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Docosahexaenoic Acid-Enriched Fish Oil Attenuates Kidney Disease and Prolongs Median and Maximal Life Span of Autoimmune Lupus-Prone Mice

Ganesh V. Halade,*† Md Mizanur Rahman,*† Arunabh Bhattacharya,*† Jeffrey L. Barnes,*‡ Bysani Chandrasekar,§*† and Gabriel Fernandes*†

The therapeutic efficacy of individual components of fish oils (FOs) in various human inflammatory diseases still remains unresolved, possibly due to low levels of n-3 fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) or lower ratio of DHA to EPA. Because FO enriched with DHA (FO-DHA) or EPA (FO-EPA) has become available recently, we investigated their efficacy on survival and inflammatory kidney disease in a well-established animal model of human systemic lupus erythematosus. Results show for the first time that FO-DHA dramatically extends both the median (658 d) and maximal (848 d) life span of (NZB X NZW)F1 (B X W) mice. In contrast, FO-EPA fed mice had a median and maximal life span of ~384 and 500 d, respectively. Investigations into possible survival mechanisms revealed that FO-DHA (versus FO-EPA) lowers serum anti-dsDNA Abs, IgG deposition in kidneys, and proteinuria. Further, FO-DHA lowered LPS-mediated increases in serum IL-18 levels and caspase-1-dependent cleavage of pro–IL-18 to mature IL-18 in kidneys. Moreover, FO-DHA suppressed LPS-mediated PI3K, Akt, and NF-κB activations in kidney. These data indicate that DHA, but not EPA, is the most potent n-3 fatty acid that suppresses glomerulonephritis and extends life span of systemic lupus erythematosus-prone short-lived B X W mice, possibly via inhibition of IL-18 induction and IL-18-dependent signaling. The Journal of Immunology, 2010, 184: 5280–5286.
The study was carried out in two phases. In the first phase, survival, systemic anti-dsDNA Abs, IgG deposition in kidneys, and proteinuria were studied. In the second phase, to emphasize the mechanisms of improved survival by DHA and LPS-evoked IL-18 signaling (22, 23), 5-mo-old mice were challenged with LPS (5 mg/kg body weight; i.p.). PBS served as a vehicle control. Both serum and kidneys were collected after 4 h, and analyzed for immunologic, biochemical, and molecular changes. All studies were approved by the Institutional Animal Care and Use Committee of University of Texas Health Science Center, San Antonio, TX.

**Serum FA analysis**

FA composition was analyzed by gas chromatography as described previously (24). Briefly, 100 μl serum were subjected to lipid extraction. FA methyl esters were derived by heating at 75°C for 1 h in 5% hydrochloric acid–methanol reagent. FA methyl esters were analyzed by gas chromatography using a fully automated HP5890A series II system equipped with a flame-ionization detector. Peaks of resolved FAs were identified by comparison with FA standards (Matreya, Pleasant Gap, PA), and area percentage for all resolved peaks was analyzed using a HP 3396 series II integrator.

**IgG deposition in kidneys**

Kidney tissues were snap-frozen in Optimal Cutting Temperature Compound (Miles Scientific, Naperville, IL) and sectioned (4 μm thick). To examine IgG deposits within renal glomeruli, the sections were incubated with FITC-conjugated goat anti-mouse IgG Ab (Sero tec, Oxford, U.K.). Fluorescence intensity within glomerular capillary walls was scored on a scale of 0–3 (0, none; 1, weak; 2, moderate; 3, strong). At least 10 glomeruli per section were analyzed by two independent investigators in a blinded fashion, and scored.

**Proteinuria**

Proteinuria was assessed using chemstrips (Roche Diagnostic, Indianapolis, IN). Consistent with the criteria applied in previous studies of murine lupus, proteinuria 100 mg/dl, 2+ to 100–500 mg/dl, and 3+ to 500 mg/dl was interpreted as an evidence of significant end-stage renal disease.

**Anti-dsDNA Abs**

Serum anti-dsDNA Ab titers were assessed as previously described using a solid-phase ELISA (16).

**Serum IL-18 levels**

Serum IL-18 levels were quantified by ELISA (Bender MedSystems, Burlingame, CA). The sensitivity of the assay is 10.0 pg/ml.

**Caspase-1 activity**

Caspase-1 activity in kidney homogenates was determined by the caspase-1/ICE colorimetric Protease assay kit (BioVision Research Products, Mountain View, CA). The assay is based on the spectrophotometric detection of the chromophore p-nitroanilide (pNA) after cleavage from the labeled substrate YVAD-pNA. The pNA light emission was quantified spectrophotometrically at 405 nm, and the results were expressed in fold-increase from controls.

**Pro– and mature IL-18 levels**

IL-18 protein levels were quantified by Western blotting using Abs specific for pro (R&D Systems, Minneapolis, MN) and mature (Santa Cruz Biotechnology, Santa Cruz, CA) forms of IL-18.

**Measurement of PI3-kinase**

PI3K lipid kinase assays were performed as described previously (25) using p85 immunoprecipitates.

**Akt levels and Akt kinase activity**

We used two independent but complimentary methods to quantify activation of Akt: immunoblotting using whole cell homogenates and activation-specific Abs, and immune-complex kinase assays using a commercially available nonradioactive Akt kinase assay kit (Cell Signaling Technology, Danvers, MA). The assay is based on Akt-induced phosphorylation (Ser21/9) of glycogen synthase kinase-3.

**NF-κB activation**

NF-κB DNA binding activity was analyzed by EMSA using nuclear protein extracts and double stranded consensus (sense, 5’-AGT TGA GGC GAC TTT CCC AGG C-G3’) or mutant (sense, 5’-AGT TGA GCC GAC TTT CCC AGG C-3’) NF-κB oligonucleotides (Santa Cruz Biotechnology). Nuclear p65 levels were quantified by Western blotting (Cell Signaling Technology). Actin served as a loading control.

**Statistical analysis**

Data are expressed as mean ± SEM. Results were analyzed by ANOVA, followed by Newman-Keuls test using Graph Prism 4 software (GraphPad, San Diego, CA) and p < 0.05 was considered statistically significant. Survival data were analyzed by Logrank, followed by χ² test.

**Results**

**Delayed onset of kidney disease and maximal life span in FO-DHA mice**

We have previously demonstrated that Menhaden FO attenuates kidney disease and moderately extends life span of B × W mice (26). We now investigated whether enriching FO with DHA (60% DHA, 5% EPA) or EPA (5% DHA, 55% EPA) will further extend life span and delay progression of renal disease (Fig. 1). Female B × W mice were fed regular FO (18% EPA, 12% DHA; FO-18/12), FO-DHA, and FO-EPA. CO that contains neither DHA nor EPA served as a control (Table I). Results show that the median life span of CO-fed control animals was 372 d, and FO-18/12 moderately extended median life span to 414 d. In contrast, FO-DHA significantly increased median life span to 658 d. Interestingly, FO-EPA had minimal effect on life span (384 d), and was comparable to that of CO-fed mice. Similarly, maximal life span was significantly higher nearly doubled in FO-DHA fed mice (848 d) compared with FO-EPA (500 d), FO-18/12 (539 d), and CO-fed (444 d) mice. These results indicate that DHA, but not
EPA-enriched FO, significantly extends both median and maximal life span of the short-lived B × W mice.

Serum FA profile

To verify whether dietary oils influence serum FA profile, we analyzed serum for PUFA by gas chromatography (24). FO-fed mice exhibited higher levels of n-3 FA as compared with CO-fed mice. Although there was no difference in 20:5n-3 (EPA) levels analyzed serum for PUFA by gas chromatography (24). FO-fed mice.

AFO-DHA fed mice. In contrast, FO-EPA failed to significantly lower the serum anti-dsDNA Ab titer as compared with CO-fed mice. Serum anti-dsDNA Abs are reduced in FO-DHA fed mice (Table II).

Consequently, n-6/n-3 FA ratio was lowest in FO-DHA fed mice compared with other FO and CO diet fed mice. Total n-6 FA content was highest and total n-3 FA content was lowest in FO-DHA fed mice with compared with other FO and CO diet fed mice. Consequently, n-6/n-3 FA ratio was lowest in FO-DHA fed mice (Table II).

Serum anti-dsDNA Abs are reduced in FO-DHA fed mice

Anti-dsDNA Abs are implicated in the pathogenesis of SLE. FO-18/12 significantly lowered the serum anti-dsDNA Ab titer as compared with CO-fed mice (Fig. 2A). These effects were more pronounced in DHA-fed mice. In contrast, FO-EPA failed to significantly modulate anti-dsDNA Ab titer, and the levels were comparable to that seen in CO-fed mice, indicating that DHA, but not EPA, significantly lowers systemic anti-dsDNA Abs in SLE-prone B × W mice (Fig. 2A).

IgG deposition and proteinuria are decreased in kidneys of FO-DHA fed mice

Elevated proteinuria and deposition of IgG in glomeruli are characteristic features of renal disease in B × W mice (27). When compared with CO-fed mice, histological evaluation of IgG deposition in kidneys (Fig. 2B) and proteinuria levels (Table III) were both significantly decreased in FO-18/12 and FO-DHA fed mice, but to a greater extent in the latter group. There was, however, no difference in IgG deposition between CO and FO-EPA fed mice, indicating that DHA, but not EPA, potently downregulates IgG deposition in kidneys of SLE-prone B × W mice (Fig. 2C).

Serum IL-18 levels are lowered in FO-DHA fed mice

IL-18 plays a causal role in SLE (5), and LPS is a potent inducer of IL-18 (22). Therefore, we investigated serum IL-18 levels after LPS challenge. Results in Fig. 3A show that FO-18/12 and FO-DHA both significantly lowered LPS-induced IL-18 in serum as compared with CO-fed mice. Once again, FO-EPA failed to modulate LPS-mediated IL-18 expression, indicating that FO-DHA significantly lowers proinflammatory IL-18 levels in serum of SLE-prone B × W mice (Fig. 3A).

Mature, but not pro-IL-18 expression, is lowered in kidneys of FO-DHA-fed mice

IL-18 is synthesized as a proform, and is cleaved by caspase-1 to a mature biologically active 18 kDa secreted form. Because FO-DHA significantly attenuated serum IL-18 levels (Fig. 3A), we investigated whether decreased serum IL-18 levels reflect reduced IL-18 expression in the kidneys. Western blot analysis of kidney homogenates revealed detectable levels of pro-IL-18, and LPS administration failed to significantly modulate its expression. In contrast, mature IL-18 was detected at low levels in vehicle (PBS)-treated animals, and was increased in all groups after LPS administration. However, LPS-mediated increase in mature IL-18 expression was attenuated in both FO-18/12 and FO-DHA groups, and once again, FO-DHA was the most potent. Because caspase-1 cleaves pro-IL-18 to mature IL-18, we further investigated whether decreased levels of mature IL-18 were due to reduced

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### Table I. Composition of semipurified CO and FO diets enriched with EPA or DHA

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>CO</th>
<th>FO-18/12</th>
<th>FO-EPA</th>
<th>FO-DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>14.00</td>
<td>14.00</td>
<td>14.00</td>
<td>14.00</td>
</tr>
<tr>
<td>Corn starch</td>
<td>42.43</td>
<td>42.43</td>
<td>42.43</td>
<td>42.43</td>
</tr>
<tr>
<td>Dextranized corn starch</td>
<td>14.50</td>
<td>14.50</td>
<td>14.50</td>
<td>14.50</td>
</tr>
<tr>
<td>Sucrose</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
<td>9.00</td>
</tr>
<tr>
<td>Cellulose</td>
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<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
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<tr>
<td>AIN-93 mineral mix</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
<td>3.50</td>
</tr>
<tr>
<td>AIN-93 vitamin mix</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>t-cystine</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
<td>0.18</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>TBHQ</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>FO</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>FO⁹</td>
<td>0</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

*All diet ingredients were purchased from MP Biomedicals. FOs enriched in either EPA or DHA: 1) FO-18/12, 2) 55/5 FO-EPA, and 3) 5/60 FO-DHA (Ocean Nutrition). CO, corn oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil.

### Table II. Profiles of PUFA n-6 and n-3 FAs in serum of (NZB × NZW)F1 mice fed with specialized CO and FO diets enriched with EPA or DHA

<table>
<thead>
<tr>
<th>FAs</th>
<th>CO</th>
<th>FO-18/12</th>
<th>FO-EPA</th>
<th>FO-DHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2n-6</td>
<td>30.33 ± 0.70⁹</td>
<td>13.69 ± 0.31⁹</td>
<td>9.79 ± 0.49⁹</td>
<td>8.85 ± 0.37⁹</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>0.28 ± 0.03</td>
<td>0.27 ± 0.00</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>19.52 ± 0.36⁹</td>
<td>9.59 ± 0.25⁹</td>
<td>12.51 ± 0.48⁹</td>
<td>5.21 ± 0.22⁹</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>ND</td>
<td>20.17 ± 1.33</td>
<td>22.69 ± 1.17</td>
<td>20.76 ± 0.43</td>
</tr>
<tr>
<td>22:5n-6</td>
<td>0.34 ± 0.12</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>3.33 ± 0.40⁹</td>
<td>0.39 ± 0.02⁹</td>
<td>0.96 ± 0.15⁹</td>
<td>0.70 ± 0.07⁹</td>
</tr>
<tr>
<td>PUFA</td>
<td>53.79 ± 0.74</td>
<td>56.48 ± 0.56</td>
<td>55.84 ± 0.92</td>
<td>59.40 ± 0.62</td>
</tr>
<tr>
<td>n-3 FA</td>
<td>33.54 ± 0.74</td>
<td>33.11 ± 1.20⁹</td>
<td>33.54 ± 1.01⁹</td>
<td>45.34 ± 0.66⁹</td>
</tr>
<tr>
<td>n-6 FA</td>
<td>50.46 ± 0.48⁹</td>
<td>23.37 ± 0.64⁹</td>
<td>22.30 ± 0.24⁹</td>
<td>14.06 ± 0.57⁹</td>
</tr>
<tr>
<td>n-6/n-3</td>
<td>15.85 ± 1.90</td>
<td>0.71 ± 0.05</td>
<td>0.67 ± 0.03</td>
<td>0.31 ± 0.02</td>
</tr>
</tbody>
</table>

Total lipids of (NZB × NZW)F1 mice serum were extracted, methylated and subjected to analysis by gas chromatography. The values (% of total FAs) are means of three independent measurements ± SEM. *p < 0.05. Significant difference (*p < 0.05) is indicated with different superscripted letters analyzed by ANOVA, followed by Newman-Keuls test. Ratio of n-6/n-3 fatty acids is expressed as (18:2n-6 + 20:3n-6 + 20:4n-6 + 22:5n-6)/(20:5n-3 + 22:5n-3 + 22:6n-3). CO, corn oil; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acids; FO, fish oil; ND, not detected; PUFA, polyunsaturated fatty acids.
subunit nuclear translocation was observed in FO-18/12 fed mice and it was even higher in DHA-enriched FO-fed mice as compared with CO-fed mice. There was, however, no inhibition in EPA-enriched FO fed mice (Fig. 5A, 5B).

**Discussion**

We previously reported that n-3 FA (20–25% EPA and DHA) present in Menhaden FO extends median and maximal life span of short-lived lupus-prone B × W mice compared with a diet supplemented with n-6 FA-rich CO (15, 16, 28). Our subsequent studies demonstrated that a combination of n-3 FA and caloric restriction (CR) further extends the life span of B × W mice even more than n-6 FA fed ad libitum or with CR (29), suggesting that source of dietary fat (n-3 FA versus n-6 FA) was an important determinant of disease progression and severity in B × W mice. Although these findings are encouraging, there are obvious concerns that a dietary regimen of 30–40% CR may be impractical for SLE patients, and that FO with low EPA/DHA content would have only moderate beneficial effects in the same patients. Because EPA and DHA are the principal biologically active FAs in FO, and as their levels/ratio affect the anti-inflammatory effects, in the current study we investigated the efficacy of FO enriched with DHA (60% DHA/55% EPA; FO-DHA) or EPA (5% DHA/55% EPA; FO-EPA) on disease severity and longevity in B × W mice. Our results demonstrate for the first time that DHA is a potent inhibitor of autoantibody production, IL-18 expression and kidney disease, and that DHA significantly extends life span of short-lived B × W mice.

FO-DHA significantly attenuated serum and kidney IL-18 expression. IL-18 is a proinflammatory cytokine and its systemic levels are significantly elevated in SLE patients (5, 10). Similarly, SLE-prone MRL/lpr mice also express high levels of serum IL-18 (10). Further, B × W mice repeatedly exposed to LPS develop an early and accelerated form of lupus nephritis (23), with enhanced polyclonal B cell activation and persistence of exacerbated nephritis, even after LPS clearance (30). In the current study, we found that LPS treatment significantly increased serum IL-18 levels in both CO and FO-EPA fed mice. However, a significant inhibition in serum and kidney IL-18 levels was observed in FO-DHA and FO-18/12 fed mice (inhibitory activity: FO-DHA > FO-18/12 > FO-EPA). Because caspase-1 cleaves pro–IL-18 to mature IL-18, and as caspase-1 activity was lower in the LPS-treated FO-DHA fed mice, our results suggest that lower levels of mature IL-18 in serum and kidneys were due to the reduced cleavage of pro–IL-18 to mature IL-18.

Because PI3 kinase plays a critical role in IL-18 induction as well as in IL-18 signaling (31, 32), we next analyzed the activation status of PI3K in kidneys after LPS treatment. Compared with vehicle-treated mice, a robust increase in PI3K activation was noted in kidneys from LPS-injected CO and FO-EPA fed mice. In contrast, FO-DHA and FO-18/12 fed mice showed lower levels of PI3K-dependent PI3P activation levels. Of note, PI3Kγ is an important target for inhibition of glomerulonephritis and extension of life span in MRL/lpr mice (33). Because FO-DHA attenuated PI3K activation, and because inhibition of PI3Kγ was shown to prolong life span and decrease glomerulonephritis, our results also suggest that dietary supplementation of FO-DHA is a viable therapeutic strategy to ameliorate chronic inflammation (33). Because the serine/threonine kinase Akt/protein kinase B is one of the major downstream targets of PI3K, we also analyzed total and phospho-Akt (Ser473) levels in kidney homogenates. Our results indicate that although total Akt remained similar in the kidneys of all four groups, phospho-Akt levels were reduced in FO-DHA and FO-18/12 fed mice. Once again, FO-DHA was more potent in inhibiting caspase-1 activity (Fig. 3B). Indeed, FO-DHA was more potent in inhibiting LPS-mediated increase in caspase-1 activity. These results indicate that FO-DHA attenuates IL-18 expression by inhibiting caspase-1 activity and caspase-1-dependent pro–IL-18 to IL-18 processing (Fig. 3C, 3D).

**FO-DHA inhibits LPS-mediated PI3K, Akt, and NF-κB activations in kidneys**

Because LPS signals via PI3K, we measured PI3P in kidneys of PBS and LPS-injected mice. Although there was no difference in basal levels of PI3P FO-18/12 and to a higher extent DHA-enriched FO prevented LPS-stimulated PI3P activation status as compared with CO feeding. There was no difference in PI3P activation between CO-fed and EPA-enriched FO-fed mice (Fig. 4A).

Akt is downstream of PI3K and its phosphorylation at Ser473 denotes activation. Similar to its inhibition of PI3K, a significant inhibition of phospho-Akt levels (Fig. 4B) as well as Akt kinase activity (Fig. 4C) were observed in FO-18/12 fed mice, and to a much higher extent in DHA-enriched FO-fed mice as compared with CO-fed mice. No inhibition was observed in EPA-enriched FO fed mice.

Because NF-κB is involved in IL-18 induction, and is downstream of PI3K and Akt, we next analyzed NF-κB DNA binding activity by EMSA using kidney nuclear extracts. We also analyzed nuclear translocation of NF-κB p65 by immunoblotting. Significant inhibition of NF-κB DNA binding activity and NF-κB p65
Akt activation. These results were further confirmed by immune-
complex kinase assays, which revealed reduced Akt kinase ac-
tivity in FO-DHA fed mice, suggesting that FO-DHA poten-
tially inhibits Akt activation in kidneys in vivo. Although DHA has
been shown to inhibit LPS-induced Akt phosphorylation in RAW264.7
macrophages in vitro (34), our studies are the first to show that
DHA, but not EPA, inhibits PI3K/Akt signaling in vivo in the
kidneys of SLE-prone mice.

NF-κB is a ubiquitous stress-responsive transcription factor and
plays a role in inflammation. Because NF-κB is a downstream
mediator of PI3K and Akt pathways, and plays a role in IL-18
induction and signaling (31, 32), we analyzed NF-κB activation
by EMSA and nuclear translocation of p65 by immunoblotting in
kidneys of SLE-prone mice. As shown in Table II, p65 activa-
tion, and suggests that reduced NF-κB activation might be a
contributing factor in the inhibition of LPS-induced renal disease
in FO-DHA fed mice.

Activations of PI3K, Akt, and NF-κB play a role in both IL-18
induction and IL-18–dependent signaling. Importantly, our results
show that FO-DHA is a potent inhibitor of these three critical
players of inflammation, thus inhibiting perpetuation of inflam-
matory signaling during SLE. Although we demonstrated that FO-
DHA inhibits LPS-mediated NF-κBp65 nuclear translocation,
recently it has also been shown that IkB degradation contributes to
LPS-mediated NF-κB activation and IL-18 signaling (22, 39, 40),
suggesting that the observed p65 nuclear translocation follows IkB
degradation, and FO-DHA might activate NF-κB via classic IkB
degradation and NF-κB activation in LPS-treated mice. Further,
PI3K, which is now considered a potential therapeutic target in
SLE (33, 41), has also been shown to be a target of DHA in
neuronal cells, thus its inhibition improves their survival (42).
Because, FO-DHA fed mice exhibited reduced levels of LPS-
induced NF-κB activation, and serum and kidney IL-18 expres-
sion, it is reasonable to speculate that FO-DHA inhibits NF-κB via
classic IkB degradation and NF-κB activation in LPS-treated mice.
Further, PI3K, which is now considered a potential therapeutic target in
SLE (33, 41), has also been shown to be a target of DHA in
neuronal cells, thus its inhibition improves their survival (42).

Circulating autoantibodies to DNA is one of the hallmarks of
SLE in humans (1) and B × W mice (3, 4). Anti-dsDNA Abs form
immune complexes and their deposition results in arthritis and
nephritis. B × W mice on CO and FO-EPA diets exhibited higher
anti-dsDNA Ab levels in serum and higher IgG deposition in the
kidneys. In correlation with their survival data, FO-DHA fed mice
had lower levels of serum anti-dsDNA Ab and kidney IgG de-
position. It was previously reported that MRL/lpr mice deficient in IL-18 administration (10). In contrast,
lower total IgG and IgG2 anti-dsDNA Abs and higher IgG
deposition in kidneys after IL-18 administration (10). In contrast,
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SLE-prone mice, and inhibition of IL-18 and its signaling by DHA may alleviate clinical features of SLE. It should be pointed out that FO doses used in this study are relatively higher compared with human consumption via food or dietary supplementation. Our recent findings, using prescribed FO (Lovaza) 1% as a human equivalent dose in B × W mice also revealed significant decrease in proteinuria and improved survival compared with 1% placebo (unpublished data). Future clinical trials with purified DHA enriched FO or Lovaza in lupus patients is warranted.

In summary, our studies demonstrate for the first time that FO enriched in DHA attenuates glomerulonephritis and significantly extends life span of short-lived SLE-prone B × W mice, compared with EPA enriched FO or FO with lower EPA+ DHA levels. This beneficial effect of DHA may be attributed to its anti-inflammatory activity through inhibition of IL-18 expression and IL-18–dependent signaling.

FIGURE 3. Effect of CO, FO-18/12, FO-EPA, and FO-DHA diets on LPS-induced serum IL-18 levels, kidney caspase-1 activity, and pro/mature IL-18 expression in (NZB × NZW)F1 female mice. At 2 mo of age, mice were switched to semipurified diets containing 10% CO (control), and FOs enriched in EPA or DHA: 1) FO-18/12, 2) 55/5 FO-EPA, and 3) 5/60 FO-DHA. At 5 mo of age, mice (n = 5) were challenged with LPS i.p. to evaluate the IL-18 expression profile in serum and kidney. PBS served as a solvent control. A, LPS-injected FO-DHA fed mice (†*) showed significantly lower LPS-induced serum IL-18 levels compared with CO, FO-18/12, FO-EPA, and FO-DHA fed mice. B, LPS-induced caspase-1 activity in kidneys was significantly decreased in FO-DHA fed mice compared with CO, FO-18/12 and FO-EPA fed mice. C, Pro–IL-18 and mature IL-18 expression compared with CO, FO-18/12, and FO-EPA fed mice. D, Densitometric analysis of pro–IL-18 and mature IL-18 in kidneys compared with CO, FO-18/12, and FO-EPA fed mice. Results are representative of two independent experiments. Data are expressed as mean ± SEM. Results were analyzed by ANOVA, followed by Newman-Keuls test. †Ps-treated FO-DHA fed mice showed significantly (p < 0.05) decreased pro IL-18 levels, Caspase-1 activity and mature IL-18 expression compared with CO, FO-18/12, and FO-EPA fed mice. Different signs (†, †, and ††) indicate a significant difference (p < 0.05 ANOVA, followed by Newman-Keuls test) in LPS-injected groups. Similar sign assigned groups indicates no significant difference between the groups.

FIGURE 4. Effect of CO, FO-18/12, FO-EPA, and FO-DHA diets on PI3K/Akt kinase pathway in (NZB × NZW)F1 female mice. LPS challenged kidney PI3K and Akt activations were determined in mice fed with CO, FO-18/12, FO-EPA, and FO-DHA for 3 mo. PI3K activation was analyzed by PI3K lipid kinase assays using p85 immunoprecipitates. Akt activation was analyzed by immunoblotting using whole cell homogenates and activation-specific Abs, and by using a commercially available kinase assay that quantifies Akt-induced glycogen synthase kinase-3 phosphorylation at Ser21/9. A, Our results indicated that FO-DHA diet significantly suppressed PI3K activation in kidneys of LPS-injected mice. B, Western blot showing significant inhibition of phospho-Akt in kidneys of LPS-injected FO-DHA fed mice. C, Similarily, immune-complex kinase assays demonstrated significant inhibition of Akt kinase activity in kidneys of LPS-injected FO-DHA fed mice. Results are representative of three independent experiments.

FIGURE 5. Effect of CO, FO-18/12, FO-EPA, and FO-DHA diets on NF-κB activation. Female (NZB × NZW)F1 mice were fed with CO, FO-18/12, FO-EPA, and FO-DHA for 3 mo, and then challenged with LPS (5 mg/kg body weight, i.p.) for 4 h. Kidneys were harvested and analyzed for NF-κB DNA binding activity by EMSA using nuclear protein extracts (A). Activation of NF-κB was confirmed by analyzing nuclear p65 levels immunoblotting. Immunoblotting (B). Actin served as a loading control. The results show significant inhibition of LPS-mediated NF-κB DNA binding activity (A) and nuclear p65 translocation (B) in FO-DHA fed mice.
Disclosures
The authors have no financial conflicts of interest.

References