Docosahexaenoic Acid Attenuates Mycotoxin-Induced Immunoglobulin A Nephropathy, Interleukin-6 Transcription, and Mitogen-Activated Protein Kinase Phosphorylation in Mice

Qunshan Jia,*† Hui-Ren Zhou,*† Maurice Bennink,*, and James J. Pestka* ** 2

*Department of Food Science and Human Nutrition, †Center for Integrative Toxicology, and **Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48823

ABSTRACT The purpose of this investigation was to evaluate the dose-dependent effects of docosahexaenoic acid (DHA) on deoxynivalenol (DON)-induced IgA nephropathy in mice and their relation to proinflammatory gene expression and mitogen-activated protein kinase (MAPK) activation. Consumption of a modified AIN-93G diet containing 1, 5, and 30 g/kg DHA resulted in dose-dependent increases of DHA in liver phospholipids with concomitant decreases in arachidonic acid compared with control diets. DHA dose dependently inhibited increases in serum IgA and IgA immune complexes (IC) as well as IgA deposition in the kidney in DON-fed mice; the 30 g/kg DHA diet had the earliest detectable effects and maximal efficacy. Both splenic interleukin-6 (IL-6) mRNA and heterogeneous nuclear RNA (hnRNA), an indicator of IL-6 transcription, were significantly reduced in DON-fed mice that consumed 5 and 30 g/kg DHA; a similar reduction was observed for cyclooxygenase (COX-2) mRNA. In a subsequent study, acute DON exposure (25 mg/kg body weight) induced splenic IL-6 mRNA and hnRNA as well as COX-2 mRNA in mice fed the control diet, whereas induction of both RNA species was significantly inhibited in mice fed 30 g/kg DHA. These latter inhibitory effects corresponded to a reduction in DON-induced phosphorylation of p38, extracellular-signal related kinase 1/2, and c-Jun N-terminal kinase 1/2 MAPKs in the spleen. Taken together, the results indicate that DHA dose-dependently inhibited DON-induced IgA dysregulation and nephropathy, and that impairment of MAPK activation and expression of COX-2 and IL-6 are potential critical upstream mechanisms. J. Nutr. 134: 3343–3349, 2004.

KEY WORDS: IgA • interleukin 6 • deoxynivalenol • n-3 fatty acid • mitogen-activated protein kinase

Immunoglobulin A nephropathy, the most common form of human primary glomerulonephritis, has as its diagnostic hallmark marked kidney mesangial IgA deposition (1). Children and young adults are mainly affected by this disease (2) with 20–40% developing end-stage renal disease (3). High serum IgA and IgA immune complex (IgA-IC)3 concentrations are potential early contributory factors for IgA nephropathy (3,4), and these bind receptors on mesangial cells, thereby inducing proliferation and cytokine production (5). Deposition of polymeric IgA might also activate complement via the alternative pathway, causing glomerular damage (6).

Dietary supplementation with (n-3) PUFA, particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), has potential human health benefits (7) mainly with regard to inflammatory diseases (8,9). Dietary (n-3) PUFAs are negatively associated with the risk of IgA nephropathy (10), whereas high (n-6) PUFA intake increases the risk of the disease (11). IgA nephropathy patients are deficient in α-linolenic acid [18:3(n-3)], a precursor of DHA and EPA, but supplementation with (n-3) PUFAs suppresses arachidonic acid (AA) synthesis and decreases nephritogenesis in these patients (12). Consistent with these findings, there is substantial clinical evidence that (n-3) PUFA supplementation without adjusting (n-6) PUFA intake retards renal disease progression in IgA nephropathy patients (13–15).

Deoxynivalenol (DON) is a trichothecene mycotoxin produced by Fusarium graminearum, often encountered in cereal grains (16). DON binds to eukaryotic ribosomes; it inhibits protein translation and induces multiple stress signaling pathways that involve the mitogen-activated protein kinases (MAPKs). Immunoxicologic studies revealed that mice chronically exposed to 2–25 mg/kg DON in the diet have elevated serum IgA and IgA-IC concurrently with mesangial IgA deposition in kidneys, thus mimicking the early stages of human IgA nephropathy and suggesting that the mucosal immune system appears to be a primary target (17–21). DON upregulates proinflammatory gene expression both in vivo and in vitro (22–26), and a role for elevated interleukin (IL)-6 in DON-induced IgA nephropathy has been established (27–29).
DON also induces cyclooxygenase-2 (COX-2) and this enzyme might also contribute IgA nephropathy by promoting expression of IL-6 via prostaglandin E2 (PGE2) production (30–33).

Menhaden fish oil consumption attenuates DON-induced IgA nephropathy in mice (34) as well as induction of IL-6 after acute DON exposure (35). In the latter study, fish oil ingestion also suppressed DON-induced phosphorylation of extracellular-signal regulated protein kinases (ERK) 1/2 and c-Jun N-terminal kinases (JNK) 1/2, which are critical upstream MAPK regulators of IL-6 expression. We observed recently that at 10 g/kg, consumption of DHA and, to a lesser extent, EPA significantly impaired DON-induced serum IgA elevation and IgA deposition, whereas high α-linolenic acid–containing flax seed oil did not (36). The purpose of this study was to test the hypotheses that DHA dose-dependently attenuates DON-induced IgA nephropathy in mice and that its effects correspond to attenuation of IL-6 and COX-2 gene expression as well as MAPK activation.

MATERIALS AND METHODS

Materials. All chemicals (reagent grade or better) were purchased from Sigma Chemical unless otherwise noted. DON was produced in F. graminearum R6576 cultures and purified by silica gel chromatography (37). Purified DON was added to powdered diets as detailed by Pestka et al. (17).

Animals. Female B6C3F1 mice (7 wk old), weighing between 20 and 25 g, were obtained from Charles River. Mice were housed in environmentally protected transparent polypropylene cages with stainless steel wire tops for 1 wk before the introduction of the different treatments. Mice had free access to water and food. Cages were filter-bonneted and kept in a laminar flow cage rack under 23–25°C temperature, 45–55% relative humidity, and a 12-h artificial photoperiod. Housing, handling, and sample collection procedures were in accordance with NIH guidelines.

Diets and experimental design. Experimental diets employed the basal AIN-93G formulation (Dyets) of Reeves et al. (38) with a modified lipid content. In Study 1, corn oil (Dyets), oleic acid (Dyets), and MEG3™ DHA-enriched oil (containing DHA 483 g/kg and 113 g/kg EPA; Ocean Nutrition) were used to amend the basal diet to yield 5 experimental diet groups (n = 9) (Table 1). Diets were prepared every 2 wk, stored at −20°C, and provided fresh to mice each day. Mice were fed the diets for 16 wk. Body weight and food intake were monitored weekly. Blood was collected every 4 wk from the saphenous leg vein (39) into heparinized vacutainers and plasma analyzed for IgA and IgA-IC. At wk 16, mice were anesthetized with methoxyfluorane and killed by cervical dislocation. Peyer’s patches were removed aseptically and used to prepare cell cultures. Spleens were removed for total RNA extraction and IL-6 mRNA, heterogenous nuclear RNA (hnRNA) and COX-2 mRNA determination. Kidneys of each mouse were removed for immunofluorescence examination. The liver was used as a surrogate for assessing (n-3) PUFA incorporation into cellular phospholipids.

In Study 2, Mice were fed DHA-enriched oil for 4 wk and then orally gavaged with a single DON dose (25 mg/kg body weight) at the end of experiment. After 3 h, mice were killed and then splenic IL-6 mRNA, hnRNA, and COX-2 mRNA expression quantified.

In Study 3, Mice were fed DHA-enriched oil for 4 wk and then gavaged with DON (25 mg/kg body weight). Mice were killed 30 min later and spleens removed for MAPK phosphorylation assessment.

Tissue analyses. Serum IgA and IgA-IC were measured by ELISA as described previously (34,40). IgA deposition was quantified in cryostat sections of kidney using immunofluorescence (34) and image analysis (41). Fatty acids in liver phospholipids were measured by GC (36,42).

Cell cultures. Briefly, tissues were passed through a sterile 100-mesh stainless screen in harvest buffer (34). Cells (1 × 10⁶/L) were plated at 100 mL/L fetal bovine serum (Gibco) 1 mmol/L sodium pyruvate, 1 × 10⁻⁴ U/L penicillin, 100 mg/L streptomycin, 0.1 mmol/L nonessential amino acid, and 50 μmol/L 2-mercaptoethanol in flat-bottomed 24-well tissue culture plates (Fisher Scientific) at 37°C under 7% CO₂ in a humidified incubator. Supernatants were collected after 5 d and stored in aliquots at −20°C for IgA analysis.

Real-time PCR. Total RNA was extracted from mouse spleens using Trizol reagent (Life Technologies) and RNease Min Elute Cleanup Kit (Qiagen). IL-6 mRNA, IL-6 hnRNA, and COX-2 mRNA expressions were measured by real-time PCR. Probe and primers for mouse IL-6 mRNA and endogenous control (18S RNA) were purchased as TaqMan assay reagents (PE Applied Biosystems). Taqman Universal PCR Master Mix (PE Applied Biosystems) was used to quantify IL-6 and 18S RNA following manufacturer’s instructions on an ABI Prism 7700 (PE Applied Biosystems). Real-time PCR Primer Express software (PE Applied Biosystems) was employed to design primer pairs for mouse IL-6 hnRNA (forward primer: gttcacaactgttctgctactcctggtcttcaatgtt). SYBER Green PCR Master Mix (PE Applied Biosystems) was used to detect IL-6 and 18S RNA following manufacturer’s instructions on an ABI Prism 7700 (PE Applied Biosystems). Real-time PCR Primer Express software (PE Applied Biosystems) was employed to design primer pairs for mouse IL-6 hnRNA (forward primer: gttcacaactgttctgctactcctggtcttcaatgtt). SYBER Green PCR Master Mix (PE Applied Biosystems) was used to detect IL-6 hnRNA and COX-2 mRNA. 18S RNA was used to normalize target gene expression. Target gene expression levels were calculated relative to the control group.

Western analysis. After determination of protein concentration using the Bio-Rad DC protein assay kit, 40 μg spleen protein per lane was loaded and fractionated by SDS-PAGE using a 10% (wt/v) acrylamide separation gel, then transferred to polyvinylidene fluoride membrane (Du Pont) and analyzed by Western blotting using antibodies specific for p42/44 (ERK 1/2), phospho-p42/44, p46/54 (JNK 1/2), phospho p42/44, p46/54, p38, and phospho-p38 (Cell Signaling) in conjunction with an Enhanced Chemiluminescence Kit (from Amersham Biosciences) as described by Zhou et al. (25). Relative phosphorylation was measured with Kodak ID Image Analysis Software and normalized against expression of nonphosphorylated forms of these MAPK families.

### TABLE 1

**Experimental groups for assessing the effects of feeding mice diets containing DHA-enriched oil on DON-induced IgAN**

<table>
<thead>
<tr>
<th>Experimental diet</th>
<th>DON</th>
<th>Linoleic acid</th>
<th>Oleic acid</th>
<th>EPA</th>
<th>DHA</th>
<th>Total (n-6)</th>
<th>Total (n-3)</th>
<th>(n-6)/(n-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>8.2</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>8.3</td>
<td>0.18</td>
<td>46/1</td>
</tr>
<tr>
<td>Control + DON</td>
<td>0.02</td>
<td>8.2</td>
<td>48</td>
<td>0</td>
<td>0</td>
<td>8.3</td>
<td>0.18</td>
<td>46/1</td>
</tr>
<tr>
<td>1 g/kg DHA + DON</td>
<td>0.02</td>
<td>8.2</td>
<td>48</td>
<td>0.2</td>
<td>12</td>
<td>8.3</td>
<td>1.2</td>
<td>6.9/1</td>
</tr>
<tr>
<td>5 g/kg DHA + DON</td>
<td>0.02</td>
<td>7.8</td>
<td>40</td>
<td>1.1</td>
<td>4.8</td>
<td>7.9</td>
<td>6.0</td>
<td>1.3/1</td>
</tr>
<tr>
<td>30 g/kg DHA + DON</td>
<td>0.02</td>
<td>6</td>
<td>0</td>
<td>6.8</td>
<td>29.0</td>
<td>7.9</td>
<td>35.8</td>
<td>1/4.5</td>
</tr>
</tbody>
</table>

1 All diets had a final oil content of 70 g/kg diet.
TABLE 2
Fatty acid composition of liver phospholipid fraction from mice fed AIN-93G diets containing DON (20 mg/kg) for 16 wk (Study 1).

<table>
<thead>
<tr>
<th>Experimental diet</th>
<th>16:0</th>
<th>18:0</th>
<th>18:1</th>
<th>18:2(n-6)</th>
<th>20:4(n-6)</th>
<th>20:5(n-3)</th>
<th>22:6(n-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>26.8 ± 1.2a</td>
<td>14.9 ± 0.4a</td>
<td>22.1 ± 1.0a</td>
<td>5.9 ± 0.4a</td>
<td>9.9 ± 1.7d</td>
<td>0.1 ± 0.1a</td>
<td>1.9 ± 1.0a</td>
</tr>
<tr>
<td>Control + DON</td>
<td>25.5 ± 0.8a</td>
<td>14.7 ± 0.4a</td>
<td>21.3 ± 0.8a</td>
<td>6.4 ± 0.4a</td>
<td>9.6 ± 1.6d</td>
<td>0.2 ± 0.1a</td>
<td>1.7 ± 0.8a</td>
</tr>
<tr>
<td>1 g/kg DHA + DON</td>
<td>32.4 ± 0.7b</td>
<td>16.7 ± 0.7b</td>
<td>17.0 ± 0.4b</td>
<td>6.5 ± 0.5a</td>
<td>7.1 ± 1.1c</td>
<td>0.2 ± 0.1a</td>
<td>5.0 ± 0.6b</td>
</tr>
<tr>
<td>5 g/kg DHA + DON</td>
<td>32.0 ± 1.3b</td>
<td>15.9 ± 0.4a</td>
<td>17.4 ± 0.5b</td>
<td>5.7 ± 0.3a</td>
<td>5.0 ± 0.5b</td>
<td>1.6 ± 0.3b</td>
<td>6.5 ± 0.8c</td>
</tr>
<tr>
<td>30 g/kg DHA + DON</td>
<td>36.8 ± 0.6c</td>
<td>19.1 ± 0.6c</td>
<td>9.6 ± 0.4c</td>
<td>4.6 ± 0.2b</td>
<td>2.4 ± 0.4a</td>
<td>4.1 ± 0.5c</td>
<td>9.7 ± 0.7d</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 6. Means in a column without a common letter differ, P < 0.05.
2 Only the major fatty acids are shown.

**Statistical Analysis.** Data were analyzed using Sigma Stat for Windows (Jandel Scientific). Data were subjected to one-way ANOVA and pairwise comparisons made by Bonferroni or Student-Newman-Keuls methods. If data were not normally distributed, they were subjected to Kruskal-Wallis ANOVA on Ranks and pairwise comparisons made by Dunn’s or Student-Newman-Keuls methods. Differences were considered significant at P < 0.05.

**RESULTS**

**Study 1.** Consumption of DON at 20 mg/kg reduced daily food intake (P < 0.05) and impaired body weight gain (P < 0.05) compared with the control group over the 16-wk period. Consumption of DHA did not modulate these effects. GC analysis of liver phospholipids after 16 wk revealed that DON alone did not affect the concentrations of AA, DHA, and EPA. In livers of mice fed diets containing DHA-enriched oil, both DHA and EPA concentrations were increased, whereas AA was reduced (Table 2).

Serum IgA concentration in control + DON mice rose over control mice beginning at wk 8 and was increased 9-fold over the control group at wk 16 (P < 0.05) (Fig. 1A). DON at 30 g/kg significantly attenuated DON-induced serum IgA elevation as early as wk 8, whereas suppression by diets containing 1 and 5 g/kg DHA was first detectable at wk 16 (P < 0.05). DHA at 30 g/kg also significantly suppressed serum IgA-IC elevation after 16 wk, but 1 and 5 g/kg DHA had no effect (Fig. 1B).

Supernatant IgA concentrations in cultures from DON-fed mice tended to be higher than in controls (P = 0.44). Feeding mice 30 g/kg DHA reduced ex vivo IgA secretion compared with the control + DON group (P < 0.05). Mice fed 5 g/kg DHA-enriched oil, tended to secrete less IgA than the control + DON group (P = 0.06).

When kidney sections were analyzed by immunofluorescence (Fig. 2A–E), image analysis revealed that DON induced IgA deposition compared with control (P < 0.05) (Fig. 2F). As with IgA-ICs, DHA at 30 g/kg blocked IgA deposition (P < 0.05); 5 g/kg tended to have this effect (P = 0.13), whereas 1 g/kg DHA had no effect compared with the control + DON group.

Relative IL-6 mRNA expression in spleens of the DON-fed control group did not differ from that in the control group (Fig. 3A). However, IL-6 mRNA was reduced in mice fed DON with 5 or 30 g/kg DHA compared with DON-fed control mice (P < 0.05). Analogous effects were found for IL-6 hnRNA, an indicator of gene transcription activity (Fig. 3B). There were also trends toward increased splenic COX-2 mRNA expression in DON-fed control mice (P = 0.12) compared with control mice as well as significant suppression by 30 g/kg DHA (Fig. 3C). COX-2 mRNA expression in the 30 g/kg DHA group was also lower than in the 1 and 5 g/kg groups (P < 0.05).

**Study 2.** Acute DON (25 mg/kg body weight) exposure induced IL-6 mRNA and hnRNA in spleen and Peyer’s patches after 3 h (P < 0.05) (Fig. 4A,B). Consumption of 30 g/kg DHA suppressed induction of IL-6 mRNA and hnRNA in spleen and IL-6 mRNA in Peyer’s patches by 54, 71, and 89%, respectively (P < 0.05). DHA (30 g/kg) consumption also inhibited DON-induced COX-2 mRNA expression by 64% (P < 0.05) (Fig. 4C).

![FIGURE 1](https://academic.oup.com/jn/article-abstract/134/12/3343/4688547) Effects of dietary DHA on serum IgA(A) and IgA-IC (B) in mice fed AIN-93G diets containing DON (20 mg/kg) for 16 wk (Study 1). Values for IgA-IC (B) represent data from wk 16. Values are means ± SEM, n = 9. Means at a time without a common letter differ, P < 0.05.
Study 3. Western blot analysis indicated that 30 min after acute DON treatment, phosphorylation of p38, ERK 1/2, and JNK 1/2 was induced in mouse spleen (P < 0.05) (Table 3). Consumption of 30 g/kg DHA suppressed DON-induced activation of all 3 MAPK families (P < 0.05).

DISCUSSION

Potential beneficial effects of fish oil or (n-3) PUFA supplementation for human IgA nephropathy patients were demonstrated by clinical studies (13,15). As shown here and previously (34,36), (n-3) PUFAs impair production and accumulation of potentially nephritogenic IgA in a mycotoxin-induced IgA nephropathy model. DHA at 30 g/kg completely abrogated increases in serum IgA as early as 8 wk, whereas at 1 and 5 g/kg DHA, efficacy was detectable only at wk 16. The

FIGURE 2  Effect of dietary DHA on mesangial IgA deposition in mice fed modified AIN-93G diets containing DON (20 mg/kg) for 16 wk (Study 1). Mesangial IgA in control (A), DON (B), 1 g/kg DHA + DON (C), 5 g/kg DHA + DON (D), and 30 g/kg DHA + DON (E) was detected by immunofluorescence and analyzed by image analysis (F). Values are mean ± SEM, n = 9. Bars without a common letter differ, P < 0.05.

FIGURE 3  Effects of dietary DHA on spleen IL-6 mRNA (A), hnRNA (B) and COX-2 (C) expression in mice fed the AIN 93G diet containing DON (20 mg/kg) for 16 wk (Study 1). Data were normalized against 18S RNA and expressed relative to control value. Values are mean ± SEM, n = 6–9. Bars without a common letter differ, P < 0.05.

FIGURE 4  Effects of dietary DHA on IL-6 and COX-2 expression in mice induced by acute oral exposure to DON. Mice were fed diet containing DHA at 60 g/kg for 4 wk (Study 2). At experiment cessation, mice were gavaged with DON (25 mg/kg body weight). After 3 h, total RNA was extracted and analyzed by real-time PCR. Analysis included splenic IL-6 mRNA (A) and hnRNA (B), Peyer's patch IL-6 mRNA (C), and splenic COX-2 mRNA (D). Data were normalized against 18S RNA and expressed relative to the control group. Values are mean ± SEM, n = 3. Bars without a common letter differ, P < 0.05.
TABLE 3

Inhibition of DON-induced MAPK phosphorylation in spleens of mice fed AIN-93G diet containing 30 g/kg DHA, (Study 3)

<table>
<thead>
<tr>
<th>MAPK</th>
<th>Control</th>
<th>DON</th>
<th>30 g/kg DHA + DON</th>
</tr>
</thead>
<tbody>
<tr>
<td>phospho-ERK1</td>
<td>1.38 ± 0.08a</td>
<td>2.14 ± 0.09c</td>
<td>1.57 ± 0.16b</td>
</tr>
<tr>
<td>phospho-JNK1</td>
<td>1.06 ± 0.05a</td>
<td>2.02 ± 0.03c</td>
<td>1.58 ± 0.15b</td>
</tr>
<tr>
<td>phospho-JNK2</td>
<td>0.80 ± 0.08a</td>
<td>0.98 ± 0.03c</td>
<td>0.87 ± 0.03b</td>
</tr>
<tr>
<td>phospho-p38</td>
<td>0.25 ± 0.12a</td>
<td>1.47 ± 0.09c</td>
<td>1.07 ± 0.05b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 5. Means in a row without a common letter differ, P < 0.05.
2 Relative value refers to ratio of band density for the phosphorylation form of MAPK divided by the band density to the nonphosphorylated MAPK as determined by Western analysis.

highest DHA concentration used here was also the most effective at inhibiting serum IgA-IC elevation and mesangial IgA deposition. Consistent with these findings, we showed previously that DHA at 10 g/kg partially inhibits DON-induced IgA nephropathy beginning after 12 wk (36). Taken together, these preclinical findings are valuable because they suggest that consumption of (n-3) PUFA has potential benefits for early intervention and prophylaxis in persons with a familial history of IgA nephropathy or in patients diagnosed to be at an early stage of the disease.

DHA treatment significantly increased DHA and EPA content in liver phospholipids, while decreasing AA (Table 2). A significant correlation existed between the DHA in liver phospholipids, while decreasing AA (Table 2). A significant correlation existed between the DON-induced MAPK phosphorylation trend was also found between liver DHA and IgA-IC (r = -0.99, P = 0.0033). A correlation trend was also found between liver DHA and IgAIC (r = -0.85, P = 0.148) and IgA deposition (r = 0.90, P = 0.09). This suggested that DHA dose affected tissue levels of this fatty acid, which ultimately affected the 3 IgA parameters. Correlations were not found between the IL-6 mRNA level and the 3 IgA nephropathy parameters possibly because IgA, IgA-IC, and mesangial IgA represent cumulative outcomes of the entire 16 wk feeding period, whereas changes in IL-6 mRNA and hnRNA are transient responses.

Because hnRNA is a precursor species observed in cells before RNA splicing to mRNA, its abundance can be used as a surrogate for the run-on assay in the detection of gene transcriptional activity (44). The finding that DHA consumption significantly blocked the accumulation of IL-6 hnRNA as well as IL-6 mRNA suggests that DHA blocks IL-6 gene expression at the transcriptional level. These results might be clinically relevant because IL-6 may play a contributory role in human IgA nephropathy (1). In addition, IL-6 production by peripheral blood mononuclear cells is decreased in persons consuming (n-3) PUFA, which corresponds to increased plasma and cell membrane (n-3) PUFA incorporation (45).

IL-6 is a critical cytokine in differentiation of B cells to high rate IgA secretion (43). Differences between IL-6 mRNA expression after chronic and acute DON exposure (i.e., no induction vs. induction) might arise from 2 factors. DON-fed mice (20 mg/kg in diet) ingest ~3 mg/(kg body weight · d) of this toxin, which is much lower than the acute bolus dose (25 mg/kg body weight). Also, because IL-6 is an early-response gene with rapid turnover (46), its induction at the local sites by ingested DON in nocturnally feeding mice might have been missed when experiments were conducted in the morning. Nevertheless, sufficient IL-6 induction likely occurred during chronic exposure to promote B cell differentiation to IgA secretion in response to commensal and self-antigens (21,47) with attendant cumulative effects.

The effects of DHA might be coordinated in part by attenuated expression of COX-2, an essential enzyme that mediates functions of (n-3) and (n-6) fatty acids (48) and upregulates IL-6 expression via increased PGE2 production. In contrast, AA, a risk factor in IgA nephropathy (12,49) was significantly depressed in liver phospholipid after DHA consumption. Reduced AA would decrease PGE2 generation (50,51) thus further attenuating IL-6 expression (33).

DHA impairment of DON-induced IL-6 and COX-2 expression may relate to (n-3) PUFA inhibition of MAPKs, which couple cell-surface receptors to critical regulatory targets and transcription of many genes including COX-2 and IL-6 (52,53). DON activation of ERK, JNK, and p38 both in macrophages and mice contributes to transcriptional and post-transcriptional upregulation of proinflammatory genes (25,32,54). DHA, but not EPA, decreased ERK activation in mesangial cells, whereas JNK activity was increased and p38 activity was not significantly affected (55). Recently, Moon and Pestka (35) found that consumption of 60 g/kg menhaden fish oil by mice suppressed DON-induced ERK 1/2 and JNK 1/2 phosphorylation but not p38 in spleen. In contrast, the results presented here demonstrated that earlier consumption of 30 g/kg DHA for 4 wk impaired phosphorylation of all 3 MAPK families. The differences between our 2 investigations may relate to the (n-3) and (n-6) PUFA concentrations as well as (n-6)/(n-3) PUFA ratios that were employed. These newly observed effects on p38 activation are particularly intriguing because this MAPK is essential for both increased transcription and mRNA stabilization of inflammatory cytokine genes (56).

DHA inhibition of DON activation of MAPKs was quite modest relative to the rather extensive IL-6 inhibition. MAPK signaling modules interact via a series of sequential binary interactions to create a protein kinase cascade (57). DHA might affect one or more specific modules that control induction of IL-6 by DON, but this cannot be resolved by Western analysis of the entire spleen cell population. We further speculate that the effects of DHA on MAPKs and IL-6 might be limited to macrophages and dendritic cells, but this also cannot be discriminated by Western analysis from such effects in B, T, epithelial, and endothelial cells.

Alternatively, it is possible that DHA alters leukocyte surface receptor protein function and signal transduction by altering the composition of phospholipid rafts, which are important signaling platforms for T-cell and macrophage activation. Sphingomyelin, which facilitates raft formation, is a signaling platforms for T-cell and macrophage activation. Sphingomyelin, which facilitates raft formation, is a
concentrations are consistent with human diet recommendations in terms of energy percentage in the diet (62) and other clinical studies employing (n-3) PUFAs (63–66).

Taken together, the results presented here suggest that consumption of diets containing DHA significantly inhibited DON-induced IgA nephropathy in dose-dependent fashion. These effects were correlated with impairment of IL-6 and COX-2 expression as well as MAPK activation. Further elucidation of the upstream effects of (n-3) PUFA on signal transduction in experimental IgA nephropathy and other inflammation models is warranted.

ACKNOWLEDGMENTS

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LITERATURE CITED

37. Reeves, P. G., Nielsen, F. H. & Fahey, G. C., Jr. (1993) AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition a...