to qRT-PCR relative to the expression of mRNA-encoding mouse *Actb* to examine the expression of *Rorc* at 72 hours following activation (left). qRT-PCR analysis of *Rorc* expression and ELISA of IL-17 in supernatants of T_H17 cells polarized with or without DHA, EPA (eicosapentaenoic acid, n-3 PUFA), or AA (arachidonic acid, n-6 pufa) on day 3 of culture (right). B, naïve $CD4^+$ T cells from RORγt-eGFP reporter mice were differentiated into T_H17 cells for 72 hours in the presence of indicated escalating doses of DHA and the proportion of RORγt-expressing cells was assessed by flow cytometry. Numbers beside outlined areas indicate percentage cells in each gate. C and E, T_H17 cells were generated as in A and the expression of the *Rora*, *AhR*, *il17f*, *il21*, *il22*, and *CD4* genes (C) and *Tbx21*, *Gata3*, and *Foxp3* genes (E) was assessed by qRT-PCR. D, T_H17 cells were generated with or without DHA as in A, first round, and reactivated for further 48 hours in presence of IL-23 with an additional DHA treatment or not (second round), then the levels of IL-17 released from these cells after the second round of stimulation were determined by ELISA. F, human T_H17 cells were generated from naïve peripheral blood $CD4^+$ T cells isolated from healthy volunteers polarized for 72 hours *in vitro* and treated or not with DHA, then IL-17 secreted in supernatants and *Rorc* mRNA level were assessed, respectively, by ELISA (left) and qRT-PCR (right). G, same analyses as in F but carried out from differentiated T_H17 cells generated before or after a 3-week long DHA supplementation diet. Representative data from 1 of 3 experiments are shown. ****, $P < 0.0001$; NS, not significant.

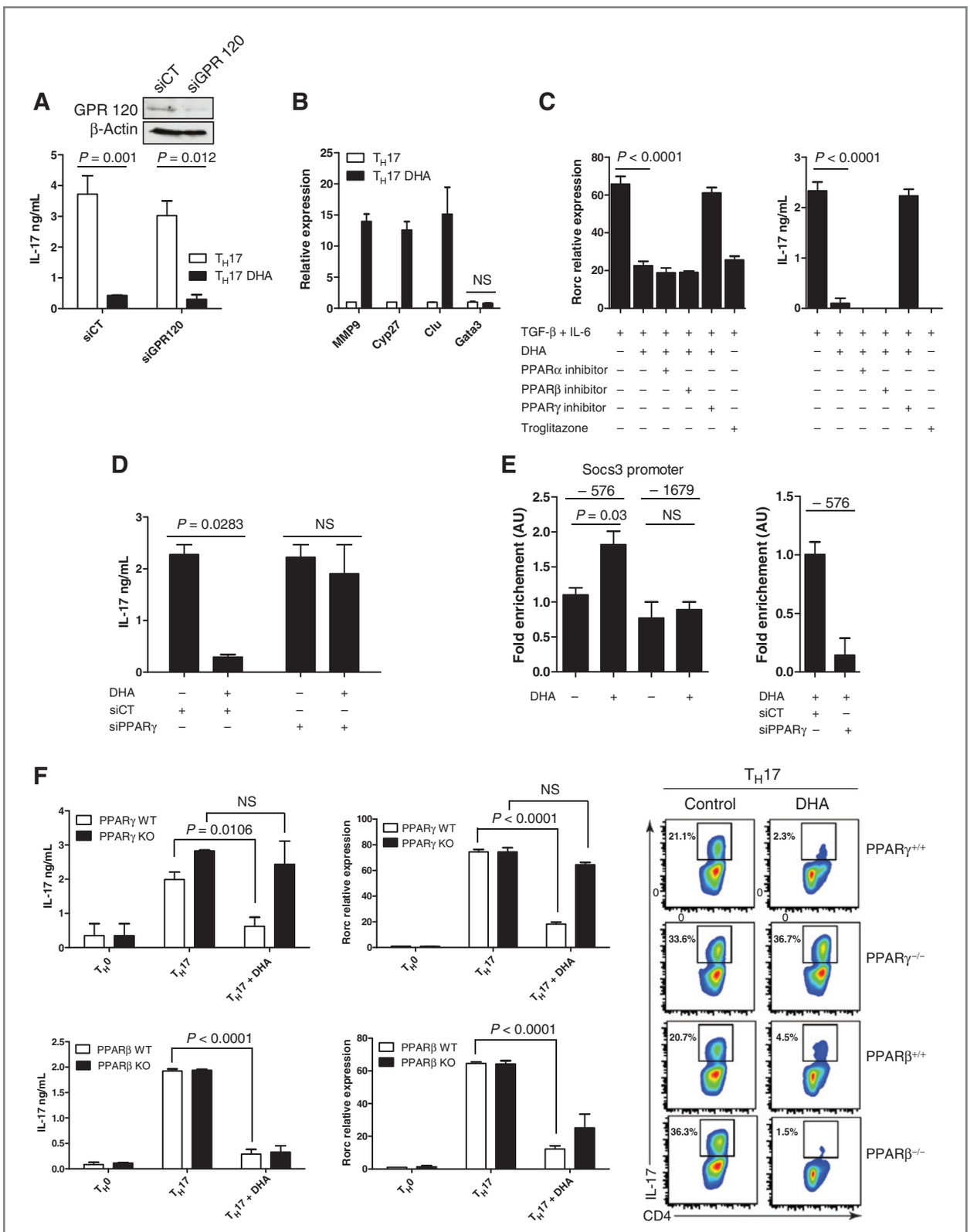


Figure 4. PPARγ transactivates *Socs3* upon activation by DHA. A, expression of mouse GPR120 (top) and IL-17 release from control or GPR120 siRNA-transfected T_H17 cells (bottom) were detected after 48 hours of polarization with or without DHA by Western blotting and ELISA, respectively. B, relative

isolated from mice fed a control or DHA-enriched diet for 3 weeks to differentiate into effector T-cell subsets following activation in absence of antigen-presenting cells. DHA-enriched diet led to accumulation of DHA within splenocytes as previously described (Supplementary Fig. S2; ref. 18). Upon examining T-cell polarization, DHA failed to significantly alter the expression of the transcription factors *Tbx21*, *Gata3*, and *Foxp3* that respectively specify the T_H1, T_H2, and regulatory T cell (Treg) lineages (Fig. 1A). However, naïve $CD4^+$ T cells isolated from mice under a DHA-enriched diet featured a poor capacity to differentiate into T_H17 cells under T_H17-polarizing conditions, as illustrated by their weak expression of the T_H17-cell transcription factor *Rorc* (Fig. 1B). By assessing IL-17a secretion from T_H17 cells generated from mice under a DHA-enriched diet by ELISA, we found reduced IL-17a secretion in comparison with controls, whereas IFN-γ or IL-4 production from T_H1 and T_H2 cells were not affected (Fig. 1C). Intracellular staining data confirmed dampened IL-17a secretion from T_H17 cells from mice receiving DHA *in vivo*, whereas the respective production of IFN-γ or IL-4 from T_H1 and T_H2 cells remained unaffected (Fig. 1D). Overall, these results suggest that DHA selectively affects T_H17 cell differentiation *in vivo*.

DHA inhibits mouse and human T_H17 cell differentiation in a cell-intrinsic manner

To test whether DHA directly affects T_H17 cell development, we differentiated naïve $CD4^+$ T cells without antigen-presenting cells under T_H17 cell-polarizing conditions in the presence of DHA. We observed a marked reduction of *Rorc* expression in T_H17 cells upon DHA treatment, confirming that DHA impairs T_H17 cell differentiation (Fig. 2A). This inhibition is specific for DHA as other PUFAs such as eicosapentaenoic or arachidonic acids did not impair T_H17 cell differentiation (Fig. 2A). To study further the impact of DHA on T_H17 differentiation, we generated T_H17 cells from Rorγt-eGFP reporter mice with increasing doses of DHA. DHA reduced the proportion of Rorγt-expressing cells in a dose-dependent manner, corroborating the DHA capacity to inhibit T_H17 cell differentiation (Fig. 2B). In addition, we also explored the ability of DHA to affect other T_H17-related genes and found that DHA impaired the expression of *Rora*, *Ahr*, *il17f*, *il21*, and *il22*, suggesting that DHA globally affects the T_H17 cell differentiation program (Fig. 2C). IL-23 is essential to maintain the proinflammatory T_H17 cell program both *in vitro* and *in vivo* (23). Because DHA blunted the primary differentiation of naïve CD4 T cells into effector T_H17 cells, we explored the ability of DHA to influence IL-23 capacity to restimulate engaged T_H17 cells. We found that DHA was also able to reduce IL-17a secretion from engaged T_H17 cells restimulated with IL-23 (Fig. 2D). To test whether DHA skewed T_H17 cell differentiation toward another CD4 effector T-cell subset, we monitored the expression of *Tbx21*, *Gata3*, and *Foxp3* genes in T_H17 cells treated with DHA. We found that DHA failed to induce any of the transcription factors defining the T_H1, T_H2, or Treg lineages, indicating that DHA restricts but does not skew T_H17 cell differentiation *in vitro* (Fig. 2E). We tested whether DHA also affects human T_H17 cell differentiation. For this, we first differentiated naïve human $CD4^+$ T cells isolated from the blood of healthy volunteers into T_H17 cells in the presence of DHA. In line with our findings with mouse T cells, we found that DHA also prevented human T_H17 cell differentiation (Fig. 2F). Similarly, upon assessing T_H17 cell induction from naïve human CD4 T cells obtained from healthy individuals before or after 3 weeks of DHA intake as a diet supplement, we found that oral DHA intake decreased T_H17 cell differentiation (Fig. 2G). Altogether, these findings indicate that DHA restrains mouse and human T_H17 cell differentiation.

DHA interferes with T_H17 cell differentiation by inducing Socs3

The transcription factor STAT3 was shown to transactivate the *Il17a/f* and the *Rorc* promoters (24, 25). Because DHA dampened IL-17a/f secretion and *Rorc* expression in developing T_H17 cells, we hypothesized that DHA negatively regulates STAT3 activation during T_H17 cell differentiation. To test this, we induced T_H17 cells from naïve $CD4^+$ T cells *in vitro* in presence or absence of DHA. As expected, we noted a robust STAT3 (p-STAT3) phosphorylation in T_H17 cells generated without DHA (Fig. 3A). In contrast, CD4 T cells incubated under T_H17-polarizing conditions with DHA featured a weak level of p-STAT3, confirming that DHA reduces STAT3 signaling in T_H17 cells (Fig. 3A). Because SOCS3 is a major regulator of STAT3 phosphorylation in T_H17 cells (24), we hypothesized that the DHA-mediated downregulation of p-STAT3 may be due to increased SOCS3 expression. We found that SOCS3 mRNA and protein expression was induced by DHA treatment in mouse and human T_H17 cells, suggesting that the DHA-mediated downregulation of T_H17 cell differentiation could be related to SOCS3 induction (Fig. 3B). To investigate the involvement of SOCS3 in the capacity of DHA to control T_H17 cell differentiation, we downregulated SOCS3 expression in T cells differentiated under T_H17-cell skewing conditions with DHA using siRNA. We found that SOCS3 silencing blunted the capacity of DHA

expression of PPARγ target genes (*MMP9*, *Cyp27*, and *Clu*) and *Gata3* as control within T_H17 cells exposed or not to DHA *in vitro*. C, mouse T_H17 cells were differentiated as described in Materials and Methods with control or DHA in presence or absence of specific PPARs ligands (PPARs inhibitors and troglitazone). *Rorc* mRNA level was monitored in these cells (left) and the amount of IL-17 secreted was quantified by ELISA (right) at 3 days following activation. D, ELISA of IL-17 in supernatants of T_H17 cells treated or not with DHA for 48 hours after transfection with control (siCT) or PPARγ siRNA (siPPARγ). E, ChIP analysis of the interaction between PPARγ and Socs3 promoter in *in vitro* differentiated T_H17 cells transfected either with siCT or siPPARγ after DHA treatment or not. Analysis on the 2 putative binding sites for PPARγ on *Socs3* promoter (−1679 and −576) is shown. F, naïve $CD4^+$ T cells from PPARγ- (top) and PPARβ (bottom)–deficient mice or WT counterparts were polarized into T_H0, T_H17, and DHA-treated T_H17 lymphocytes. At day 3 of culture, IL-17 secretion and *Rorc* expression levels were monitored by ELISA and qRT-PCR, respectively. At day 5 and after restimulation, flow cytometry of the intracellular production of IL-17 by these cells in presence or not of DHA was assessed (right). Representative data from 1 of 3 experiments are shown. NS, not significant; KO, knockout.

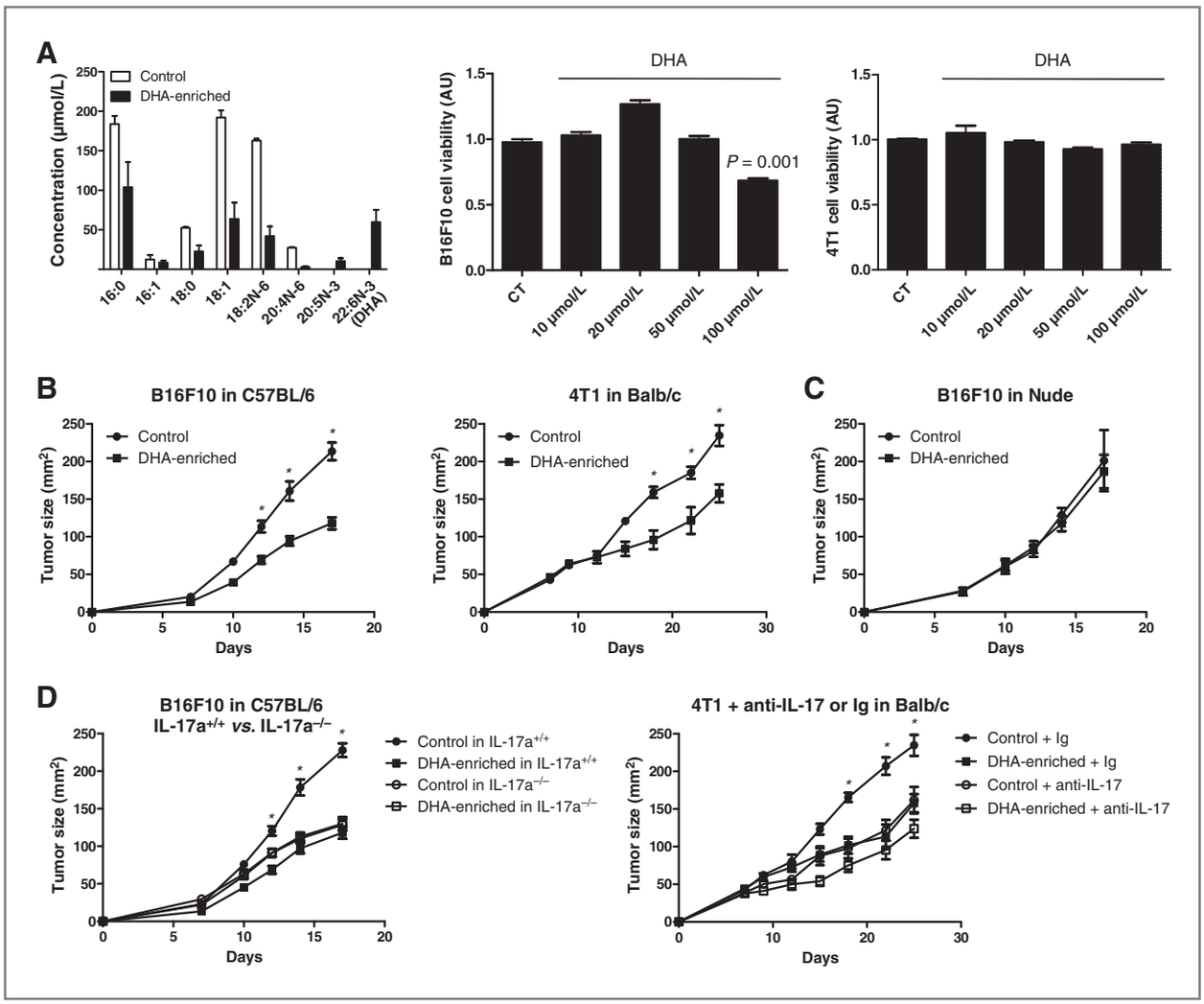


Figure 5. DHA prevents tumor outgrowth *in vivo* in an IL-17–dependent manner. A, plasma major fatty acids concentration of mice under control or DHA-enriched diet was assessed by gas chromatographic analysis (left). B16F10 melanoma and 4T1 mammary adenocarcinoma tumor cells (respectively, middle and right) were incubated with increasing doses of DHA for 72 hours and the cell viability was assessed by MTT assay. B, C57BL/6 (left) and BALB/c mice (right) under control or DHA-enriched diet were respectively inoculated with B16F10 and 4T1 tumor cells and tumor growth was monitored over 3 weeks. C, same as in B, but B16F10 were injected subcutaneously in Nude mice. D, monitoring of tumor growth of respectively B16F10 melanoma cells in C57BL/6 and *IL17a*$^{-/-}$ mice (left) and of 4T1 in BALB/c mice injected with an anti-IL-17 antibody (200 μg/day injected on day 0, 1, 2, 3, 4, and 6) or control rat immunoglobulin G (IgG). All along these experiments, animals were either given control or DHA-enriched diet *, $P < 0.05$.

to restrain IL-17 and RORγt expression in T_H17 cells (Fig. 3C and D). Collectively, our findings suggest that DHA restrains T_H17 cell differentiation by interfering with STAT3 signaling through SOCS3 induction.

DHA prevents T_H17 cell differentiation in a PPARγ-dependent manner

To unravel the mechanism accounting for SOCS3 induction in developing T_H17 cells upon DHA treatment, we investigated the intracellular signaling pathways engaged by DHA on T_H17 cells. GPR120 was recently identified as an omega-3 fatty acid receptor (26). To test the involvement of GPR120 in the DHA-driven impairment of T_H17 cell differentiation, we downregulated GPR120 expression with siRNA in naïve T cells and analyzed the effect of DHA during T_H17 differentiation. Our results showed no significant differences in T_H17 polarization between cells transfected with control or GPR120 siRNA, indicating that the decreased T_H17 cell induction upon DHA treatment does not depend on GPR120 (Fig. 4A). PPARγ is another proposed receptor for omega-3 fatty acid. Synthetic PPARγ ligands have been reported to induce *Socs3* expression and inhibit STAT3 activation (27). Given that DHA interfered with T_H17 cell differentiation through STAT3 signaling pathway blockade, we hypothesized that the effects of DHA on T_H17 cells relied on the transcription factor PPARγ. Gene expression analysis revealed that the known PPARγ target genes *MMP9*, *Cyp27*, and *Clu* were induced in DHA-treated T_H17 cells, suggesting

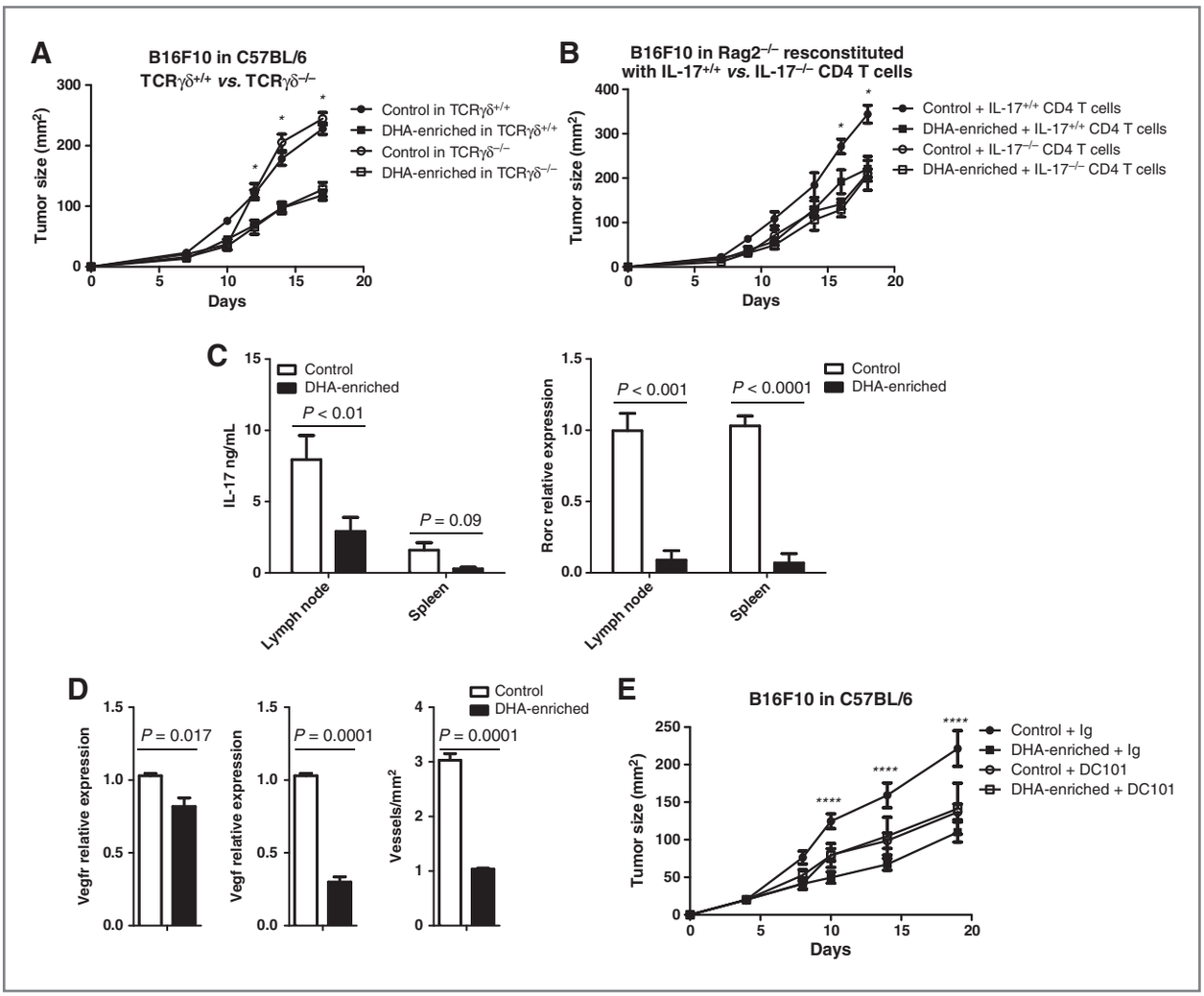


Figure 6. DHA limits CD4 T cell–derived IL-17 secretion and tumor angiogenesis. A, C57BL/6 γδTCR-deficient mice under control or DHA-enriched diet were respectively inoculated with B16F10 tumor cells and tumor growth was monitored over 3 weeks. B, Rag2$^{-/-}$ mice received intravenous αβ CD4 T cells isolated from either IL-17$^{+/+}$ or IL-17$^{-/-}$ mice and were subsequently fed a control or DHA-enriched diet. Five weeks later, mice were injected with B16F10 melanoma cancer cells and tumor growth was monitored. Pooled data from 2 experiments are shown. C, CD4^{+} T cells isolated from draining lymph nodes and spleen of B16F10 tumor-bearing immunocompetent mice were activated 3 days with anti-CD3 and anti-CD28 antibodies. Then, IL-17 release was controlled by ELISA (left) and Rorc mRNA expression level was determined by qRT-PCR analysis (right). D, qRT-PCR analysis of *Vegfr* and *Vegf* mRNA level in B16F10 tumor extracts from immunocompetent tumor-bearing mice treated as in Fig. 5B and number of CD31-positive blood vessels in these tumors. E, same as in Fig. 5B but C57BL/6 mice were treated or not with an anti-VEGFR2 (clone DC101) antibody (200 μg/day injected on day 1 and 5). Representative data from 1 of 3 experiments are shown. *, $P < 0.05$; ****, $P < 0.0001$.

that PPARγ signaling is activated by DHA treatment on T_H17 cells (Fig. 4B). In addition, the DHA effect on T_H17 cell differentiation was markedly reduced in the presence of a PPARγ inhibitor GW9662, but not with a PPARβ inhibitor GSK0660 or PPARα inhibitor GW6471, as measured by IL-17 secretion and *Rorc* expression (Fig. 4C). Moreover, PPARγ agonist troglitazone neutralized IL-17 secretion from T_H17 cells to a comparable extent to DHA, suggesting that PPARγ activation is involved in DHA effects on T_H17 cells (Fig. 4C). To validate the elective involvement of PPARγ in the intracellular effects of DHA on T_H17 cells, we downregulated PPARγ expression with siRNA and tested the ability of DHA to inhibit T_H17 cell differentiation in absence of PPARγ. We found that silencing PPARγ completely abolished the ability of DHA to restrain T_H17 cell induction (Fig. 4D). Although PPARγ agonists induce *Socs3* expression (27, 28), the direct link between PPARγ and SOCS3 remains unclear. To test whether the DHA-driven *Socs3* induction is directly due to PPARγ activation, we conducted a bioinformatic analysis of the *Socs3* promoter and found 2 putative binding sites for PPARγ at positions −1679 and −576 from the transcription start site. By conducting ChIP, we found that PPARγ binds to the *Socs3* promoter only at position −576 in DHA-treated T_H17 cells as compared with control T_H17 cells (Fig. 4E). We further checked the specificity of this interaction by downregulating PPARγ expression by siRNA and confirmed that

the amount of PPARγ-binding *Socs3* promoter was accordingly reduced (Fig. 4E). Finally, we tested the effects of DHA on naïve T cells from WT-, PPARγ-, or PPARβ-deficient mice. In line with our results obtained with the PPAR inhibitors, we found that while DHA could suppress T_H17 cell differentiation from WT- and PPARβ-deficient T cells, PPARγ-deficient T cells were totally unaffected by DHA treatment (Fig. 4F). Altogether, these findings show that the ability of DHA to hamper T_H17 cell differentiation is strictly dependent on PPARγ-dependent Socs3 expression.

DHA prevents tumor growth *in vivo* in an IL-17–dependent manner

Although DHA was proposed to promote cancer cell death (29), we found that DHA did not exert direct cytotoxic effects on mouse melanoma B16F10 and in 4T1 mammary adenocarcinoma tumor cells *in vitro* at concentrations that could be reached *in vivo* (60 μmol/L; Fig. 5A). However, we noted that the DHA-enriched diet delays subcutaneous tumor growth *in vivo* in both cancer models (Fig. 5B). In contrast, DHA did not show any antitumor efficacy in nude mice in both models suggesting that DHA mediates its anticancer function through T cells (Fig. 5C and Supplementary Fig. S3). Given that DHA impedes proinflammatory cytokine release, which has been proposed to contribute to B16F10 and 4T1 tumor growth (8, 30–32), we tested whether DHA affected tumor growth *in vivo* in these 2 tumor models by affecting IL-1, IL-6, or IL-17 release. DHA still exhibited *in vivo* anticancer activity against B16F10 tumors in absence of IL-1 and IL-6 (Supplementary Fig. S4). However, DHA failed to exhibit any effect on tumor progression in *IL-17a*$^{-/-}$ mice, suggesting that the anticancer effects of DHA are dependent on IL-17 (Fig. 5D). These results were confirmed by neutralizing IL-17 *in vivo* in the 4T1 tumor model (Fig. 5D). As a control, IL-17 mAb did not exert any direct cytotoxic effect on tumor cells, ruling out any direct effect of IL-17 on tumor cell growth (Supplementary Fig. S5).

Because γδ T cells and T_H17 cells are major IL-17 sources *in vivo*, we investigated whether DHA anticancer efficacy relied on γδ T cells and IL-17–producing CD4 T cells. We found that the DHA-enriched diet could prevent tumor growth in γδTCR-deficient mice, ruling out any significant contribution of these cells in DHA anticancer activity (Fig. 6A). However, upon testing DHA antitumor effect in T-cell–deficient Rag2$^{-/-}$ mice reconstituted with either IL-17$^{+/+}$ or IL-17$^{-/-}$ αβ CD4 T cells, we failed to note any therapeutic benefit of the DHA diet in mice that had received IL-17–deficient CD4 T cells (Fig. 6B). We also measured T_H17 cell responses from WT mice under the control or DHA-enriched diet. In line with our previous findings, draining lymph node CD4 T cells from B16F10 tumor-bearing mice under the DHA-enriched diet expressed lower amounts of IL-17 and *Rorc* than controls (Fig. 6C). Together these data suggest that DHA may affect tumor growth via this capacity to regulate IL-17 secretion from T_H17 cells.

IL-17A is a proangiogenic factor that favors neovascularization and tumor growth *in vivo* via its capacity to induce VEGF production (8, 33). In line with the documented angiogenic properties of IL-17A, we noted that B16F10 tumors from mice under DHA-enriched diet featured decreased tumor vasculature reflected by reduced *vegfr* and *vegf* expression and reduced number of CD31-positive blood vessels (Fig. 6D). Finally, to determine the role of VEGF in the antitumor effect of DHA, we treated tumor-bearing mice fed or not a DHA-enriched diet with the VEGF receptor (VEGFR)-blocking mAb, DC101. We observed that while DHA and anti-VEGF therapy exhibited similar antitumor effects, these treatments failed to synergize (Fig. 6E). Collectively, these data suggest that the activity of DHA relies on the prevention of IL-17–dependent tumor neoangiogenesis.

Discussion

Although activation of the transcription factor PPARγ has been associated with reduced inflammation, the intracellular series of events consecutive to PPARγ activation remain incompletely understood and the actual involvement of PPARγ in the anti-inflammatory effects of PPARγ ligands remains debated (34, 35). In this study, we propose what we believe to be a novel molecular pathway by which PPARγ contributes to limit the secretion of the proinflammatory cytokine IL-17. DHA activates PPARγ, which binds to the *Socs3* promoter and drives *Socs3* expression in nascent T_H17 cells, leading to reduced STAT3 phosphorylation, *Rorc* expression and IL-17 secretion. Finally, DHA prevents tumor growth *in vivo* through the downregulation of IL-17 production.

Although our data indicate that DHA inhibits T_H17 cell induction through downregulating the STAT3 signaling pathway, the strong inhibitory effect of DHA on T_H17 cell differentiation suggests that DHA could act at several levels on the T_H17 cell differentiation program. Indeed, activation of PPARγ has been shown to prevent the clearance of corepressors from the *Rorγt* promoter (36), resulting in repressed T_H17 cell generation. We cannot rule out that this signaling pathway plays an additional role in DHA effect on differentiating T_H17 cells.

The links between inflammation and cancer have been thoroughly documented as illustrated by data showing an increased risk of patients suffering from inflammatory bowel disease to develop cancer compared with healthy individuals (37). Chronic inflammation can indeed contribute to cell transformation and cancer formation. Dietary supplementation of DHA has been shown to alleviate the severity of intestinal inflammation (38, 39) in experimental models of colitis and in inflammatory bowel disease in humans (40, 41). These findings possibly explain why dietary (n-3) PUFAs intake was associated with decreased risk of cancer (42, 43). However, recent data indicate that DHA also improves outcome of chemotherapy in patients with metastatic breast cancer, suggesting that it not only affects cancer incidence, but can be therapeutically used to prevent tumor growth (44). Our study brings up evidence that the anticancer effects of DHA could rely on its ability to dampen IL-17 levels *in vivo*.

The role of T_H17 cells during cancer growth remains intensely debated. Adoptive transfer experiments have shown the anticancer potential of both mouse and human T_H17 cells (45, 46). It was further shown recently that the remarkable ability of T_H17 cells to eliminate tumors in these settings relied on their ability to self-renew and transdifferentiate into IFN-γ–producing cells *in vivo* (46). However, in some human

cancers IL-17 was shown to be associated with poor prognosis (47–49). Mouse and human studies revealed an association between IL-17 levels in the tumor bed, VEGF, and blood vessels number (33, 50). In addition, IL-17 has the capacity to directly trigger VEGF release from VEGFR-expressing cells such as cancer cells and stromal cells (33). These data support the contention that IL-17 is an important factor, which drives VEGF-dependent neoangiogenesis during cancer growth. Anti-VEGF therapies are being implemented for cancer treatment in humans but are expensive. In this regard, our study supports the antiangiogenic property of a DHA-enriched diet via its capacity to regulate IL-17 production and VEGF-related angiogenesis. If such results could be confirmed in patients with cancer, DHA should be considered as a potential antiangiogenic candidate for future clinical trials.

Altogether, our study unraveled that DHA-mediated PPARγ activation limits T_H17 cell differentiation by inducing SOCS3 expression. Because our results suggest that the activity of DHA is relevant in humans, DHA administration might prove beneficial in diseases where IL-17 cytokine secretion is undesirable.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. Ladoire, A. Hichami, F. Ghiringhelli, L. Apetoh
Development of methodology: F. Végran, B. Ryffel, A. Hichami
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H. Berger, F. Végran, M. Chikh, F. Gilardi, S. Ladoire, H. Bugaut, G. Mignot, M. Bruchard, B. Ryffel, C. Pot, B. Desvergne, L. Apetoh
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): H. Berger, F. Végran, S. Ladoire, L. Apetoh
Writing, review, and/or revision of the manuscript: H. Berger, F. Végran, M. Chikh, F. Gilardi, G. Mignot, C. Rébé, C. Pot, B. Desvergne, F. Ghiringhelli, L. Apetoh
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): F. Chalmin, V. Derangère, A. Chevriaux, C. Rébé
Study supervision: L. Apetoh

Grant Support

The authors are supported by grants from the Ligue Nationale contre le Cancer (F. Ghiringhelli and F. Végran), the Fondation de France (F. Ghiringhelli), the Institut National du Cancer (F. Ghiringhelli), the Association pour la recherche sur le cancer (F. Ghiringhelli and G. Mignot), the Conseil Régional Bourgogne/INSERM (H. Berger), the Etat de Vaud and the Swiss National Funds for Research (B. Desvergne), the CNRS, FEDER, Le Studium, Orléans and Fondation pour la Recherche Médicale (B. Ryffel), the Agence Nationale de la Recherche (ANR-10-PDOC-014-01; L. Apetoh), the Ligue Régionale contre le cancer Comité Grand-Est (L. Apetoh) and the European Community (Marie Curie Fellowship PCIG10-GA-2011-303719; L. Apetoh). This work was supported by a French Government grant managed by the French National Research Agency under the program "Investissements d'Avenir" with reference ANR-11-LABX-0021.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 31, 2012; revised March 25, 2013; accepted April 4, 2013; published OnlineFirst April 25, 2013.

References

1. Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. Nat Med 2007;13:1050–9.
2. Ghiringhelli F, Apetoh L, Tesniere A, Aymeric L, Ma Y, Ortiz C, et al. Activation of the NLRP3 inflammasome in dendritic cells induces IL-1beta-dependent adaptive immunity against tumors. Nat Med 2009;15:1170–8.
3. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature 2008;454:436–44.
4. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol 2009;27:485–517.
5. Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17$^+$ T helper cells. Cell 2006;126:1121–33.
6. Harris TJ, Grosso JF, Yen HR, Xin H, Kortylewski M, Albesiano E, et al. Cutting edge: an *in vivo* requirement for STAT3 signaling in TH17 development and TH17-dependent autoimmunity. J Immunol 2007;179:4313–7.
7. Yang XO, Panopoulos AD, Nurieva R, Chang SH, Wang D, Watowich SS, et al. STAT3 regulates cytokine-mediated generation of inflammatory helper T cells. J Biol Chem 2007;282:9358–63.
8. Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H. IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. J Exp Med 2009;206:1457–64.
9. Bang HO, Dyerberg J, Nielsen AB. Plasma lipid and lipoprotein pattern in Greenlandic West-coast Eskimos. Lancet 1971;1:1143–5.
10. Bang HO, Dyerberg J, Sinclair HM. The composition of the Eskimo food in north western Greenland. Am J Clin Nutr 1980;33:2657–61.
11. Finocchiaro C, Segre O, Fadda M, Monge T, Scigliano M, Schena M, et al. Effect of n-3 fatty acids on patients with advanced lung cancer: a double-blind, placebo-controlled study. Br J Nutr 2012;108:327–33.
12. Li H, Ruan XZ, Powis SH, Fernando R, Mon WY, Wheeler DC, et al. EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: evidence for a PPAR-gamma-dependent mechanism. Kidney Int 2005;67:867–74.
13. Siddiqui RA, Harvey KA, Xu Z, Bammerlin EM, Walker C, Altenburg JD. Docosahexaenoic acid: a natural powerful adjuvant that improves efficacy for anticancer treatment with no adverse effects. Biofactors 2011;37:399–412.
14. Lochner M, Peduto L, Cherrier M, Sawa S, Langa F, Varona R, et al. *In vivo* equilibrium of proinflammatory IL-17$^+$ and regulatory IL-10$^+$ Foxp3$^+$ RORgamma t$^+$ T cells. J Exp Med 2008;205:1381–93.
15. Nakae S, Komiyama Y, Nambu A, Sudo K, Iwase M, Homma I, et al. Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. Immunity 2002;17:375–87.
16. Nadra K, Quignodon L, Sardella C, Joye E, Mucciolo A, Chrast R, et al. PPARgamma in placental angiogenesis. Endocrinology 2010;151:4969–81.
17. Nadra K, Anghel SI, Joye E, Tan NS, Basu-Modak S, Trono D, et al. Differentiation of trophoblast giant cells and their metabolic functions are dependent on peroxisome proliferator-activated receptor beta/delta. Mol Cell Biol 2006;26:3266–81.
18. Triboulot C, Hichami A, Denys A, Khan NA. Dietary (n-3) polyunsaturated fatty acids exert antihypertensive effects by modulating calcium signaling in T cells of rats. J Nutr 2001;131:2364–9.
19. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37:911–7.
20. Pace-Asciak CR. One-step rapid extractive methylation of plasma nonesterified fatty acids for gas chromatographic analysis. J Lipid Res 1989;30:451–4.
21. Almallah YZ, El-Tahir A, Heys SD, Richardson S, Eremin O. Distal procto-colitis and n-3 polyunsaturated fatty acids: the mechanism (s) of natural cytotoxicity inhibition. Eur J Clin Invest 2000;30:58–65.

22. Kong W, Yen JH, Ganea D. Docosahexaenoic acid prevents dendritic cell maturation, inhibits antigen-specific Th1/Th17 differentiation and suppresses experimental autoimmune encephalomyelitis. Brain Behav Immun 2011;25:872–82.
23. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005;201:233–40.
24. Chen Z, Laurence A, Kanno Y, Pacher-Zavisin M, Zhu BM, Tato C, et al. Selective regulatory function of Socs3 in the formation of IL-17-secreting T cells. Proc Natl Acad Sci U S A 2006;103:8137–42.
25. Durant L, Watford WT, Ramos HL, Laurence A, Vahedi G, Wei L, et al. Diverse targets of the transcription factor STAT3 contribute to T cell pathogenicity and homeostasis. Immunity 2010;32:605–15.
26. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell 2010;142:687–98.
27. Yu JH, Kim KH, Kim H. SOCS 3 and PPAR-gamma ligands inhibit the expression of IL-6 and TGF-beta1 by regulating JAK2/STAT3 signaling in pancreas. Int J Biochem Cell Biol 2008;40:677–88.
28. Park EJ, Park SY, Joe EH, Jou I. 15d-PGJ2 and rosiglitazone suppress Janus kinase-STAT inflammatory signaling through induction of suppressor of cytokine signaling 1 (SOCS1) and SOCS3 in glia. J Biol Chem 2003;278:14747–52.
29. Molinari R, D'Eliseo D, Manzi L, Zolla L, Velotti F, Merendino N. The n3-polyunsaturated fatty acid docosahexaenoic acid induces immunogenic cell death in human cancer cell lines via pre-apoptotic calreticulin exposure. Cancer Immunol Immunother 2011;60:1503–7.
30. Nam JS, Terabe M, Kang MJ, Chae H, Voong N, Yang YA, et al. Transforming growth factor beta subverts the immune system into directly promoting tumor growth through interleukin-17. Cancer Res 2008;68:3915–23.
31. Garcia de G, Boyano D, Smith-Zubiaga I, Alvarez A, Canton I, Canavate L. Involvement of interleukin-6 in the biology and metastatic activity of B16F10 melanoma cells. Eur Cytokine Netw 1998;9:187–92.
32. Bunt SK, Yang L, Sinha P, Clements VK, Leips J, Ostrand-Rosenberg S. Reduced inflammation in the tumor microenvironment delays the accumulation of myeloid-derived suppressor cells and limits tumor progression. Cancer Res 2007;67:10019–26.
33. Numasaki M, Fukushi J, Ono M, Narula SK, Zavodny PJ, Kudo T, et al. Interleukin-17 promotes angiogenesis and tumor growth. Blood 2003;101:2620–7.
34. Yki-Jarvinen H. Thiazolidinediones. N Engl J Med 2004;351:1106–18.
35. Shibata N, Kawaguchi-Niida M, Yamamoto T, Toi S, Hirano A, Kobayashi M. Effects of the PPARgamma activator pioglitazone on p38 MAP kinase and IkappaBalpha in the spinal cord of a transgenic mouse model of amyotrophic lateral sclerosis. Neuropathology 2008;28:387–98.
36. Klotz L, Burgdorf S, Dani I, Saijo K, Flossdorf J, Hucke S, et al. The nuclear receptor PPAR gamma selectively inhibits Th17 differentiation in a T cell-intrinsic fashion and suppresses CNS autoimmunity. J Exp Med 2009;206:2079–89.
37. Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. Am J Physiol Gastrointest Liver Physiol 2004;287:G7–17.
38. Belluzzi A, Boschi S, Brignola C, Munarini A, Cariani G, Miglio F. Polyunsaturated fatty acids and inflammatory bowel disease. Am J Clin Nutr 2000;71:339S–42S.
39. Almallah YZ, Ewen SW, El-Tahir A, Mowat NA, Brunt PW, Sinclair TS, et al. Distal proctocolitis and n-3 polyunsaturated fatty acids (n-3 PUFAs): the mucosal effect *in situ*. J Clin Immunol 2000;20:68–76.
40. Nieto N, Torres MI, Rios A, Gil A. Dietary polyunsaturated fatty acids improve histological and biochemical alterations in rats with experimental ulcerative colitis. J Nutr 2002;132:11–9.
41. Shimizu T, Igarashi J, Ohtuka Y, Oguchi S, Kaneko K, Yamashiro Y. Effects of n-3 polyunsaturated fatty acids and vitamin E on colonic mucosal leukotriene generation, lipid peroxidation, and microcirculation in rats with experimental colitis. Digestion 2001;63:49–54.
42. Fradet V, Cheng I, Casey G, Witte JS. Dietary omega-3 fatty acids, cyclooxygenase-2 genetic variation, and aggressive prostate cancer risk. Clin Cancer Res 2009;15:2559–66.
43. Thiebaut AC, Chajes V, Gerber M, Boutron-Ruault MC, Joulin V, Lenoir G, et al. Dietary intakes of omega-6 and omega-3 polyunsaturated fatty acids and the risk of breast cancer. Int J Cancer 2009;124:924–31.
44. Bougnoux P, Hajjaji N, Ferrasson MN, Giraudeau B, Couet C, Le Floch O. Improving outcome of chemotherapy of metastatic breast cancer by docosahexaenoic acid: a phase II trial. Br J Cancer 2009;101:1978–85.
45. Kryczek I, Zhao E, Liu Y, Wang Y, Vatan L, Szeliga W, et al. Human TH17 cells are long-lived effector memory cells. Sci Transl Med 2011;3:104ra0.
46. Muranski P, Borman ZA, Kerkar SP, Klebanoff CA, Ji Y, Sanchez-Perez L, et al. Th17 cells are long lived and retain a stem cell-like molecular signature. Immunity 2011;35:972–85.
47. Zhang JP, Yan J, Xu J, Pang XH, Chen MS, Li L, et al. Increased intratumoral IL-17-producing cells correlate with poor survival in hepatocellular carcinoma patients. J Hepatol 2009;50:980–9.
48. Chen X, Wan J, Liu J, Xie W, Diao X, Xu J, et al. Increased IL-17–producing cells correlate with poor survival and lymphangiogenesis in NSCLC patients. Lung Cancer 2010;69:348–54.
49. Tosolini M, Kirilovsky A, Mlecnik B, Fredriksen T, Mauger S, Bindea G, et al. Clinical impact of different classes of infiltrating T cytotoxic and helper cells (Th1, th2, treg, th17) in patients with colorectal cancer. Cancer Res 2011;71:1263–71.
50. Liu J, Duan Y, Cheng X, Chen X, Xie W, Long H, et al. IL-17 is associated with poor prognosis and promotes angiogenesis via stimulating VEGF production of cancer cells in colorectal carcinoma. Biochem Biophys Res Commun 2011;407:348–54.