B Cell Activity Is Impaired in Human and Mouse Obesity and Is Responsive to an Essential Fatty Acid upon Murine Influenza Infection

Rasagna Kosaraju,*,†,1 William Guesdon,*,†,1 Miranda J. Crouch,*,†,1
Heather L. Teague,*,†,2 E. Madison Sullivan,*,† Erik A. Karlsson,‡ Stacey Schultz-Cherry,‡
Kymberly Gowdy,§ Lance C. Bridges,*,†,3 Lauren R. Reese,†,¶ P. Darrell Neufer,†,¶
Michael Armstrong,‖ Nichole Reisdorph,‖ J. Justin Milner,#,4 Melinda Beck,# and
Saame Raza Shaikh*,†

Obesity is associated with increased risk for infections and poor responses to vaccinations, which may be due to compromised B cell function. However, there is limited information about the influence of obesity on B cell function and underlying factors that modulate B cell responses. Therefore, we studied B cell cytokine secretion and/or Ab production across obesity models. In obese humans, B cell IL-6 secretion was lowered and IgM levels were elevated upon ex vivo anti-BCR/TLR9 stimulation. In murine obesity induced by a high fat diet, ex vivo IgM and IgG were elevated with unstimulated B cells. Furthermore, the high fat diet lowered bone marrow B cell frequency accompanied by diminished transcripts of early lymphoid commitment markers. Murine B cell responses were subsequently investigated upon influenza A/Puerto Rico/8/34 infection using a Western diet model in the absence or presence of docosahexaenoic acid (DHA). DHA, an essential fatty acid with immunomodulatory properties, was tested because its plasma levels are lowered in obesity. Relative to controls, mice consuming the Western diet had diminished Ab titers whereas the Western diet plus DHA improved titers. Mechanistically, DHA did not directly target B cells to elevate Ab levels. Instead, DHA increased the concentration of the downstream specialized proresolving lipid mediators (SPMs) 14-hydroxydocosahexaenoic acid, 17-hydroxydocosahexaenoic acid, and protectin DX. All three SPMs were found to be effective in elevating murine Ab levels upon influenza infection. Collectively, the results demonstrate that B cell responses are impaired across human and mouse obesity models and show that essential fatty acid status is a factor influencing humoral immunity, potentially through an SPM-mediated mechanism.   *The Journal of Immunology*, 2017, 198: 4738–4752.

O besity is associated with impaired immunity, which contributes toward a variety of comorbidities (1–4). Many factors compromise innate and adaptive immunity in the obese population, which include oxidative stress, hormonal imbalances, and nutrient overload (5–7). A considerable amount of work has defined the cellular and molecular mechanisms by which obesity promotes an inflammatory profile, particularly in adipose tissue (8, 9). In contrast, far less is known about how

obesity influences humoral immunity. This is an essential gap in knowledge to address given that obesity is associated with increased susceptibility to infections and poor responses to vaccinations (10–13).

There is some evidence that humoral immunity is impaired in the obese, although there is no clear consensus. For example, hemagglutination inhibition (HAI) titers, a standard assay used to determine Ab levels to influenza virus, were reported normal 30 d

*Department of Biochemistry and Molecular Biology, Brody School of Medicine, East Carolina University, Greenville, NC 27834; †East Carolina Diabetes and Obesity Institute, East Carolina University, Greenville, NC 27834; ‡Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN 38105; §Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville, NC 27834; ¶Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC 27834; ‖Department of Pharmaceutical Sciences, University of Colorado, Denver, CO 80045; and #Department of Nutrition, Gillings School of Global Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599

¹R.K., W.G., and M.J.C. contributed equally to this work.

²Current address: National Institutes of Health, Bethesda, MD.

³Current address: Biochemistry, Molecular and Cell Sciences, Arkansas College of Osteopathic Medicine, Arkansas Colleges of Health Education, Fort Smith, AR.

⁴Current address: Division of Biological Sciences, University of California, La Jolla, CA.

ORCIDs: 0000-0001-6013-4252 (H.L.T.); 0000-0001-6004-5671 (E.A.K.); 0000-0003-2483-5064 (L.C.B.); 0000-0002-0752-2130 (J.J.M.).

Received for publication June 14, 2016. Accepted for publication April 10, 2017.

This work was supported by National Institutes of Health Grant R01AT008375 (to S.R.S.), a Brody Brothers Foundation award (to S.R.S.), a foundation award from

Address correspondence and reprint requests to Dr. Saame Raza Shaikh, Brody School of Medicine, East Carolina University, East Carolina Diabetes and Obesity Institute, 115 Heart Drive, Mail Stop 743, Greenville, NC 27858. E-mail address: Shaikhsa@ecu.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: BMI, body mass index; DHA, docosahexaenoic acid; GPR, G protein–coupled receptor; HAI, hemagglutination inhibition; HAU, hemagglutinating unit; HDHA, hydroxydocosahexaenoic acid; HF, high fat; HOMA-IR, homeostasis model assessment for insulin resistance; ODN, oligodeoxynucleotide; PDX, protectin DX; p.i., postinfection; PPAR, peroxisome proliferator–activated receptor; PUFA, polyunsaturated fatty acid; RDE, receptor-destroying enzyme; Rv, resolvin; SPM, specialized proresolving lipid mediator; WD, Western diet.

postvaccination but were lowered 12 mo postvaccination in obese humans compared with nonobese subjects (13). In another study, the ability to mount influenza-specific IgM and IgG responses 8 wk after influenza vaccination was normal in obese humans compared with lean controls, although the Ab response was diminished relative to an obese diabetic cohort (14). Mouse models also suggest that obesity impairs Ab production (15). For instance, murine HAI titers were lowered 7 d postinfection (p.i.) upon influenza infection and were completely blunted by 35 d p.i. (16). Moreover, the effects of obesity are not just limited to viral infection because obese mice also have diminished Ab production upon *Staphylococcus aureus* infection (17).

There is strong evidence that B cells, which have a central role in humoral immunity, regulate adipose tissue inflammation in obesity (18–21). For instance, in obese mice, IgG2c is elevated in adipose tissue and the B regulatory/B1 subsets improve adipose tissue inflammation (22–25). In contrast, much less is known about the influence of obesity on B cell cytokine secretion and Ab production outside of the context of adipose tissue inflammation (26). There are some conflicting reports suggesting that B cell activity could be impaired with type 2 diabetes, a comorbidity associated with obesity (20, 27). In obese type 2 diabetic mice, B cells secrete proinflammatory cytokines, similar to diabetic and/or obese patients with elevated fasting glucose (20, 28). Alternatively, newly diagnosed patients with diabetes have suppressed B cell inflammatory cytokines upon stimulation whereas Ab production is reported to be normal upon influenza vaccination (27, 29).

If B cell function is potentially compromised in the obese, then it is essential to define those factors that modulate B cell activity. Essential fatty acid status is a neglected variable in studies of humoral immunity. Essential n-3 polyunsaturated fatty acids (PUFAs) are of interest given their immunomodulatory properties (30). Furthermore, plasma levels of long-chain n-3 PUFAs are low in obese individuals compared with lean controls, which could contribute toward impairments in humoral immunity (31–33). The two major long-chain n-3 PUFAs of interest are eicosapentaenoic and docosahexaenoic acids (DHA), which can have anti-inflammatory effects but their influence on B cell activity is far less known (30). Our laboratory, in addition to other investigators, has recently discovered that n-3 PUFAs, particularly DHA, may improve B cell–driven responses, warranting more in-depth studies (34, 35).

The objectives of this study were to investigate whether obesity impairs B cell responses across three models and whether essential fatty acid status has a role in modulating Ab levels. B cell cytokine secretion and Ab production upon ex vivo stimulation were first investigated in a cohort of obese humans relative to lean controls. We next examined whether a high fat (HF) diet–induced model of obesity impaired murine ex vivo Ab production and B cell frequency in the bone marrow. Subsequently, the effects of a murine Western diet (WD) model (that provides moderate levels of fat) in the absence or presence of DHA was tested on Ab responses to influenza infection. Influenza infection, which allowed for the stimulation of B cells in vivo, is a significant burden in the obese population (4, 36). Finally, selected mechanistic underpinnings by which DHA could improve primary B cell responses in obese mice upon influenza infection were investigated. We focused on downstream D-series–specialized proresolving lipid mediators (SPMs) that have potent immune-enhancing properties on B cells (37, 38).

Materials and Methods

Human subjects and PBMC/B cell analyses

Human blood samples were procured after informed consent and under approval by the East Carolina University Institutional Review Board.

Cells from peripheral blood were analyzed from nonobese (body mass index [BMI], <25 kg/m^2) and obese (BMI, >30 kg/m^2) male subjects (Table I). The cohort included subjects who were nonsmokers with exclusion criteria to be free of chronic inflammatory/autoimmune disease, not taking supplements enriched in n-3 PUFAs, and free of infection during the past month. Subjects were fasted overnight before obtaining blood.

Peripheral blood taken in vacutainer tubes was diluted 1:1 in PBS followed by separation of PBMCs using Ficoll Paque (GE Healthcare, Washington, NC) gradient centrifugation. All fluorophore Ab markers used were obtained from BioLegend (San Diego, CA) or Miltenyi Biotec (San Diego, CA) and consisted of: Zombie NIR, CD45 (PE), CD3 (Pacific Blue), CD4 (FITC), CD8 (PE-Cy5), CD14 (FITC), and CD19 (allophycocyanin). Isolated PBMCs from gradient centrifugation were stained for viability using Zombie NIR. The following subsets were analyzed using a BD LSR II flow cytometer: CD45$^+$CD3$^+$CD4$^+$, CD45$^+$CD3$^+$CD8$^+$, CD45$^+$CD3$^-$CD14$^+$, and CD45$^+$CD14$^-$CD19$^+$.

B cells were isolated from PBMCs using a B cell isolation kit II (Miltenyi Biotec) with a resulting purity of >99%. Purified human B cells were cultured in RPMI 1640 with 5% FBS, 2 mM L-glutamine, 5 × 10^{-5} M 2-ME, 10 mM HEPES, and 50 μg/ml gentamicin at a concentration of 3 × 10^6 cells/ml. B cells were stimulated with: 1) CpG oligodeoxynucleotide (ODN) 2395 (a TLR9 agonist) at 1 μg/ml plus BCR stimulation using rabbit anti-human IgM Ab fragment at a concentration of 2 μg/ml; 2) Pam3CSK4 (a TLR1/2 agonist) at a concentration of 10 μg/ml. B cells were plated in round-bottom inert grade 96-well plates and cultured in duplicate for differing time points after activation. Supernatants IL-6, IL-10, and TNF-α were measured on day 2 postactivation using Luminex assay kits (Thermo Fisher Scientific) as per the manufacturer's instructions. Supernatants IgM and IgG were measured on day 7 postactivation using ELISAs (Abcam, Cambridge, MA) as per the manufacturer's instructions.

Obesity mouse models

Murine experiments fulfilled the guidelines established by the East Carolina University for euthanasia and humane treatment. Male C57BL/6 mice, ~5–7 wk old, were fed experimental diets (Envigo, Indianapolis, IN) for 15 wk. In the first murine model, animals were fed a control (10% of total kilocalories from lard) or HF diet (Research Diets, New Brunswick, NJ) (60% of total kilocalories from fat) for 15 wk. In the second murine model, mice were fed a low fat control diet, a WD, or a WD supplemented with DHA ethyl esters (Cayman Chemical, Ann Arbor, MI) of >93% purity as previously described (39). The WD provided 45% of total kilocalories from milk fat. DHA accounted for 2% of total energy, which is easily achievable for humans through intake of over-the-counter or prescription supplements (40). The composition of the experimental diets is provided in Supplemental Table I.

Infection model

Animals were infected intranasally with 0.03 hemagglutinating unit (HAU) of influenza A/PR/8/34 virus. All experiments with mice were in accordance with the guidelines set forth by East Carolina University. Mice were euthanized with CO$_2$ inhalation followed by cervical dislocation. Tissues were harvested following euthanasia.

SPM studies in vivo

SPMs were prepared as described (41). Six-week-old C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) were lightly anesthetized with isoflurane and then vaccinated with A/California/04/2009 (pdmH1N1) in the absence or presence of 1 μg of D-series SPMs (Cayman Chemical). Three weeks following vaccination, mice were bled and serum was analyzed for HAI and microneutralization titers against pdmH1N1 virus. In other studies, mice were given 1 μg of D-series SPMs in PBS/ethanol concomitant with 0.03 HAU of influenza A/PR/8/34 virus. PBS/ethanol alone served as the vehicle control.

Metabolic studies

Mice were fasted for 6 h and the baseline glucose value was established with a glucometer. An i.p. injection provided 2.5 g of dextrose (Hospira, Lake Forest, IL) per kilogram of lean mass, and glucose measurements were made from the tail vein. The homeostasis model assessment for insulin resistance (HOMA-IR) index was calculated as previously described (42). Echo-MRI was used as previously shown for fat/lean mass (43).

PCRs

Quantitative RT-PCR analysis was conducted on total bone marrow cells and B cells as previously described (39). The fold change from the control was calculated by the $2^{-\Delta\Delta Ct}$ method. Primer sequences used in the study are provided in Supplemental Table II. For PCR analyses, total RNA was isolated from sorted splenic B220$^+$ cells using a B220$^+$ FITC (BioLegend) Ab. cDNA (0.5 μg) was synthesized using a first-strand synthesis kit (Invitrogen, Carlsbad, CA). Both random hexamer and oligonucleotide primers were used in the reaction. Following synthesis of cDNA, PCR was conducted in a 50-μl reaction mix using Platinum PCR SuperMix High Fidelity (Invitrogen). A 300 nM concentration of primer solutions was used followed by the addition of cDNA. PCR products were visualized on a 1% agarose gel.

Flow cytometry

Bone marrow was extracted as previously demonstrated (39). For consistency, the left leg was used for each mouse. The fluorescently labeled Abs were obtained from BioLegend and consisted of CD19 (PerCP-Cy5.5), CD43 (allophycocyanin), CD24 (Pacific Blue), IgM (PE), IgD (allophycocyanin), and CD138 (allophycocyanin). The following B cell subsets were analyzed: CD19$^-$CD43$^+$CD24$^-$IgM$^-$ (prepro), CD19$^+$CD43$^+$CD24$^+$IgM$^-$ (pro), CD19$^+$CD43$^-$CD24$^+$IgM$^-$ (pre), CD19$^+$CD24$^+$IgM$^+$IgD$^-$ (immature), CD19$^+$IgM$^+$IgD$^+$ (mature), and CD19$^+$CD138$^+$ (plasma cells).

ELISAs, HAI, and microneutralization assays

IgM and IgG levels (Abcam) were analyzed from serum with ELISAs (34). For the HAI assay, serum was treated overnight with receptor-destroying enzyme (RDE; Denka Seiken, Campbell, CA), followed by heat inactivation for 1 h and then diluted 1:10 in PBS (16). HAI assays were performed according to World Health Organization guidelines (Global Influenza Surveillance Network Manual for the laboratory diagnosis and virological surveillance of influenza). RDE-treated sera were incubated with 4 HAU of influenza virus for 15 min at room temperature followed by a 1-h incubation at 4°C with 0.5% turkey RBCs. HAI was calculated by taking the reciprocal of the highest dilution of serum that completely inhibited hemagglutination of the turkey RBCs (16).

For microneutralization assays, RDE-treated sera were diluted in microneutralization media (DMEM, 2 mM glutamine, 1% BSA) at a 1:2 dilution. Sera were then incubated with 100 tissue culture-infective dose (50%) virus for 1 h at 37°C on white polystyrene plates. Following incubation, 3×10^4 Madin–Darby canine kidney cells were added to each well and plates were incubated at 37°C overnight. The bulk of the media was removed and plates were frozen for at least 30 min at −80°C. Luminescence was determined by adding 25 μl of Nano-Glo substrate solution (Promega) according to the manufacturer's instructions, and the plates were placed in a Synergy H1 hybrid reader. Neutralization was considered to be any well below half the luminescence generated by a well infected with 100 tissue culture-infective dose (50%) A/Puerto Rico/8/34 NLuc virus (44).

Lipidomic sample preparation

All standards and internal standards used for liquid chromatography–tandem mass spectrometry analysis were purchased from Cayman Chemical. All solvents were HPLC grade or better. Tissue homogenate samples were pretreated for solid phase extraction as follows. Briefly, a volume of tissue homogenate equivalent to 500 μg was brought to a volume of 1 ml in 10% methanol along with 10 μl of 10 pg/μl internal standard solution [100 pg total per each of 5(S)-HETE-d$_8$, 8-iso-PGF2α-d$_4$, 9(S)-HODE-d$_4$, LTB4-d$_4$, LTD4-d$_5$, LTE4-d$_5$, PGE$_2$-d$_4$, PGF2α-d$_9$, and RvD2-d$_5$ in ethanol]. Serum samples were pretreated for solid phase extraction. Proteins were precipitated from 50 μl of serum by adding 200 μl of ice-cold methanol and 10 μl of the internal standard solution, followed by vortexing and then incubating on ice for 15 min. The samples were then placed in a microcentrifuge for 10 min at 4°C at 14,000 rpm. A 200-μl portion of the supernatant was diluted to 10% methanol by adding 1.4 ml of water. Lipid mediators were isolated from the pretreated samples by solid phase extraction as described (45). Lipid mediators were extracted using Strata-X 33-μm 30 mg/1 ml SPE columns (Phenomenex, Torrance, CA) on a Biotage positive pressure SPE manifold. Columns were washed with 2 ml of methanol followed by 2 ml of H$_2$O. After applying the sample, the columns were washed with 1 ml of 10% methanol and the lipid mediators were then eluted with 1 ml of methanol directly into a reduced surface activity/maximum recovery glass autosampler vial. The methanol solvent was then evaporated to dryness under a steady stream of nitrogen directly on the SPE manifold. The sample was immediately reconstituted with

15–20 μl of ethanol and analyzed immediately or stored at −70°C until analysis for ≤1 wk.

Lipid mediators in whole spleen samples were isolated as described by Yang et al. (46). Spleen samples were preweighed and transferred into a 1.5-ml microcentrifuge tube. One microliter of methanol and 10 μl of internal standard solution were added and then the sample was vortexed and stored overnight at −20°C. The sample was transferred to a DUALL all-glass size 21 tissue homogenizer and ground until completely homogenized. The homogenate was transferred to a 1.5-ml centrifuge tube and centrifuged at 14,000 rpm for 10 min at 4°C. The supernatant was diluted to 10 ml with water adjusted to pH 3.5. The samples were then applied to HyperSep C18 500 mg/6 ml SPE columns (Thermo Fisher Scientific) that were prewashed with 20 ml of methanol followed by 20 ml of water. The SPE columns were washed with 10 ml of water followed by 10 ml of hexane. Lipid mediators were eluted with 8 ml of methyl formate (eicosanoids and docosanoid fraction) followed by 10 ml of methanol (cysteinyl leukotriene fraction). Both fractions were dried under a stream of nitrogen and reconstituted with ethanol and combined into a reduced surface activity/maximum recovery glass autosampler vial analyzed immediately or stored at −70°C until analysis for ≤1 wk.

Liquid chromatography–mass spectrometry

Quantitation of lipid mediators was performed using two-dimensional reverse phase HPLC tandem mass spectrometry (liquid chromatography–tandem mass spectrometry). The HPLC system consisted of an Agilent 1260 autosampler (Agilent Technologies, Santa Clara, CA), an Agilent 1260 binary loading pump (pump 1), an Agilent 1260 binary analytical pump (pump 2), and a six-port switching valve. Pump 1 buffers consisted of 0.1% formic acid in water (solvent A) and 9:1 v/v acetonitrile/water with 0.1% formic acid (solvent B). Pump 2 buffers consisted of 0.01% formic acid in water (solvent C) and 1:1 v/v acetonitrile/isopropanol (solvent D).

Ten microliters of extracted sample was injected onto an Agilent SB-C18 2.1 × 5 mm, 1.8-μm trapping column using pump 1 at 2 ml/min for 0.5 min with a solvent composition of 97% solvent A/3% solvent B. At 0.51 min the switching valve changed the flow to the trapping column from pump 1 to pump 2. The flow was reversed and the trapped lipid mediators were eluted onto an Agilent Eclipse Plus C18 2.1 × 150 mm, 1.8-μm analytical column using the following gradient at a flow rate of 0.3 ml/min: hold at 75% solvent A/25% solvent D from 0 to 0.5 min, then a linear gradient from 25 to 75% D during 20 min followed by an increase from 75 to 100% D from 20 to 21 min, then holding at 100% D for 2 min. During the analytical gradient, pump 1 washed the injection loop with 100% B for 22.5 min at 0.2 ml/min. Both the trapping column and the analytical column were re-equilibrated at starting conditions for 5 min before the next injection.

Mass spectrometric analysis was performed on an Agilent 6490 triple quadrupole mass spectrometer in negative ionization mode. The drying gas was 250°C at a flow rate of 15 ml/min. The sheath gas was 350°C at 12 ml/min. The nebulizer pressure was 35 psi. The capillary voltage was 3500 V. Data for lipid mediators was acquired in dynamic MRM mode using experimentally optimized collision energies obtained by flow injection analysis of authentic standards. Calibration standards for each lipid mediator were analyzed over a range of concentrations from 0.25 to 250 pg on column. Calibration curves for each lipid mediator were constructed using Agilent MassHunter quantitative analysis software. Tissue and serum samples were quantitated using the calibration curves to obtain the column concentration, followed by multiplication of the results by the appropriate dilution factor to obtain the concentration in picograms per microgram of protein (tissue homogenates) or picograms per milliliter (serum).

Proliferation assays

B cell pellets were stored for a minimum of 24 h at −80°C. A CyQUANT cell proliferation assay kit (Molecular Probes, Eugene, OR) was used to quantify number of cells per well and as per the manufacturer's instructions.

In vitro studies

Mouse splenic B cells were plated in 96-well plates at 1×10^6/ml and activated with: 1) 1 μg/ml CpG 1826 (Novus Biologicals, Littleton, CO) plus 2 μg/ml anti-IgM (Jackson ImmunoResearch Laboratories, West Baltimore Pike, PA), or 2) 1 μg/ml LPS (Sigma-Aldrich, St. Louis, MO). Cells were also treated with 5 μM rosiglitazone (Sigma-Aldrich) or 5 μM DHA (Cayman Chemical) and then activated 30 min later with 1 μg/ml CpG 1826 plus 2 μg/ml anti-IgM. DHA stocks were conjugated to BSA as previously described (47).

All murine results are from multiple cohorts of mice. Data were analyzed with GraphPad Prism version 5.0b. IgM and IgG levels assayed in human studies were fit using linear regression analysis. Most data sets displayed normalized distributions as determined by a Kolmogorov–Smirnov test. Statistical significance for human studies, in vitro experiments, and those experiments comparing lean versus HF-fed mice were analyzed with a two-tailed unpaired Student t test. All other analyses relied on one-way or two-way ANOVAs followed by a post hoc Bonferroni multiple comparison tests. Those data sets that did not display normalized distributions were analyzed with a Kruskal–Wallis test followed by a Dunn multiple comparison test. A p value of <0.05 was considered significant.

Results

B cell IL-6 secretion is lowered and IgM levels are elevated in obese humans

We first studied B cell responses in a cohort of age-matched nonobese and obese males that considerably differed in BMI (Table I). Flow cytometry analysis of PBMCs (Fig. 1A) generally revealed an increased percentage of B cells ($p = 0.05$) in obese individuals with no effect on monocytes, CD4$^+$ cells, or CD8$^+$ T cells (Fig. 1B). When B cells were enumerated with trypan blue exclusion upon purification of the cells, there was a significant increase in B cell frequency in the obese subjects compared with nonobese controls (Fig. 1C).

Highly purified B cells, as assessed by CD14 and CD19 staining (Fig. 1D), were used for measuring cytokine and Ab production. IL-6 secretion in response to activation with CpG-ODN (TLR9 agonist) plus anti-IgM (for BCR engagement) was ~35–40% lower in B cells from obese relative to nonobese subjects with no significant effect on TNF-α or IL-10 secretion (Fig. 1E, left panel). B cell cytokine secretion from obese subjects was not affected upon targeting of TLR1/2 with Pam3CSK4 relative to controls (Fig. 1E, right panel). Cytokine secretion was not detectable in the absence of CpG-ODN plus anti-IgM or Pam3CSK4 stimulation (data not shown). Activation of B cells with CpG-ODN plus anti-IgM revealed a correlation between increasing IgM levels (Fig. 1F) and increasing BMI, with no correlation between IgG levels and BMI (Fig. 1G). Thus, the studies with human subjects showed obesity dysregulated B cell cytokine secretion and Ab production upon CpG-ODN plus anti-IgM stimulation.

B cell IgM and IgG levels are elevated in the absence of stimulation for mice consuming an HF diet

Experiments were next conducted in a mouse model to determine whether murine B cell activity was also impaired in response to diet-induced obesity. Ex vivo splenic B cell Ab production was measured using lean and HF diets. Mice consuming the HF diet displayed elevated levels of ex vivo IgM (Fig. 2A, left panel) and IgG (Fig. 2B, left panel) in the absence of stimulation. There was no absolute change in IgM (Fig. 2A, left panel) or IgG (Fig. 2B, left panel) production with the HF diet upon stimulation with either CpG-ODN plus anti-IgM or LPS. However, relative to the negative controls, the increase in IgM (Fig. 2A, right panel) and IgG (Fig. 2B, right panel) levels for obese mice upon stimulation with either CpG-ODN plus anti-IgM or LPS were diminished by ≥2-fold relative to the control mice. Circulating IgM (Fig. 2C, left panel) and IgG (Fig. 2C, right panel) levels were also assayed and there was a significant increase in the levels of IgG with obese mice.

The frequency of bone marrow B cell subsets is lowered in mice consuming an HF diet

In parallel with the ex vivo studies, we probed for potential defects in B cell development (Fig. 3). The percentage (Fig. 3A, left panel) and frequency (Fig. 3A, right panel) of B cells in the bone marrow were lowered with the HF diet relative to the lean control by ~2-fold. B cell subsets were further analyzed with flow cytometry (Fig. 3B). There was attenuation in the percentage of CD19$^+$IgM$^-$CD43$^-$CD24$^+$ cells with mice consuming the HF diet compared with the control (Fig. 3C, left panel). The frequency of all the major B cell subsets was lowered with the HF diet relative to the control (Fig. 3C, right panel). Additionally, CD138$^+$ B cells were analyzed by flow cytometry (Fig. 3D). The percentage (Fig. 3E, left panel) and frequency (Fig. 3E, right panel) of CD138$^+$ B cells in the bone marrow were lowered with the HF diet compared with the control by 1.7- to 2-fold. B cell proliferation was not influenced by the HF diet relative to the lean control (data not shown).

Table I. Patient characteristics

Parameters	Nonobese ($n = 10$)	Obese ($n = 10$)
Age (y; mean and range)	34.54 (22–57)	37.63 (22–65)
BMI (kg/m^2; mean and range)	24.0 (21.7–28.8)	39.6 (30.0–65.7)****
Race, n (%)		
White	9 (90%)	7 (70%)
African American	—	3 (30%)
Indian	1 (10%)	—
Medications, n (%)		
NSAID	1 (10%)	—
PDE5 inhibitor	1 (10%)	—
Phenethylamine	1 (10%)	—
SSRI	1 (10%)	1 (10%)
Vitamin	1 (10%)	—
Benzodiazepine	—	3 (30%)
β Blocker	—	1 (10%)
Cyclopyrrolone	—	1 (10%)
HMG CoA reductase inhibitor	—	1 (10%)
Proton pump inhibitor	—	3 (30%)
Sulfonylurea	—	1 (10%)
Testosterone	—	1 (10%)

The subject who consumed nonsteroidal anti-inflammatory drug does not have a chronic inflammatory condition. Dash (—) indicates not applicable.
****$p < 0.0001$.
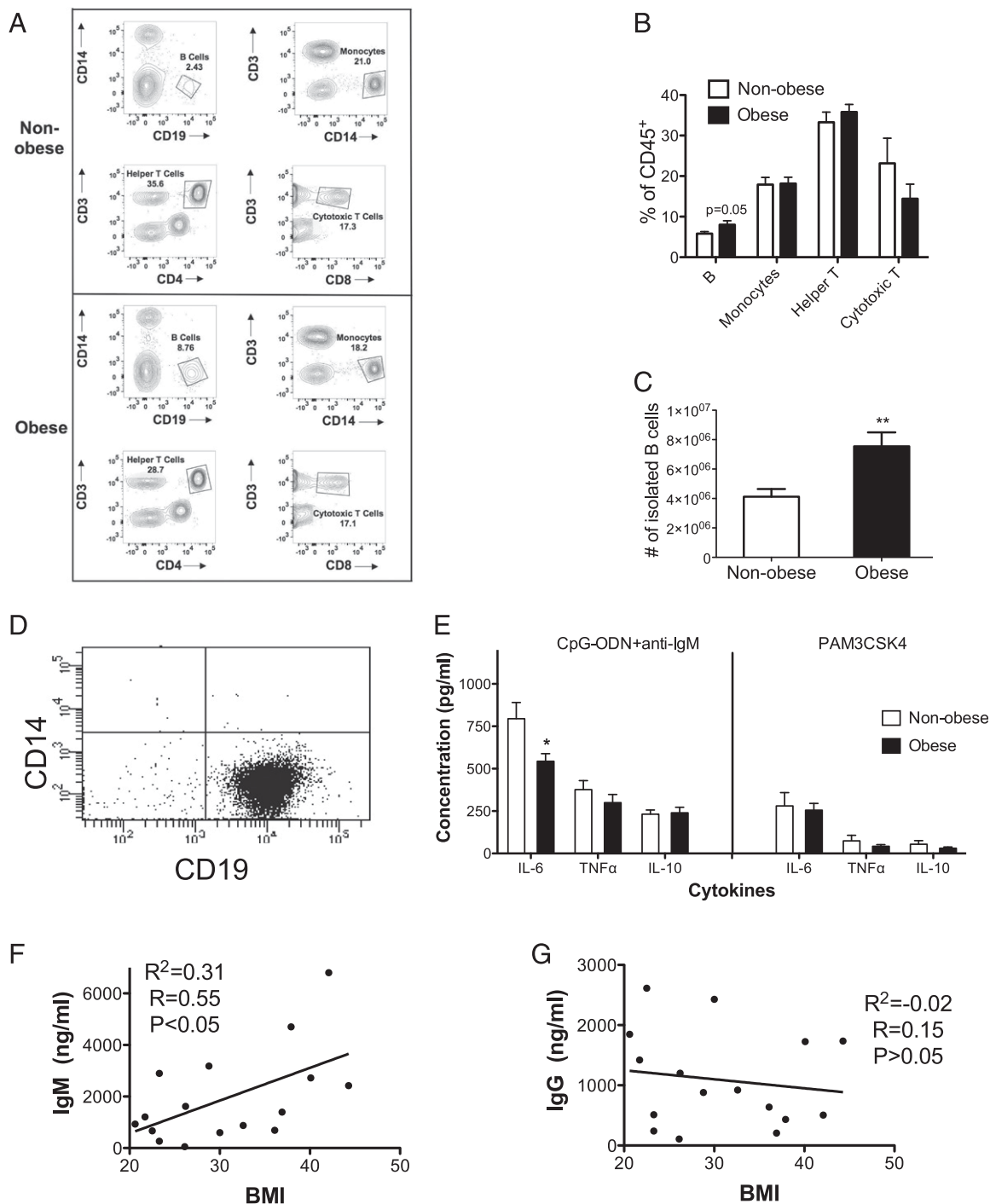NSAID, nonsteroidal anti-inflammatory drug; SSRI, selective serotonin reuptake inhibitor.

FIGURE 1. B cell IL-6 secretion and IgM production are modified in human obesity. (**A**) Sample flow cytometry data showing the percentage of B cells, monocytes, helper CD4^{+} cells, and cytotoxic CD8^{+} T cells in PBMCs. (**B**) Quantification of the percentage of CD45^{+} cells in PBMCs. (**C**) Frequency of B cells isolated via negative selection from nonobese and obese subjects and enumerated with trypan blue exclusion. (**D**) Purity of B cells as measured by CD19/CD14 staining with flow cytometry. (**E**) B cell cytokine secretion upon CpG-ODN plus anti-IgM or Pam3CSK4 stimulation. Correlation plots of (**F**) IgM and (**G**) IgG upon stimulation with CpG-ODN plus anti-IgM with BMI. Data are average $\pm$ SEM; $n = 10$ nonobese and 10 obese subjects for (B) and (D) and $n = 16$ subjects for (F) and (G). *$p < 0.05$, by unpaired Student t test (B, C, and E). Correlation plots (F and G) relied on linear regression analyses.

To explain the reduction in the proportion of B cells in the bone marrow, selected markers relevant to early lymphoid commitment were assayed at the mRNA level from isolated bone marrow cells (Fig. 3F). We specifically focused on the early lymphoid commitment markers IL-7Rα, IL-7, and STAT5 (Fig. 3F) (48, 49). With mice consuming the HF diet relative to lean controls, bone marrow transcripts of IL-7Rα and IL-7 were decreased by ∼2-fold, whereas STAT5 showed a trend toward being lowered ($p = 0.08$). Markers of intermediate (PAX5 and OCT2) and late B cell development (Blimp1) were also assayed (50, 51). These markers, which may reflect the decrease in B cell subset frequency, were also lowered by ≥2-fold in the bone marrow of mice on an HF diet compared with controls (Fig. 3F).
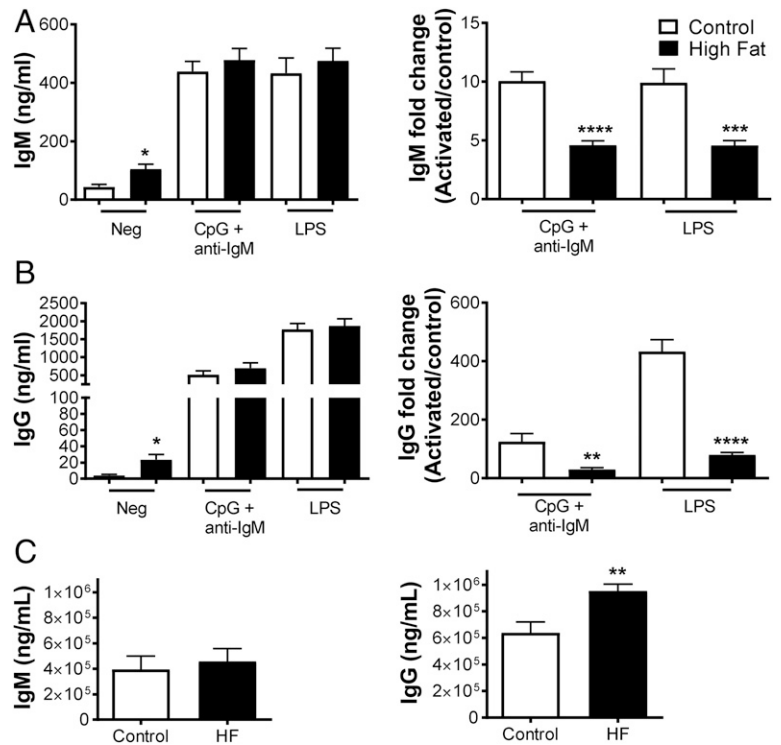
FIGURE 2. B cell IgM and IgG levels are elevated in the absence of stimulation with mice consuming HF diets. (**A**) IgM and (**B**) IgG levels from splenic B cells in the absence and presence of CpG-ODN plus anti-IgM or LPS stimulation are shown. Fold changes in IgM and IgG relative to the negative control are depicted to the right. Ex vivo antibody levels were measured on day 3 postactivation. Circulating (**C**) IgM and IgG levels for mice consuming a lean control or HF diet are shown. Mice were fed experimental diets for 15 wk. Data are average $\pm$ SEM; $n = 6$–7 mice per diet. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$, by unpaired Student t test.

The Ab response is decreased in a murine WD model and improved by DHA upon influenza infection

The next set of studies focused on how a moderate WD model in the absence or presence of DHA would influence primary B cell responses, which was assayed in response to a mouse-adapted influenza stain (18). DHA was specifically used because essential fatty acid levels are lowered in obesity (31). The composition of the diets was routinely verified to ensure the absence of DHA in the WD compared with WD plus DHA and diets were also continuously monitored for the lack of oxidation prior to and during the course of the study (data not shown). Prior to infection, body weights were elevated for both WDs compared with the control (Fig. 4A), which was driven by an increase in fat mass and not lean mass (Fig. 4B). The HOMA-IR index, a measure of glucose/insulin sensitivity, was elevated for mice fed both WDs relative to the lean mice (Fig. 4C).

Infection studies revealed that on day 7 p.i., the WD fed mice tended to have lower HAI titers, similar in magnitude to those found in a previous report (16). Inclusion of DHA in the diet increased the HAI titers relative to the WD by 4-fold (Fig. 4D). HAI titers were not assessed on day 14 p.i. given that mice consuming a WD show no impairment in Ab production at this time point (16). Notably, on day 7 p.i., only 66% of the mice consuming the WD had measurable HAI titers (Fig. 4E), consistent with previous work (16). On day 21 p.i., HAI titers were lowered in mice fed the WD by ~43% compared with the lean control (Fig. 4D). DHA improved the titers compared with the WD by 25% but not to the same level as the control (Fig. 4D). Given that DHA showed a modest effect on HAI titers at 21 d p.i., we further investigated using a microneutralization assay. This assay revealed that the WD lowered titers ($p = 0.06$) by 2.6-fold relative to the lean control and that the inclusion of DHA in the diet improved titers by 3.7-fold relative to WD (Fig. 4F).

Recovery of the mice in response to the infection was also measured. Mice consuming the WD did not have a significant difference in recovery, as measured by the total loss of body mass,

compared with the control diet in the early part of the time course; however, on day 14 p.i. the body mass of mice on the WD was significantly lower compared with the control and WD plus DHA (Fig. 4G). When calculated as the percentage weight loss, the WD plus DHA modestly improved recovery relative to the WD at days 7–10 p.i. (Fig. 4H). The results from this model further established compromised B cell responses with obesity and highlighted the importance of DHA status.

DHA is not directly targeting B cells, suggesting a role for downstream SPMs

The next objective was to establish how DHA could enhance Ab titers. We first focused on DHA binding the G protein–coupled receptor (GPR)120, a sensor for DHA reported to be on the surface of macrophages but not investigated with B cells (52). PCR analysis of sorted B cells showed very low abundance of GPR120 compared with epididymal adipose tissue, and activation of B cells with GPR120 agonists did not promote Ab production (data not shown).

A second possibility was DHA directly targeting B cells to increase Ab levels, potentially by binding peroxisome proliferator–activated receptor (PPAR)γ. Activation of B cell PPARγ with agonists boosts Ab levels in human B cells although less is known about mouse B cells (53). Thus, to first determine that PPARγ activation promotes Ab production in mouse cells, splenic B cells were pretreated with 5 μM rosiglitazone, a PPARγ agonist. Stimulation in the presence of rosiglitazone with CpG-ODN plus anti-IgM increased IgG but not IgM levels after 3 d (Supplemental Fig. 1A) and both IgM and IgG were elevated after 6 d (Supplemental Fig. 1A). The increase in Ab levels was not driven by an increase in the proliferation of the B cells (Supplemental Fig. 1B) as reported for human B cells (53). Given that PPARγ stimulation enhanced Ab production in mouse B cells, we next tested the effects of in vitro treatment with DHA, a known PPARγ agonist. When B cells were treated with 5 μM DHA for 3 d, IgM and IgG levels were dramatically lowered (Fig. 5A) in parallel with a decrease in B cell proliferation (Fig. 5B) compared with a
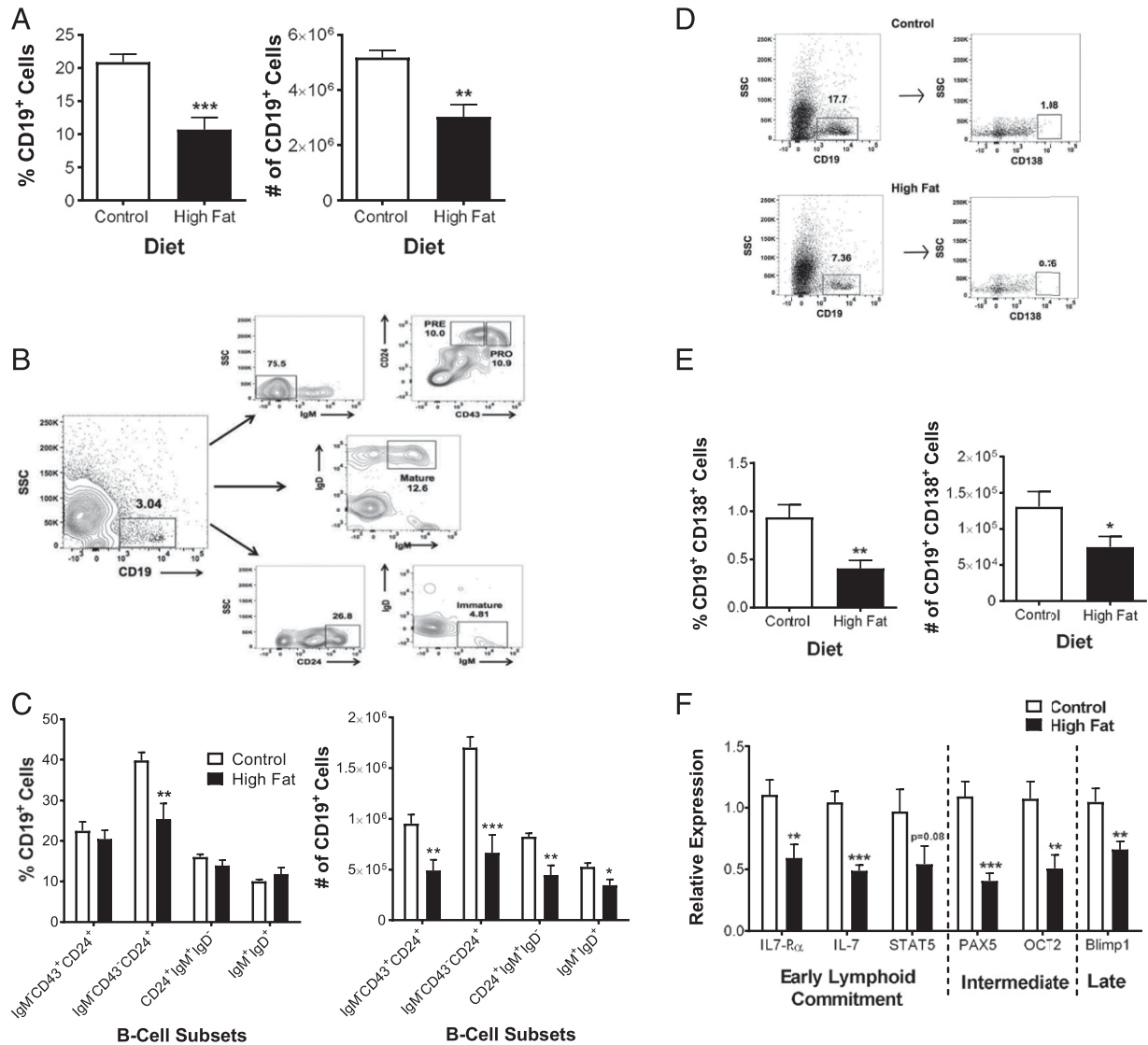
FIGURE 3. The frequency of bone marrow B cell subsets is lowered in mice consuming an HF diet accompanied by diminished early lymphoid commitment markers. (**A**) Percentage and frequency of total B cells in the bone marrow of mice consuming control and HF diets. (**B**) Sample gating strategy for analyzing B cell subsets in the bone marrow. (**C**) Percentage and frequency of B cell subsets in the bone marrow. (**D**) Sample gating strategy for analyzing CD138$^+$ B cells in the bone marrow. (**E**) Percentage and frequency of CD138$^+$ B cells for lean and obese mice. (**F**) Transcript levels of key early, intermediate, and late B cell development markers assayed on total bone marrow cells. Mice were fed a control or an HF diet for 15 wk. Data are average $\pm$ SEM; n = 5–7 mice per diet for (A), (C), and (E) and 10–11 mice per diet for (F). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, by unpaired Student t test.

BSA control. This ruled out that DHA was having a direct effect on the B cells as a PPARγ agonist. Conversely, the results suggested that DHA by itself lowered B cell proliferation and activity.

We then tested the possibility that D-series SPMs synthesized from DHA could boost Ab production (Fig. 5C). The SPM 17-hydroxydocosahexaenoic acid (HDHA) is reported to increase Ab levels in vitro and in a preclinical model of vaccination (41, 54). Given that DHA would likely give rise to multiple SPMs in vivo, several D-series SPMs downstream of 17-HDHA were tested. We used a preclinical vaccination model used for 17-HDHA to directly compare our results (54). HAI titers (Fig. 5D) were significantly elevated, compared with the vehicle control, with resolvin (Rv)D1, RvD2, and protectin DX (PDX). Micro-neutralization titers revealed a similar trend with the exception of RvD2 in boosting Ab levels (Fig. 5E).

We confirmed that B cells express multiple receptors that could respond to differing SPMs (Fig. 5F). Sorted splenic and bone marrow B cells expressed the SPM receptors ALX/FPR2 and

ChemR23 at the mRNA level (Fig. 5G). In comparison, transcript levels of BLT1, the receptor for LTB4 and RvE1, was robustly expressed at the mRNA level in B cells (Fig. 5G). Splenic B cell transcripts of ALX/FPR2 and ChemR23 were notably lower than peritoneal macrophages, which were used as a control because they expressed high levels of the SPM receptors (37).

DHA increases D-series SPMs in the WD model accompanied by an increase in the frequency of CD138$^+$ cells

The next set of experiments tested the possibility that DHA would increase downstream SPMs in mice consuming the WD at 7 d p.i. Lipid mediators (Fig. 6A–D) were first assayed in serum to assess circulatory levels. SPMs were not significantly elevated in serum in response to DHA in the diet (Fig. 6E). Because there is debate as to whether SPMs are measurable in circulation (55, 56), levels of D-series SPMs were also measured in the spleen. In the spleen, the WD plus DHA increased the levels of 14-HDHA and 17-HDHA by up to 2-fold compared with the WD and/or the control
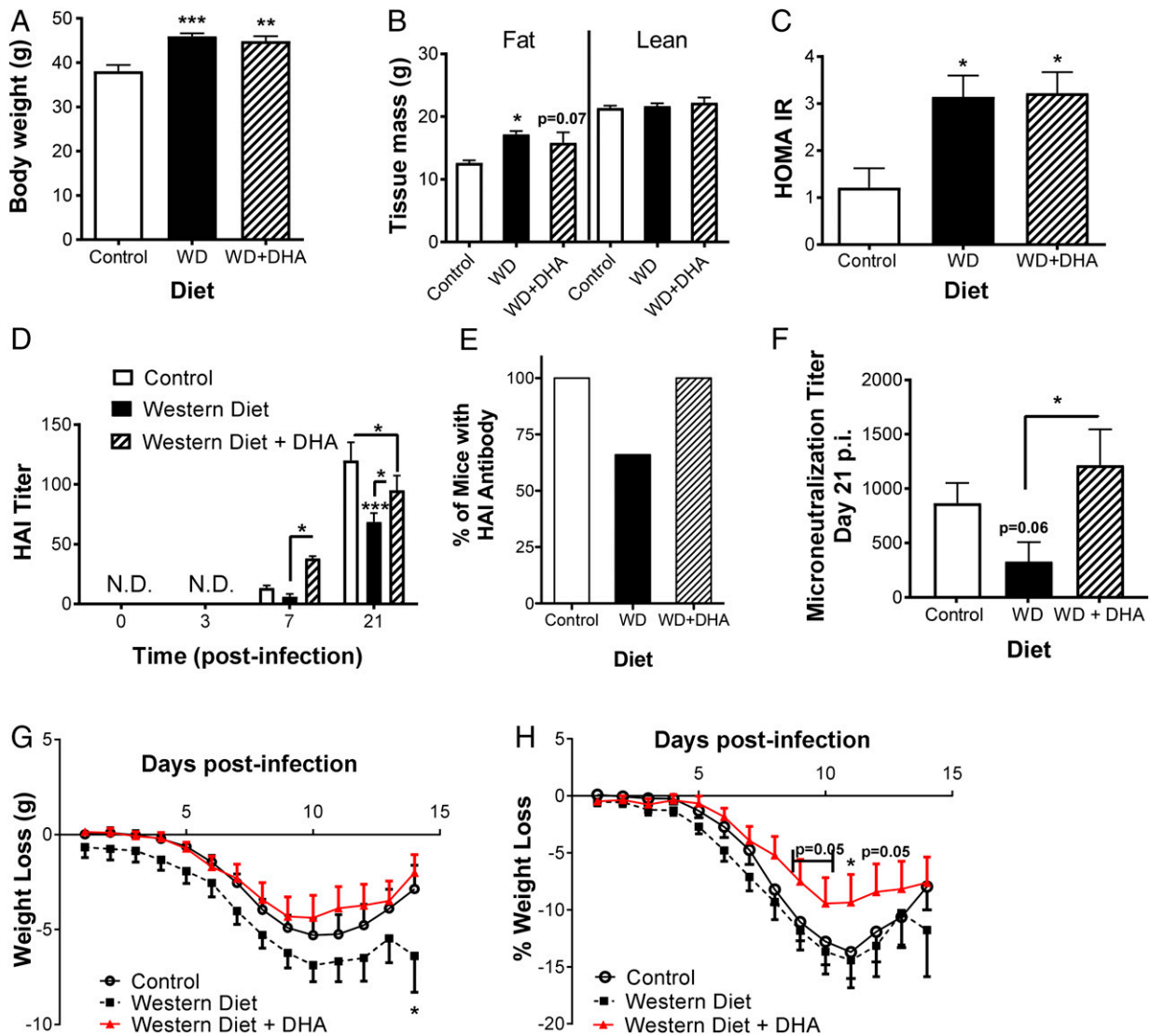
FIGURE 4. A WD suppresses Ab titers, which are improved with the essential fatty acid DHA. (**A**) Body weights of mice prior to infection after 15 wk of dietary intervention. (**B**) Lean and fat mass as measured by Echo-MRI. (**C**) HOMA-IR index calculated from fasting glucose/insulin. Mice were fasted for 6 h prior to measurements. (**D**) HAI titers for C57BL/6 mice that were fed a control, WD, or WD plus DHA diets for 15 wk followed by influenza infection. (**E**) Percentage of mice showing HAI Abs at day 7 p.i. (**F**) Microneutralization titers for C57BL/6 mice that were fed a control, WD, or WD plus DHA diets for 15 wk followed by influenza infection for 21 d. (**G**) Absolute weight loss and (**H**) percentage weight loss as a function of time are shown. Data are average ± SEM; $n = 4$–9 mice per diet for (A)–(F) and 15–20 mice per diet for (G) and (H). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, by one-way ANOVA followed by a Bonferroni posttest (A, B, C, and F) or a two-way ANOVA followed by a Bonferroni posttest (D, G, and H).

(Fig. 6F). PDX levels were lowered by 50% with the WD compared with the control. RvD2 levels trended ($p = 0.07$) in a downward fashion with the WD compared with the control (Fig. 6F). PDX and RvD2 levels were generally restored to control levels with WD plus DHA (Fig. 6F). The increase in D-series SPMs, particularly in spleen, were accompanied by a reduction in a range of *n*-6 PUFA–derived mediators, notably those derived from arachidonic acid (Supplemental Fig. 2A, 2B) with some additional changes in linoleic acid–derived mediators with both WDs (Supplemental Fig. 2C, 2D).

Because 17-HDHA is reported to increase Ab production by increasing the frequency of bone marrow CD19⁺CD138⁺ plasma cells (41, 54), and DHA elevated 17-HDHA, we determined whether DHA elevated the production of CD19⁺CD138⁺ cells at day 21 p.i. The frequency of B cells was slightly lowered by the WD relative to the control (Fig. 7A). There was no change in the percentage (Fig. 7B, left panel) or frequency (Fig. 7B, right panel) of the major B cell subsets with either WD. Upon flow cytometry

analysis of the CD138⁺ population (Fig. 7C), the WD diet tended ($p = 0.07$) to lower the percentage of CD138⁺ cells relative to the control (Fig. 7D, left panel). The percentage (Fig. 7D, left panel) and frequency (Fig. 7D, right panel) of CD138⁺ cells increased with the WD plus DHA compared with the WD. Thus, the phenotyping data revealed that DHA enhanced the frequency of CD138⁺ cells in the bone marrow, further suggesting a mechanism mediated by 17-HDHA (41, 54).

Administration of D-series SPMs after infection boosts Ab titers

The next set of experiments tested the possibility that 17-HDHA and perhaps other D-series SPMs would boost Ab titers. Mice were injected with 17-HDHA 24 h after the administration of the influenza virus and Ab titers were assayed at 14 d p.i. Given that the lipidomic data showed DHA elevated 14-HDHA and PDX, these molecules were also specifically tested. HAI (Fig. 8A, left panel) and microneutralization (Fig. 8A, right panel) titers were elevated
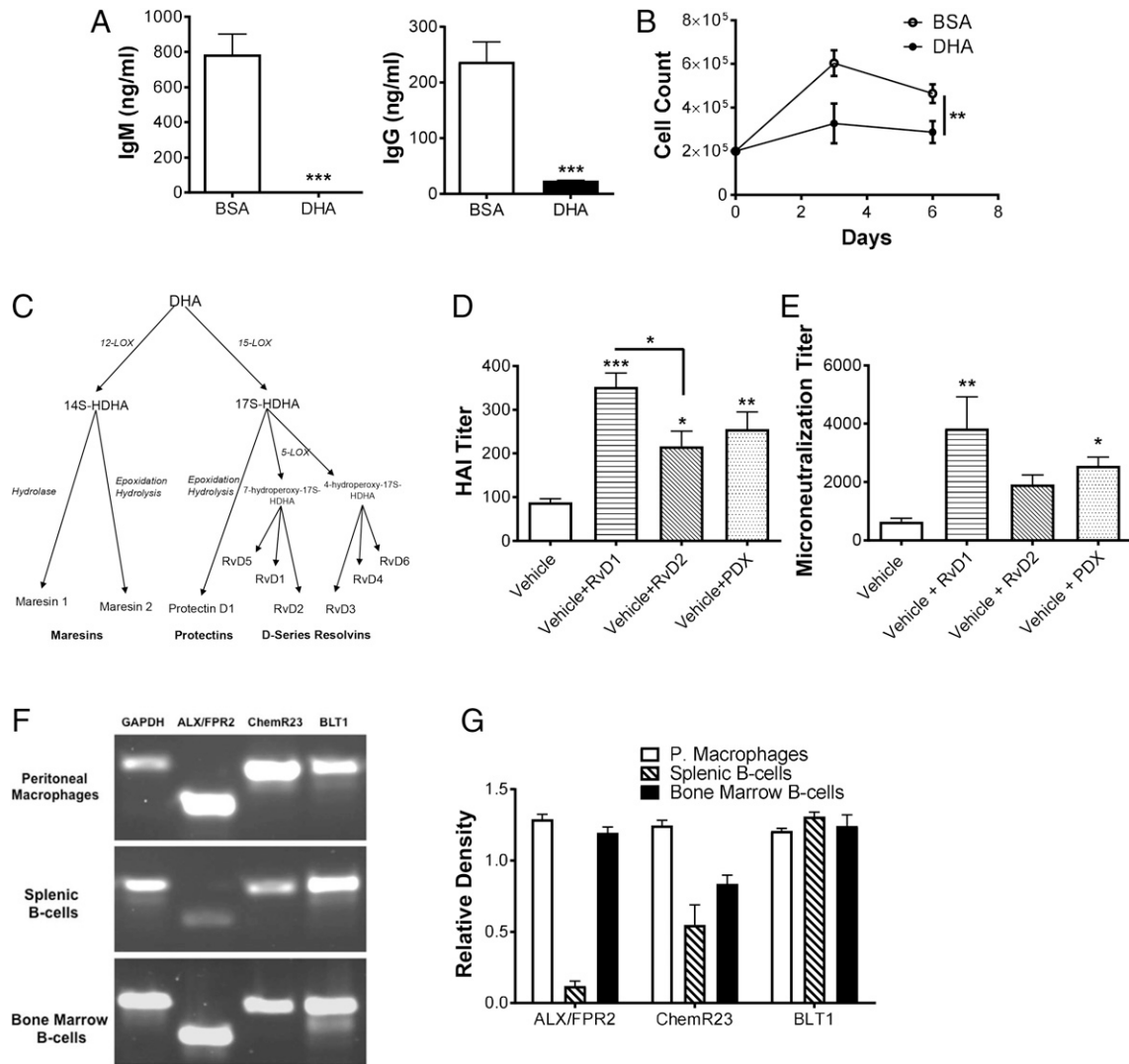
FIGURE 5. DHA is not directly targeting B cells to boost Ab production, suggesting a role for D-series SPMs. (**A**) IgM and IgG levels upon treatment of splenic B cells with 5 μM DHA. DHA was added 30 min prior to activation with CpG-ODN plus anti-IgM and was further added each day for 3 d. BSA served as a control because DHA was complexed to BSA. (**B**) Proliferation of B cells in response to DHA treatment. (**C**) Pathway by which DHA promotes production of downstream SPMs. LOX is for lipoxygenase. (**D**) HAI and (**E**) microneutralization titers of mice after vaccination with pdmH1N1 vaccine in response to vehicle control or SPM injection. (**F**) Sample PCR data showing mRNA expression of ALX/FPR2, ChemR23, and BLT1 for sorted splenic and bone marrow B cells. Peritoneal macrophages were used for comparative purposes. (**G**) Quantification of mRNA expression of ALX/FPR2, ChemR23, and BLT1. Data are average ± SEM; n = 3–5 independent experiments except (F), which is a single experiment representative of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, by unpaired Student t test (A) or two-way ANOVA followed by a Bonferroni posttest (B), or a Kruskal–Wallis test followed by a Dunn multiple comparison test (D and E).

with 14-HDHA, 17-HDHA, and PDX. Body weight analysis during the course of the infection showed no effect of 14-HDHA, 17-HDHA, or PDX on murine recovery compared with the vehicle control (data not shown).

14-HDHA administered with infection enhances Ab titers accompanied by an increase in the frequency of CD138+ cells

We finally tested whether administration of the three SPMs had the same effect when administered in parallel with the infection. Surprisingly, HAI (Fig. 8B, left panel) and microneutralization (Fig. 8B, right panel) titers were only elevated with 14-HDHA, but not 17-HDHA or PDX. We further probed whether 14-HDHA was selectively promoting the production of CD138+ cells in the bone marrow at day 21 p.i. (Fig. 8C). Flow cytometry analyses revealed a ≥2-fold elevation in the percentage (Fig. 8D, left panel) and frequency (Fig. 8D, right panel) of CD138+ cells with 14-HDHA but no effect of 17-HDHA or PDX. Thus, these results showed

that SPMs have differing effects on Ab production depending on the time of the administration and, notably, 14-HDHA increased Ab levels accompanied by an increase in CD138+ cells.

Discussion

B cell activity is modified across obesity models

The results of our study demonstrate that B cell cytokine secretion and/or Ab production are modified across mouse and human obesity models. Few studies in humans have directly investigated whether B cell function is impaired with obesity; therefore, this was first addressed by testing how ex vivo B cell cytokine secretion and Ab production were influenced in obese subjects. Notably, IL-6 secretion was lowered upon BCR/TLR9 activation, which agrees with a report revealing that B cell cytokine secretion can be suppressed in early stage patients with diabetes (27). However, the cytokine data were not in complete agreement with results
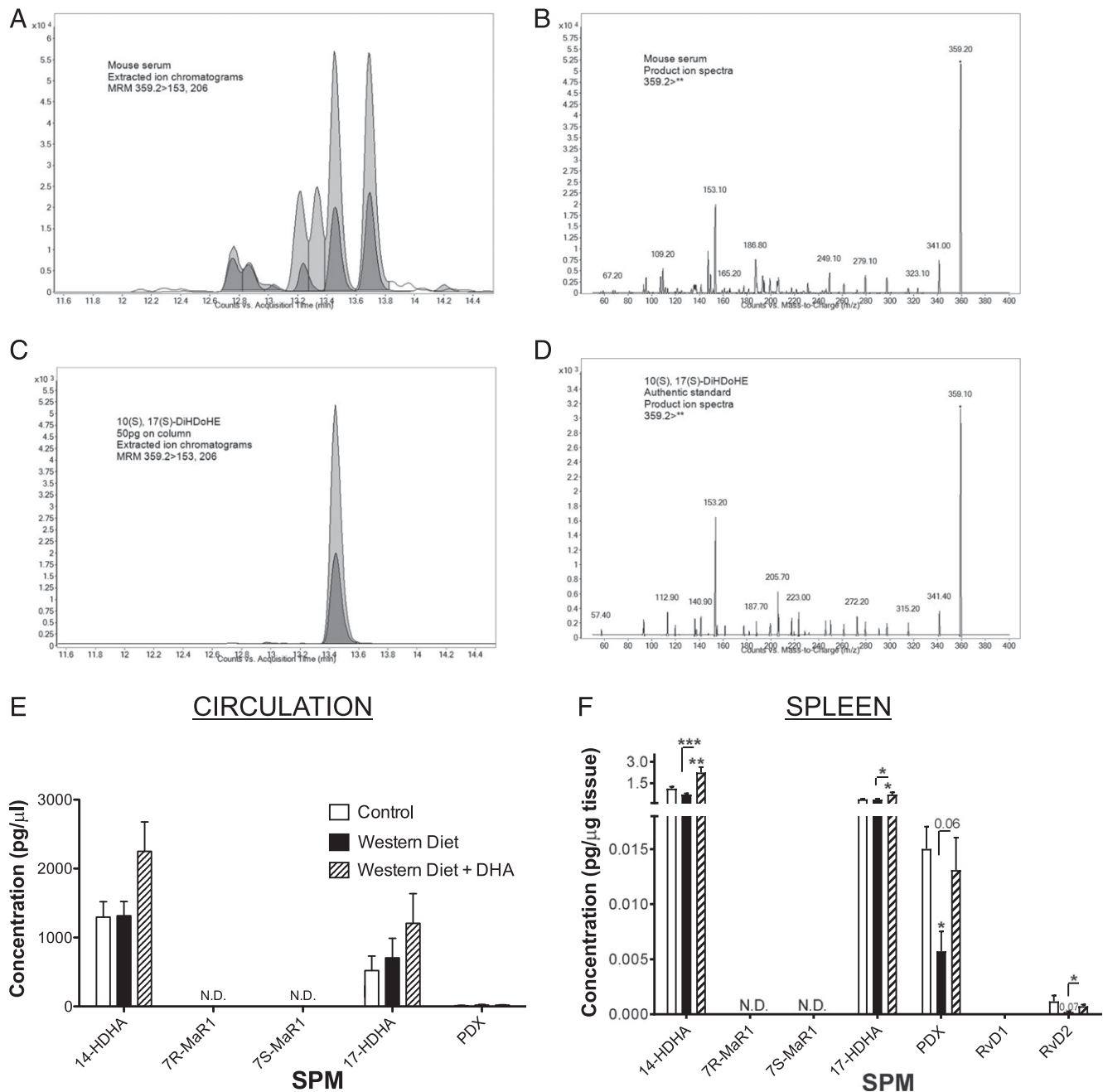
FIGURE 6. DHA enhances downstream D-series SPM levels of mice consuming a WD. Extracted ion chromatograms (**A** and **C**) and product ion spectra (**B** and **D**) for 10(*S*),17(*S*)-DiHDoHE (PDX) in mouse serum (A and B) and authentic standard (C and D) are shown. Specialized proresolving lipid mediators synthesized from DHA in murine (**E**) serum and (**F**) spleen at 7 d p.i. upon consuming a control, WD, or WD plus DHA for 15 wk. $n = 5–10$ mice per diet. Samples for serum analyses required the pooling of two mice per experiment. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, by one-way ANOVA followed by a Bonferroni posttest.

showing that LPS enhances some proinflammatory cytokines in obesity from human or mouse B cells upon ex vivo stimulation (28). We hypothesize that this is likely driven by differences in signaling pathways (i.e., TLR4 compared with BCR/TLR9), and our human subjects were mostly free of a diagnosis of type 2 diabetes and therefore may not display elevated B cell cytokines. An additional finding with human subjects was an increase in the frequency of B cells, which was consistent with data showing an enhanced percentage of B cells in obese and type 2 diabetic patients (14).

The human and mouse models of obesity revealed several changes in B cell Ab production. In humans, IgM but not IgG levels

positively correlated with increasing BMI upon BCR/TLR9 stimulation. In contrast, the HF diet elevated murine IgM and IgG levels prior to BCR/TLR9 stimulation, suggesting hyperstimulated B cells that did not respond efficiently to BCR/TLR9 or TLR4 stimulation when comparing Ab levels between the stimulated and unstimulated conditions. This result supports the emerging notion that hyperstimulated B cells function suboptimally (20, 57). The differences in Ab production between the human and mouse data were not entirely surprising given differing model systems. One clear set of distinctions between mice and humans was that diet was not controlled for in the human studies and the human population is genetically diverse. Future studies
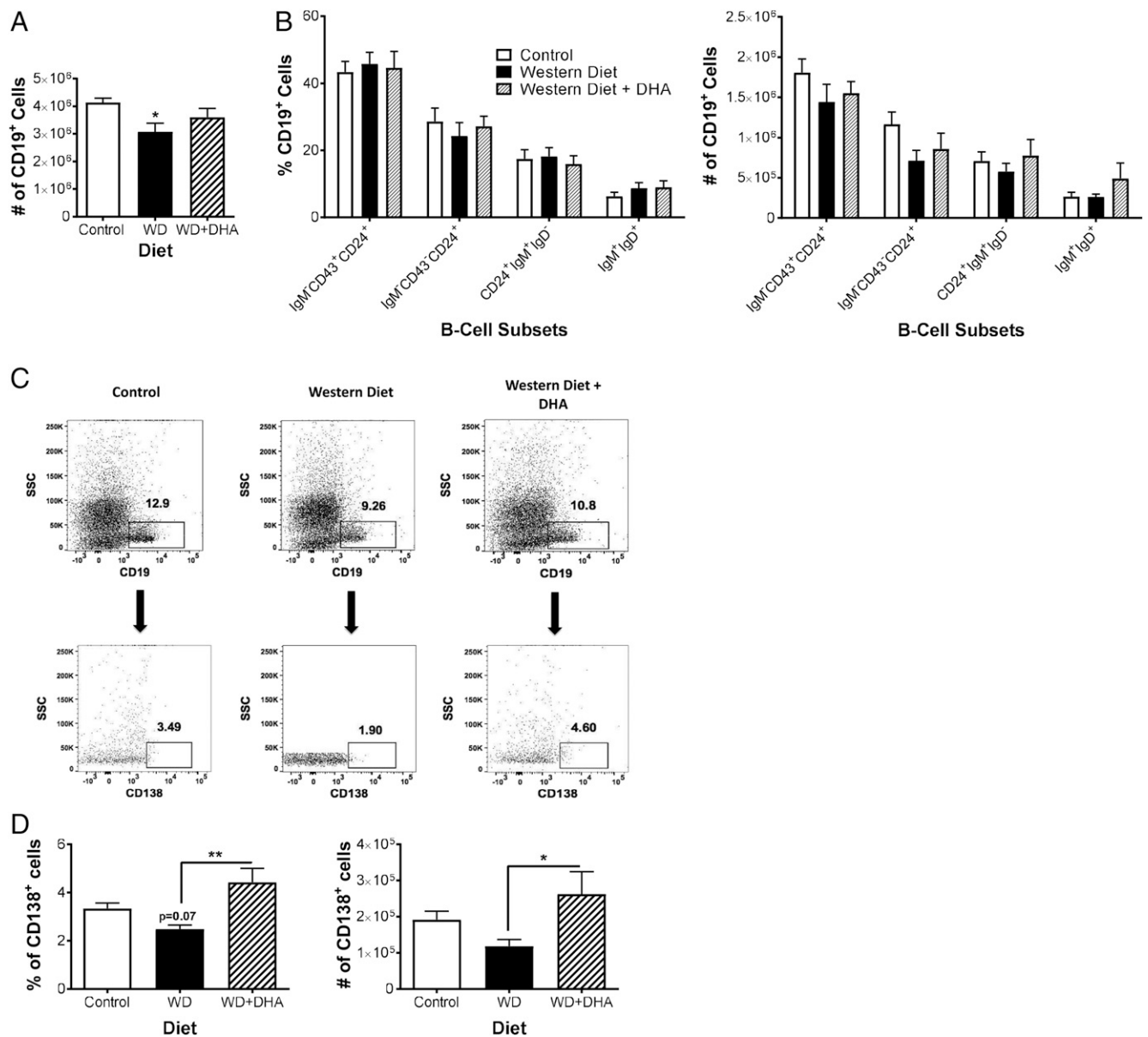
FIGURE 7. DHA increases the frequency of bone marrow CD138$^+$ plasma cells in mice consuming a WD. (**A**) Number of isolated B cells in bone marrow for mice consuming a control, WD, or WD plus DHA for 15 wk. (**B**) Percentage and frequency of B cell subsets. (**C**) Sample flow cytometry data of long-lived CD138$^+$ plasma cells in bone marrow for mice consuming a control, WD, and WD plus DHA. (**D**) Percentage and frequency of CD138$^+$ plasma cells in bone marrow. The results are from 21 d p.i. Data are average ± SEM; n = 7–8 mice per diet. *$p < 0.05$, **$p < 0.01$ by one-way ANOVA followed by a Bonferroni posttest.

will need to address differences in B cell responses between mouse and human models.

The mechanisms by which obesity impairs B cell production of Ab are likely pleiotropic. Upon affinity maturation, B cells differentiate into plasma cells that are responsible for secretion of high-affinity class-switched Abs, which are essential for prolonged protective immunity. In the HF diet model, there was a clear reduction in the percentage of bone marrow CD138$^+$ B cells, and a similar trend was observed in the WD model. Furthermore, in the HF diet model, there was a decrease in B cell frequency, and subsequent PCR analyses revealed a decrease in key transcripts for early lymphoid commitment, which was likely driven by defects in the bone marrow environment (58). To exemplify, mRNA levels of IL-7 were lowered, which is important because IL-7 is secreted by stromal cells in the bone marrow and is a key growth factor for commitment toward the lymphoid lineage (59). A de-

crease in IL-7 levels would then impair signaling through IL-7Rα and downstream activation of STAT5 (48). The decrease in plasma cells could be attributed to the reduction of Blimp1, a transcription factor necessary for plasma cell generation (51). Alternatively, the reduction in Blimp1 and other markers that were assayed for intermediate B cell development in bone marrow mRNA may simply reflect the reduction in the frequency of bone marrow CD19$^+$ cells driven by decreased IL-7Rα signaling.

The hyperstimulation of B cells observed with the HF diet may be due to several factors. Recent data suggest that circulating leptin, which is elevated in obesity, may be driving hyperstimulation of B cells (20). Specifically, B cells from obese individuals have elevated levels of intracellular TNF-α, which negatively correlates with their functional capacity (20). Therefore, leptin could be a potential factor that could be promoting Ab production under basal conditions from the B cells of obese mice. There could also

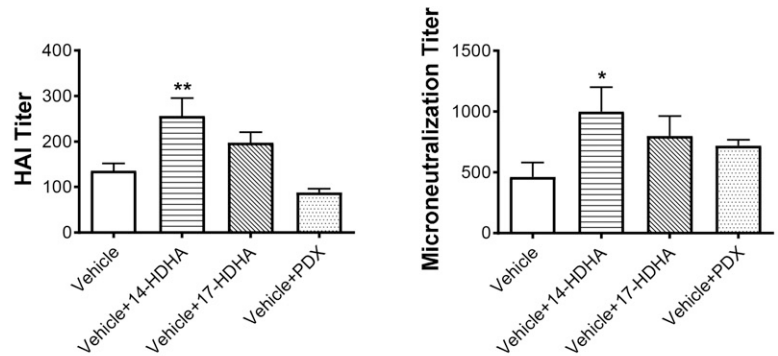## A Administration 24 hours post-infection
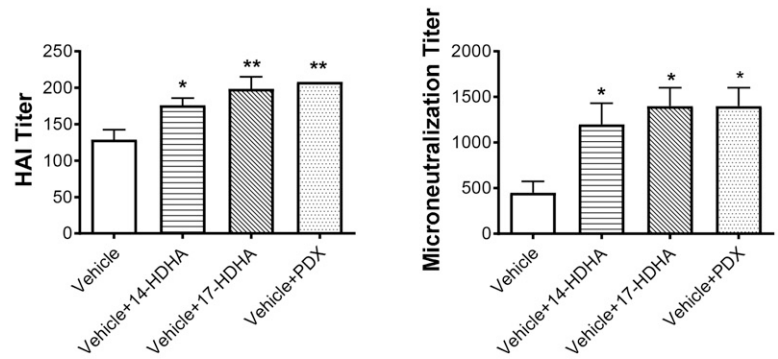
B Administration with infection

C

D

FIGURE 8. 14-HDHA enhances Ab titers accompanied by an increase in CD138^{+} cells upon influenza infection. (**A**) HAI and microneutralization titers upon administering select D-series SPMs 24 h postinfection. (**B**) HAI and microneutralization titers upon administering selected D-series SPMs in parallel with influenza infection. HAI and microneutralization titers were assayed in (A) and (B) at 14 d p.i. (**C**) Sample flow cytometry plots of CD138^{+} cells on day 21 p.i. for mice treated with 14-HDHA, 17-HDHA, or PDX. (**D**) Average percentage and frequency of CD138^{+} cells. Data are average $\pm$ SEM; n = 3–5 mice per treatment. $*p < 0.05$, $**p < 0.01$, by unpaired Student t test.
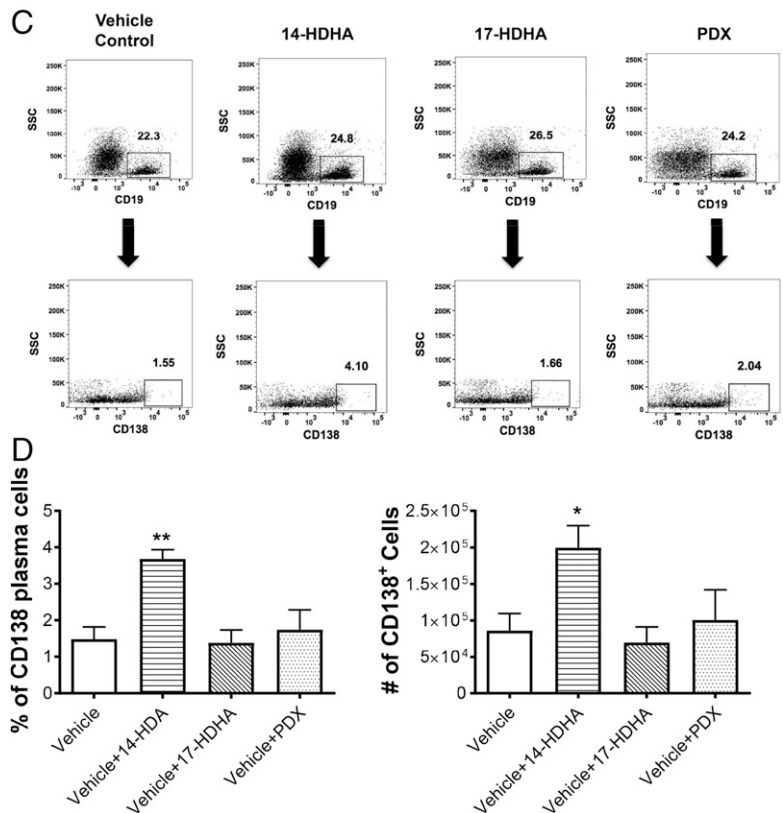
be other factors in obesity, such as elevated fasting insulin or glucose, that could be influencing Ab production.

A key mechanism that could impair B cell Ab production could be through the loss of conditional essential fatty acids and thereby SPMs in obesity. There is evidence demonstrating that obese children and adults have low levels of circulating essential fatty acids, including DHA, which could then impair many aspects of innate and adaptive immunity (30–33, 60). The reduction in DHA levels in obese subjects could be driven by low consumption of essential fatty acids in the diet and/or potential genetic modifi-

cations to key enzymes that metabolize DHA (33). We did not assay for circulating levels of essential fatty acids in this study. However, we did measure plasma D-series SPM concentration between lean and obese humans but SPM levels were at the lower limit of detection (data not shown), which could be due to sample degradation during the course of the clinical study.

The lipidomic data from the mice consuming the WD showed that PDX levels were lowered in mice consuming the WD relative to the control, and RvD2 levels showed a strong trend to be diminished compared with the lean control. Suppressed levels of PDX and other SPMs (which were not probed for such as E-series SPMs or the PDX isomer PD1) could be driving the inability to mount effective Ab responses to influenza (61). There is precedence for the lowering of specific SPM precursors and SPMs in mouse and human obesity in selected adipose tissue depots (62–64). For instance, the levels of PD1, 14-HDHA, and 17-HDHA are lowered in adipose tissue of obese mice, relative to controls, which contributes toward chronic inflammation (62, 65). Furthermore, 14-HDHA and 17-HDHA have also been shown to be decreased upon wound closure in obese diabetic mice (66). Therefore, future studies will need to address how lowering of circulating D-series SPM levels, potentially through a decrease in DHA levels or in essential fatty acid metabolism, could impair B cell responses such as class switching and production of Abs.

DHA's action through SPMs

Ramon et al. (54) demonstrated 17-HDHA boosted Ab production driven by the production of CD138$^+$ plasma cells in the bone marrow. Therefore, we investigated whether DHA was enhancing the production of CD138$^+$ plasma cells and indeed observed a modest increase in this B cell population. Contrary to our expectations, when we tested the effects of administering 17-HDHA, 14-HDHA, and PDX during the onset of infection, 14-HDHA enhanced Ab titers accompanied by an increase in the frequency of CD138$^+$ cells. However, 14-HDHA, similar to DHA, did not improve the body weights of the mice after infection. We also found no effect of DHA on survival upon infection in a preliminary study (data not shown). This suggests that perhaps the dose of DHA in the diet or a single injection of 14-HDHA is insufficient to improve body weights upon infection. Furthermore, DHA may be enhancing the production of Abs in obese mice without conferring protection. Indeed, a recent study showed that obese mice, despite generating neutralizing and nonneutralizing Abs upon adjuvanted influenza vaccination, failed to be protected upon challenge with influenza virus (67).

The effects of DHA are probably not just through 14-HDHA. Given that DHA promoted higher levels of other SPMs, the effects of DHA are likely driven by a combination of several SPMs, which may exert their effects at differing time points. This is relevant given that when 17-HDHA, 14-HDHA, and PDX were administered 24 h after the infection, Ab titers were also elevated. Furthermore, the studies with RvD1, RvD2, and PDX showed that all tested SPMs enhanced HAI titers in a preclinical vaccination model. Thus, future studies will likely require testing of combinations of SPMs to boost Ab production in obese mice. Additionally, subsequent experiments using B cell–specific knockouts and knockdowns of SPM receptors will need to be generated to establish cause-and-effect of DHA. This will require investigating whether there are additional B cell SPM receptors besides the ones analyzed in this study that may be responsive to differing SPMs. Furthermore, the effects of DHA through SPM production are likely not just through B cells but may be targeting many other cell types such as follicular helper T cells that would ultimately influence Ab production.

Several SPMs generated from DHA may also exert potential beneficial effects within the lungs where viral transcript levels were lowered upon infection in the presence of DHA (data not shown). This was not surprising given a report showing that influenza virus levels in a mouse model were lowered with PDX (68). Inclusion of DHA in the diet also promoted robust changes with *n*-6 PUFA-derived mediators, particularly in the spleen where arachidonic acid–derived mediators were lowered. Collectively, the lipidomic results provide a roadmap for future studies on how selected lipid mediators from *n*-6 fatty acids could also target humoral immunity in obesity. As an example, when PGE$_2$ is knocked down, responses to influenza infection are improved (69). Therefore, how DHA and/or SPMs target PGE$_2$ is of relevance given its role in influenza infection. Thus, the results open the door for screening additional lipid mediators that may regulate the humoral immune response (70).

A striking and unexpected finding came from the in vitro experiments with DHA. DHA in culture robustly lowered Ab production from B cells, and this finding was consistent with the notion that DHA is generally immunosuppressive when directly interacting with dendritic cells or helper CD4$^+$ T cells (71, 72). These results suggest that DHA's mechanism of action in vivo is not directly due to DHA acting on the B cells. It is likely that there are competing effects of DHA and its downstream mediators on B cell activity. There is literature precedence to show differing effects of DHA-derived lipid mediators on B cell responses. For instance, 17-HDHA boosts B cell Ab production by promoting the formation of long-lived plasma cell but dampens differentiation of naive B cells into IgE-secreting cells (73).

Essential fatty acid status is not well controlled for in immunometabolism studies

A notable conclusion from these studies is that essential fatty acid status influences humoral immunity and needs to be controlled for in mouse and human studies. In fact, various dietary fat sources are employed in HF diets and typically the fat source is lard in addition to the use of coconut oil, palm oil, milk fat, and/or soybean oil (74). Therefore, the use of different fat sources for immunological studies raises the concern that functional and mechanistic outcomes could be confounded by variations in the composition of the fat, such as essential fatty acids, provided to the rodent or human subject. A recent study even shows differences in immunological outcomes between a low fat diet and mice consuming a normal mouse chow. Mice consuming a low fat diet were more susceptible to death from influenza infection compared with mice on a normal chow (75). Therefore, it is essential to avoid confounding results by standardizing treatment regimes in relationship to fat composition.

In conclusion, the results across model systems provide evidence that B cell cytokine secretion and/or Ab production, outside of the context of adipose tissue inflammation, are modulated in obesity. Modifications to B cell function may be a major contributing factor toward the poor response to infections and vaccinations in the obese. Furthermore, the data show that essential fatty acid status, which is dysregulated in obesity, is an important variable in influencing Ab production in obese mice upon influenza infection, potentially through an SPM-mediated mechanism. This has implications for further investigating intervention strategies with a range of essential fatty acids and D-series SPMs for selected clinical populations such as the obese or even the aged that have diminished humoral immunity.

Disclosures

References

1. McGill, H. C., Jr., C. A. McMahan, E. E. Herderick, A. W. Zieske, G. T. Malcom, R. E. Tracy, and J. P. Strong, Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. 2002. Obesity accelerates the progression of coronary atherosclerosis in young men. *Circulation* 105: 2712–2718.

2. Quante, M., A. Dietrich, A. ElKhal, and S. G. Tullius. 2015. Obesity-related immune responses and their impact on surgical outcomes. *Int. J. Obes.* 39: 877–883.

3. Allott, E. H., and S. D. Hursting. 2015. Obesity and cancer: mechanistic insights from transdisciplinary studies. *Endocr. Relat. Cancer* 22: R365–R386.

4. Karlsson, E. A., and M. A. Beck. 2010. The burden of obesity on infectious disease. *Exp. Biol. Med. (Maywood)* 235: 1412–1424.

5. Furukawa, S., T. Fujita, M. Shimabukuro, M. Iwaki, Y. Yamada, Y. Nakajima, O. Nakayama, M. Makishima, M. Matsuda, and I. Shimomura. 2004. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J. Clin. Invest.* 114: 1752–1761.

6. Dixit, V. D. 2008. Adipose-immune interactions during obesity and caloric restriction: reciprocal mechanisms regulating immunity and health span. *J. Leukoc. Biol.* 84: 882–892.

7. Mishra, A. K., V. Dubey, and A. R. Ghosh. 2016. Obesity: an overview of possible role(s) of gut hormones, lipid sensing and gut microbiota. *Metabolism* 65: 48–65.

8. Lee, B. C., and J. Lee. 2014. Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance. *Biochim. Biophys. Acta* 1842: 446–462.

9. Weisberg, S. P., D. McCann, M. Desai, M. Rosenbaum, R. L. Leibel, and A. W. Ferrante, Jr. 2003. Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* 112: 1796–1808.

10. Weber, D. J., W. A. Rutala, G. P. Samsa, J. E. Santimaw, and S. M. Lemon. 1985. Obesity as a predictor of poor antibody response to hepatitis B plasma vaccine. *JAMA* 254: 3187–3189.

11. Huttunen, R., and J. Syrjänen. 2013. Obesity and the risk and outcome of infection. *Int. J. Obes.* 37: 333–340.

12. Genoni, G., F. Prodam, A. Marolda, E. Giglione, I. Demarchi, S. Bellone, and G. Bona. 2014. Obesity and infection: two sides of one coin. *Eur. J. Pediatr.* 173: 25–32.

13. Sheridan, P. A., H. A. Paich, J. Handy, E. A. Karlsson, M. G. Hudgens, A. B. Sammon, L. A. Holland, S. Weir, T. L. Noah, and M. A. Beck. 2012. Obesity is associated with impaired immune response to influenza vaccination in humans. *Int. J. Obes.* 36: 1072–1077.

14. Zhai, X., G. Qian, Y. Wang, X. Chen, J. Lu, Y. Zhang, Q. Huang, and Q. Wang. 2016. Elevated B cell activation is associated with type 2 diabetes development in obese subjects. *Cell. Physiol. Biochem.* 38: 1257–1266.

15. Arai, S., N. Maehara, Y. Iwamura, A. Honda, K. Nakashima, T. Kai, M. Ogishi, K. Morita, J. Kurokawa, M. Mori, et al. 2013. Obesity-associated autoantibody production requires AIM to retain the immunoglobulin M immune complex on follicular dendritic cells. *Cell Rep.* 3: 1187–1198.

16. Milner, J. J., P. A. Sheridan, E. A. Karlsson, S. Schultz-Cherry, Q. Shi, and M. A. Beck. 2013. Diet-induced obese mice exhibit altered heterologous immunity during a secondary 2009 pandemic H1N1 infection. *J. Immunol.* 191: 2474–2485.

17. Farnsworth, C. W., C. T. Shehatou, R. Maynard, K. Nishitani, S. L. Kates, M. J. Zuscik, E. M. Schwarz, J. L. Daiss, and R. A. Mooney. 2015. A humoral immune defect distinguishes the response to *Staphylococcus aureus* infections in mice with obesity and type 2 diabetes from that in mice with type 1 diabetes. *Infect. Immun.* 83: 2264–2274.

18. Rothaeusler, K., and N. Baumgarth. 2010. B-cell fate decisions following influenza virus infection. *Eur. J. Immunol.* 40: 366–377.

19. Shaikh, S. R., K. M. Haas, M. A. Beck, and H. Teague. 2015. The effects of diet-induced obesity on B cell function. *Clin. Exp. Immunol.* 179: 90–99.

20. Frasca, D., F. Ferracci, A. Diaz, M. Romero, S. Lechner, and B. B. Blomberg. 2016. Obesity decreases B cell responses in young and elderly individuals. *Obesity (Silver Spring)* 24: 615–625.

21. Ying, W., A. Tseng, R. C. Chang, H. Wang, Y. L. Lin, S. Kanameni, T. Brehm, A. Morin, B. Jones, T. Splawn, et al. 2016. miR-150 regulates obesity-associated insulin resistance by controlling B cell functions. *Sci. Rep.* 6: 20176.

22. Winer, D. A., S. Winer, L. Shen, P. P. Wadia, J. Yantha, G. Paltser, H. Tsui, P. Wu, M. G. Davidson, M. N. Alonso, et al. 2011. B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. *Nat. Med.* 17: 610–617.

23. Shen, L., M. H. Chng, M. N. Alonso, R. Yuan, D. A. Winer, and E. G. Engleman. 2015. B-1a lymphocytes attenuate insulin resistance. *Diabetes* 64: 593–603.

24. Harmon, D. B., P. Srikakulapu, J. L. Kaplan, S. N. Oldham, C. McSkimming, J. C. Garmey, H. M. Perry, J. L. Kirby, T. A. Prohaska, A. Gonen, et al. 2016. Protective role for B-1b B cells and IgM in obesity-associated inflammation, glucose intolerance, and insulin resistance. *Arterioscler. Thromb. Vasc. Biol.* 36: 682–691.

25. Nishimura, S., I. Manabe, S. Takaki, M. Nagasaki, M. Otsu, H. Yamashita, J. Sugita, K. Yoshimura, K. Eto, I. Komuro, et al. 2013. Adipose natural regulatory B cells negatively control adipose tissue inflammation. *Cell Metab.* 18: 759–766.

26. Nicholas, D. A., and B. S. Nikolajczyk. 2016. B cells shed light on diminished vaccine responses in obesity. *Obesity (Silver Spring)* 24: 551.

27. Madhumitha, H., V. Mohan, N. P. Kumar, R. Pradeepa, S. Babu, and V. Aravindhan. 2015. Impaired Toll-like receptor signalling in peripheral B cells from newly diagnosed type-2 diabetic subjects. *Cytokine* 76: 253–259.

28. DeFuria, J., A. C. Belkina, M. Jagannathan-Bogdan, J. Snyder-Cappione, J. D. Carr, Y. R. Nersesova, D. Markham, K. J. Strissel, A. A. Watkins, M. Zhu, et al. 2013. B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile. *Proc. Natl. Acad. Sci. USA* 110: 5133–5138.

29. Sheridan, P. A., H. A. Paich, J. Handy, E. A. Karlsson, M. Hudgens, S. Weir, T. Noah, and M. A. Beck. 2015. The antibody response to influenza vaccination is not impaired in type 2 diabetics. *Vaccine* 33: 3306–3313.

30. Calder, P. C. 2015. Marine omega-3 fatty acids and inflammatory processes: effects, mechanisms and clinical relevance. *Biochim Biophys Acta* 1851: 469–484.

31. Micallef, M., I. Munro, M. Phang, and M. Garg. 2009. Plasma *n*-3 polyunsaturated fatty acids are negatively associated with obesity. *Br. J. Nutr.* 102: 1370–1374.

32. Yu, Y., Z. Cai, J. Zheng, J. Chen, X. Zhang, X. F. Huang, and D. Li. 2012. Serum levels of polyunsaturated fatty acids are low in Chinese men with metabolic syndrome, whereas serum levels of saturated fatty acids, zinc, and magnesium are high. *Nutr. Res.* 32: 71–77.

33. Albert, B. B., J. G. Derraik, C. M. Brennan, J. B. Biggs, G. C. Smith, M. L. Garg, D. Cameron-Smith, P. L. Hofman, and W. S. Cutfield. 2014. Higher omega-3 index is associated with increased insulin sensitivity and more favourable metabolic profile in middle-aged overweight men. *Sci. Rep.* 4: 6697.

34. Teague, H., C. J. Fhaner, M. Harris, D. M. Duriancik, G. E. Reid, and S. R. Shaikh. 2013. *n*-3 PUFAs enhance the frequency of murine B-cell subsets and restore the impairment of antibody production to a T-independent antigen in obesity. *J. Lipid Res.* 54: 3130–3138.

35. Gurzell, E. A., H. Teague, M. Harris, J. Clinthorne, S. R. Shaikh, and J. I. Fenton. 2013. DHA-enriched fish oil targets B cell lipid microdomains and enhances ex vivo and in vivo B cell function. *J. Leukoc. Biol.* 93: 463–470.

36. Wong, J. Y., H. Kelly, C. M. Cheung, E. Y. Shiu, P. Wu, M. Y. Ni, D. K. M. Ip, and B. J. Cowling. 2015. Hospitalization fatality risk of influenza A(H1N1) pdm09: a systematic review and meta-analysis. *Am. J. Epidemiol.* 182: 294–301.

37. Serhan, C. N. 2014. Pro-resolving lipid mediators are leads for resolution physiology. *Nature* 510: 92–101.

38. Buckley, C. D., D. W. Gilroy, and C. N. Serhan. 2014. Proresolving lipid mediators and mechanisms in the resolution of acute inflammation. *Immunity* 40: 315–327.

39. Teague, H., M. Harris, J. Fenton, P. Lallemand, B. M. Shewchuk, and S. R. Shaikh. 2014. Eicosapentaenoic and docosahexaenoic acid ethyl esters differentially enhance B-cell activity in murine obesity. *J. Lipid Res.* 55: 1420–1433.

40. Bradberry, J. C., and D. E. Hilleman. 2013. Overview of omega-3 fatty acid therapies. *P&T* 38: 681–691.

41. Ramon, S., F. Gao, C. N. Serhan, and R. P. Phipps. 2012. Specialized pro-resolving mediators enhance human B cell differentiation to antibody-secreting cells. *J. Immunol.* 189: 1036–1042.

42. Kane, D. A., C. T. Lin, E. J. Anderson, H. B. Kwak, J. H. Cox, P. M. Brophy, R. C. Hickner, P. D. Neufer, and R. N. Cortright. 2011. Progesterone increases skeletal muscle mitochondrial H_2O_2 emission in nonmenopausal women. *Am. J. Physiol. Endocrinol. Metab.* 300: E528–E535.

43. Rockett, B. D., M. Harris, and S. Raza Shaikh. 2012. High dose of an *n*-3 polyunsaturated fatty acid diet lowers activity of C57BL/6 mice. *Prostaglandins Leukot. Essent. Fatty Acids* 86: 137–140.

44. Karlsson, E. A., V. A. Meliopoulos, C. Savage, B. Livingston, A. Mehle, and S. Schultz-Cherry. 2015. Visualizing real-time influenza virus infection, transmission and protection in ferrets. *Nat. Commun.* 6: 6378.

45. Deems, R., M. W. Buczynski, R. Bowers-Gentry, R. Harkewicz, and E. A. Dennis. 2007. Detection and quantitation of eicosanoids via high performance liquid chromatography-electrospray ionization-mass spectrometry. *Methods Enzymol.* 432: 59–82.

46. Yang, Y., C. Cruickshank, M. Armstrong, S. Mahaffey, R. Reisdorph, and N. Reisdorph. 2013. New sample preparation approach for mass spectrometry-based profiling of plasma results in improved coverage of metabolome. *J. Chromatogr. A* 1300: 217–226.

47. Shaikh, S. R., B. D. Rockett, M. Salameh, and K. Carraway. 2009. Docosahexaenoic acid modifies the clustering and size of lipid rafts and the lateral organization and surface expression of MHC class I of EL4 cells. *J. Nutr.* 139: 1632–1639.

48. Mazzucchelli, R., and S. K. Durum. 2007. Interleukin-7 receptor expression: intelligent design. *Nat. Rev. Immunol.* 7: 144–154.

49. Goetz, C. A., I. R. Harmon, J. J. O'Neil, M. A. Burchill, T. M. Johanns, and M. A. Farrar. 2005. Restricted STAT5 activation dictates appropriate thymic B versus T cell lineage commitment. *J. Immunol.* 174: 7753–7763.

50. Matthias, P., and A. G. Rolink. 2005. Transcriptional networks in developing and mature B cells. *Nat. Rev. Immunol.* 5: 497–508.

51. Shapiro-Shelef, M., K. I. Lin, D. Savitsky, J. Liao, and K. Calame. 2005. Blimp-1 is required for maintenance of long-lived plasma cells in the bone marrow. *J. Exp. Med.* 202: 1471–1476.

52. Oh, D. Y., S. Talukdar, E. J. Bae, T. Imamura, H. Morinaga, W. Fan, P. Li, W. J. Lu, S. M. Watkins, and J. M. Olefsky. 2010. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* 142: 687–698.

53. Garcia-Bates, T. M., C. J. Baglole, M. P. Bernard, T. I. Murant, P. J. Simpson-Haidaris, and R. P. Phipps. 2009. Peroxisome proliferator-activated receptor γ ligands enhance human B cell antibody production and differentiation. *J. Immunol.* 183: 6903–6912.

54. Ramon, S., S. F. Baker, J. M. Sahler, N. Kim, E. A. Feldsott, C. N. Serhan, L. Martínez-Sobrido, D. J. Topham, and R. P. Phipps. 2014. The specialized proresolving mediator 17-HDHA enhances the antibody-mediated immune response against influenza virus: a new class of adjuvant? *J. Immunol.* 193: 6031–6040.

55. Colas, R. A., M. Shinohara, J. Dalli, N. Chiang, and C. N. Serhan. 2014. Identification and signature profiles for pro-resolving and inflammatory lipid mediators in human tissue. *Am. J. Physiol. Cell Physiol.* 307: C39–C54.

56. Skarke, C., N. Alamuddin, J. A. Lawson, X. Li, J. F. Ferguson, M. P. Reilly, and G. A. FitzGerald. 2015. Bioactive products formed in humans from fish oils. *J. Lipid Res.* 56: 1808–1820.

57. Frasca, D., A. Diaz, M. Romero, A. M. Landin, and B. B. Blomberg. 2014. High TNF-α levels in resting B cells negatively correlate with their response. *Exp. Gerontol.* 54: 116–122.

58. Chan, M. E., B. J. Adler, D. E. Green, and C. T. Rubin. 2012. Bone structure and B-cell populations, crippled by obesity, are partially rescued by brief daily exposure to low-magnitude mechanical signals. *FASEB J.* 26: 4855–4863.

59. Clark, M. R., M. Mandal, K. Ochiai, and H. Singh. 2014. Orchestrating B cell lymphopoiesis through interplay of IL-7 receptor and pre-B cell receptor signalling. *Nat. Rev. Immunol.* 14: 69–80.

60. Burrows, T., C. E. Collins, and M. L. Garg. 2011. Omega-3 index, obesity and insulin resistance in children. *Int. J. Pediatr. Obes.* 6: e532–e539.

61. Balas, L., M. Guichardant, T. Durand, and M. Lagarde. 2014. Confusion between protectin D1 (PD1) and its isomer protectin DX (PDX). An overview on the dihydroxy-docosatrienes described to date. *Biochimie* 99: 1–7.

62. Clària, J., J. Dalli, S. Yacoubian, F. Gao, and C. N. Serhan. 2012. Resolvin D1 and resolvin D2 govern local inflammatory tone in obese fat. *J. Immunol.* 189: 2597–2605.

63. Spite, M., J. Clària, and C. N. Serhan. 2014. Resolvins, specialized proresolving lipid mediators, and their potential roles in metabolic diseases. *Cell Metab.* 19: 21–36.

64. Titos, E., B. Rius, C. López-Vicario, J. Alcaraz-Quiles, V. García-Alonso, A. Lopategi, J. Dalli, J. J. Lozano, V. Arroyo, S. Delgado, et al. 2016. Signaling and immunoresolving actions of resolvin D1 in inflamed human visceral adipose tissue. *J. Immunol.* 197: 3360–3370.

65. Neuhofer, A., M. Zeyda, D. Mascher, B. K. Itariu, I. Murano, L. Leitner, E. E. Hochbrugger, P. Fraisl, S. Cinti, C. N. Serhan, and T. M. Stulnig. 2013. Impaired local production of proresolving lipid mediators in obesity and 17-HDHA as a potential treatment for obesity-associated inflammation. *Diabetes* 62: 1945–1956.

66. Tang, Y., M. J. Zhang, J. Hellmann, M. Kosuri, A. Bhatnagar, and M. Spite. 2013. Proresolution therapy for the treatment of delayed healing of diabetic wounds. *Diabetes* 62: 618–627.

67. Karlsson, E. A., T. Hertz, C. Johnson, A. Mehle, F. Krammer, and S. Schultz-Cherry. 2016. Obesity outweighs protection conferred by adjuvanted influenza vaccination. *MBio* 7: e01144-16.

68. Morita, M., K. Kuba, A. Ichikawa, M. Nakayama, J. Katahira, R. Iwamoto, T. Watanebe, S. Sakabe, T. Daidoji, S. Nakamura, et al. 2013. The lipid mediator protectin D1 inhibits influenza virus replication and improves severe influenza. *Cell* 153: 112–125.

69. Coulombe, F., J. Jaworska, M. Verway, F. Tzelepis, A. Massoud, J. Gillard, G. Wong, G. Kobinger, Z. Xing, C. Couture, et al. 2014. Targeted prostaglandin E₂ inhibition enhances antiviral immunity through induction of type I interferon and apoptosis in macrophages. *Immunity* 40: 554–568.

70. Tam, V. C., O. Quehenberger, C. M. Oshansky, R. Suen, A. M. Armando, P. M. Treuting, P. G. Thomas, E. A. Dennis, and A. Aderem. 2013. Lipidomic profiling of influenza infection identifies mediators that induce and resolve inflammation. *Cell* 154: 213–227.

71. Yog, R., R. Barhoumi, D. N. McMurray, and R. S. Chapkin. 2010. *n*-3 polyunsaturated fatty acids suppress mitochondrial translocation to the immunologic synapse and modulate calcium signaling in T cells. *J. Immunol.* 184: 5865–5873.

72. Teague, H., B. D. Rockett, M. Harris, D. A. Brown, and S. R. Shaikh. 2013. Dendritic cell activation, phagocytosis and CD69 expression on cognate T cells are suppressed by *n*-3 long-chain polyunsaturated fatty acids. *Immunology* 139: 386–394.

73. Kim, N., S. Ramon, T. H. Thatcher, C. F. Woeller, P. J. Sime, and R. P. Phipps. 2016. Specialized proresolving mediators (SPMs) inhibit human B-cell IgE production. *Eur. J. Immunol.* 46: 81–91.

74. Hariri, N., and L. Thibault. 2010. High-fat diet-induced obesity in animal models. *Nutr. Res. Rev.* 23: 270–299.

75. Milner, J. J., J. Rebeles, S. Dhungana, D. A. Stewart, S. C. J. Sumner, M. H. Meyers, P. Mancuso, and M. A. Beck. 2015. Obesity increases mortality and modulates the lung metabolome during pandemic H1N1 influenza virus infection in mice. *J. Immunol.* 194: 4846–4859.