n-3 Long-chain PUFA reduce allergy-related mediator release by human mast cells in vitro via inhibition of reactive oxygen species

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Abstract

Increased n-6 and reduced n-3 long-chain PUFA (LC-PUFA) intake in Western diets may contribute to the increased prevalence of allergic diseases. Key effector cells in allergy are mast cells (MC). The aim of the present study was to investigate the effects of n-6 and n-3 LC-PUFA on MC phenotype. Human MC lines (LAD2 and HMC-1) were incubated for 24 h with either arachidonic acid (AA, n-6 LC-PUFA) or the n-3 LC-PUFA EPA or DHA. The effects of these three LC-PUFA on degranulation, mediator secretion and reactive oxygen species (ROS) generation were assessed. ROS, mitogen-activated protein kinase (MAPK) or NF-kB inhibitors were used to unravel signalling pathways involved in cytokine secretion. AA, EPA or DHA did not reduce IgE-mediated degranulation by LAD2 cells. However, AA increased PGD2 and TNF-α secretion by ionomycin/phorbol 12-myristate 13-acetate-stimulated HMC-1, whereas EPA and DHA more prominently inhibited IL-4 and IL-13 secretion. Suppression of IL-4 and IL-13 release by LC-PUFA correlated with reduced ROS generation. IL-4 and IL-13 release by activated HMC-1 was abrogated using ROS inhibitors. Inhibition of MAPK signalling, but not NF-kB, downstream of ROS reduced IL-13 secretion by activated HMC-1. Combined incubation of EPA or DHA with MAPK inhibitors further suppressed IL-13 secretion. In conclusion, the n-6 LC-PUFA AA enhanced pro-inflammatory mediator production by MC, while the n-3 LC-PUFA EPA as well as DHA more effectively suppressed ROS generation and IL-4 and IL-13 release. This suggests that dietary supplementation with EPA and/or DHA may alter the MC phenotype, contributing to a reduced susceptibility to develop and sustain allergic disease.

Key words: Long-chain PUFA: Mast cell degranulation: Mediator secretion: Reactive oxygen species: Allergy

During the last decades, the prevalence of allergic diseases has dramatically increased. Mast cells (MC) are key effector cells in allergy and play a pivotal role in initiating and maintaining allergic reactions and inflammation by the release of numerous inflammatory mediators. Upon activation, MC immediately release a plethora of preformed mediators such as histamine and proteases that are stored in secretory cytoplasmic granules. Arachidonic acid (AA)-derived eicosanoids and multiple pro-inflammatory chemokines and cytokines, such as allergy-driving IL-4 and IL-13, are generated de novo. These mediators are important in the pathogenesis of allergic responses and can increase the susceptibility to develop allergic disease and enhance allergic symptoms.

PUFA can modulate immune responses. In general, n-6 long-chain (LC) PUFA such as AA (20:4n-6) are considered to be pro-inflammatory and the n-3 LC-PUFA EPA (20:5n-3) and DHA (22:6n-3) protect against inflammation. Decreased dietary intake of n-3 LC-PUFA from fatty fish together with the high intake of n-6 PUFA from mainly vegetable oils in the Western diet is likely to contribute to the increased incidence of allergic and inflammatory disease in humans over the last decades. Dietary supplementation with n-3 LC-PUFA has been studied to some extent in allergic disease, focusing on prevention. Human pregnancy studies revealed a reduction in infant atopy when women were supplemented with fish oil during pregnancy and lactation.
n-3 LC-PUFA compete with n-6 LC-PUFA for incorporation into the cell membrane. After release from the membrane, AA can be metabolised by cyclo-oxygenases (COX) and lipoxygenases, resulting in the formation of 2,4-series eicosanoids which support inflammatory responses. By contrast, exchange of AA by n-3 LC-PUFA results in the production of 3,5-series eicosanoids which are less potent\(^{11,13,15}\). Besides modification of the generation of eicosanoids, LC-PUFA may change intracellular signalling pathways and may affect the activation of nuclear transcription factors and consequently gene transcription\(^{11,24}\). Dietary EPA and DHA have been shown to inhibit the release of pro-inflammatory cytokines (IL-1, IL-6, IL-8 and TNF-\(\alpha\)) by macrophages and mononuclear cells\(^{11,25,26}\), however, little is known about the effects of LC-PUFA in MC.

In MC, cytokine secretion is under the regulation of intracellular reactive oxygen species (ROS), mitogen-activated protein kinases (MAPK) and NF-\(\kappa\)B signalling pathways\(^{27,28}\). The pathophysiological importance of ROS such as superoxide generated from MC has not been fully elucidated. At high levels, ROS are involved in the innate immune response whereas at low levels they are involved in cell signalling\(^{28}\).

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Mast cell mediator release and cell viability

After pre-incubation with AA, EPA or DHA, in some experiments, inhibitors were used before MC stimulation. HMC-1 were incubated for 30 min with 1–10 μM of COX inhibitors (indomethacin or NS398) or for 10 min with a ROS inhibitor (superoxide dismutase; 20 or 100 U/ml (equivalent to 7 or 35 μg/ml) or 1,3-dimethyl-2-thiourea; 30, 40, 50 and 60 μM), a MAPK inhibitor (ERK inhibitor PD98059; 50 and 100 μM, p38 inhibitor SB203580; 20 and 50 μM, JNK inhibitor SP600125; 10 and 20 μM) or an NF-κB inhibitor (Bay117082; 10 and 30 μM). The inhibitors were diluted in medium (final concentration of dimethyl sulphoxide ≤0.2% (v/v), except for PD98059 with 0.5% dimethyl sulphoxide (v/v)). After pre-incubation with LC-PUFA and/or inhibitors, the cells were stimulated with 1 μM-ionomycin plus 16α-styrophorol 12-myristate 13-acetate (PMA) (both diluted in medium, final concentration of dimethyl sulphoxide 0.1% (v/v)) and incubated at 37°C. Optimal doses of ionomycin and PMA were chosen after pilot experiments. To assess inflammatory mediator production in the culture supernatants of HMC-1, supernatants were collected 30 min (PGD2), 4 h (TNF-α and IL-8) and 24 h (IL-4 and IL-13) after stimulation. Secreted cytokine concentrations were determined by ELISA cytoSet kits according to the manufacturer’s instruction (BioSource International, Inc.). PGD2 was measured by the Enzyme Immunoassay Prostaglandin D2-MOX kit (Cayman Chemical).

Effect of arachidonic acid, EPA or DHA on IgE-stimulated degranulation of LAD2 cells

β-Hexosaminidase release was used as a marker for MC degranulation. LAD2 cells were stimulated by sensitisation with IgE followed by cross-linking with α-IgE. AA at 100 μM increased FcεRI-mediated β-hexosaminidase release of LAD2 cells while EPA and DHA did not affect MC degranulation (AA 100 μM: 36.4 (SEM 6.7) v. 49.9 (SEM 5.6)%, n = 3, P < 0.05; Fig. 1(a)). Degranulation after incubation with AA did not differ significantly from EPA or DHA. The solvent of LC-PUFA (ethanol, 0.1% (v/v)) did not affect β-hexosaminidase release (data not shown).

Modulation of mediator release of HMC-1 by arachidonic acid, EPA or DHA

To determine the effects of modification of cell membrane fatty acid composition on cytokine production, HMC-1 were used. To study dose-dependency of MC mediator release, HMC-1 were incubated with 0, 1, 10, 25 and 100 μM-AA, EPA or DHA because this was expected to be more sensitive to changes in cell membrane fatty acid composition than degranulation. As shown in Fig. 1(b), the production of PGD2 increased dramatically in a dose-dependent manner after incubation with AA (13.4 (SEM 6.3) v. 71.6 (SEM 24.1) ng/ml, 25 μM-AA, n = 3, P < 0.01); in contrast, EPA and DHA did not change significantly (AA 25 μM: 14.4 (SEM 7.8) v. 21.2 (SEM 5.1) ng/ml, n = 3, P = 0.29).
DHA inhibited PGD₂ production (13·4 (SEM 6·3) v. 3·5 (SEM 1·9) ng/ml, 25 μM-DHA, n 3, P<0·05). AA (25 μM) was also found to enhance TNF-α secretion by HMC-1 (4·4 (SEM 1·0) v. 10·1 (SEM 3·5) ng/ml, n 5, P<0·05); no effects were observed for EPA and DHA (Fig. 1(c)). IL-8 secretion was not affected by AA, EPA or DHA (data not shown). However, 24 h pre-incubation with AA, EPA or DHA resulted in a decrease in ionomycin/PMA-induced IL-4 and IL-13 release (Fig. 1(d) and (e)). The effects of DHA on IL-13 production were most pronounced since DHA already reduced IL-13 secretion at 25 μM (301·1 (SEM 94·5) v. 104·6 (SEM 25·8) pg/ml, n 6, P<0·01). In addition, this was significantly lower than AA-treated cells (P<0·05). AA as well as EPA and DHA reduced IL-13 secretion at a concentration of 100 μM (AA 86·8 (SEM 19·7) pg/ml; EPA 38·5 (SEM 13·7) pg/ml; DHA 44·0 (SEM 14·6) pg/ml, n 6, P<0·01); however, the EPA and DHA treatments resulted in significantly lower IL-13 secretion than AA (P<0·01). At the highest concentration used, EPA and DHA were able to reduce IL-4 secretion (119·8 (SEM 50·6) v. 35·7 (SEM 10·2), 32·8 (SEM 8·1) pg/ml respectively, n 6, P<0·05). The solvent of LC-PUFA (ethanol, 0·1 % (v/v)) and ionomycin/PMA (0·1 % (v/v) dimethyl sulphoxide) did not affect mediator release (data not shown).

Pre-incubation for 30 min with indomethacin (general COX inhibitor; 10 μM) as well as NS398 (COX-2 inhibitor; 1 and 10 μM) effectively inhibited COX as PGD₂ release was blocked by more than 90% (data not shown). However, COX inhibitors did not affect TNF-α or IL-13 secretion; hence, these cytokines are not regulated by a COX-dependent mechanism (data not shown).

**Arachidonic acid, EPA and DHA do not affect cell viability**

AA, EPA and DHA (0, 1, 10, 25 and 100 μM) incubation up to 48 h did not affect cell viability of stimulated HMC-1 as determined by WST-1 assay. Ionomycin (1 μM) and PMA (16 nM) stimulation for 24 h slightly tended to reduce cell viability when compared with non-stimulated HMC-1 (data not shown). EPA tended to induce a slight increase in mitochondrial activity and the WST-1 signal was significantly higher with 100 μM-AA (n 6, P<0·05). Cell viability was also studied by the trypan blue exclusion test. The addition of AA, EPA or DHA did not induce cell death at the concentrations 25 and 100 μM (data not shown).

**Generation of intracellular reactive oxygen species and modulation by arachidonic acid, EPA or DHA**

ROS are known as second messengers and are related to different inflammatory diseases(27). HMC-1 showed a slight increase in ROS generation upon ionomycin/PMA stimulation (Fig. 2(a)). HMC-1 cells have high basal ROS levels (mean fluorescence intensity (MFI)) and due to limitations in the sensitivity of the method used, additional ROS production upon stimulation was not higher than ROS production in unstimulated cells (MFI 394·8 (SEM 95·3) v. 424·9 (SEM 53·7), NS) (Fig. 2(b)). To study the effect of AA, EPA or DHA on ROS production in ionomycin/PMA-stimulated HMC-1, cells were incubated with 25 and 100 μM based on the differences between 25 and 100 μM in IL-4 and IL-13 secretion. Intracellular ROS generation is dose-dependently reduced by AA, EPA or DHA in stimulated HMC-1. This effect was most pronounced for DHA, which showed a significant reduction in ROS generation at 25 μM (MFI 325·1 (SEM 47·9), P<0·05) when compared with basal ROS generation after stimulation. This also was significantly lower than the AA-treated cells (P<0·05). At a concentration of 100 μM-AA, EPA and DHA were all able to reduce ROS generation (AA: MFI 278·2 (SEM 22·6), P<0·01; EPA: MFI 267·6 (SEM 47·1), P<0·001; DHA: MFI 215·6 (SEM 32·1), P<0·001). ROS generation after stimulation (mean of n 3 for each condition, used from Fig. 2(b)) was found to correlate positively with IL-4 and IL-13 secretion (mean of n 5/6 for 3 for WST-1, 6 for ROS).

### Table 1. Membrane fatty acid composition of HMC-1 and LAD2 cells after 24 h long-chain PUFA incubation

(Mean values with their standard errors; n 4 independent experiments)

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AA, arachidonic acid. Mean values were significantly different from the control group (0 μM) after incubation with AA, EPA or DHA: *P<0·05, **P<0·01, ***P<0·001 (one-way ANOVA with post hoc Dunn’s multiple comparison test).
each condition, used from Fig. 1(d) or (e), respectively) in HMC-1 \( (P=0.006, r=0.897) \) and \( (P=0.005, r=0.907) \), respectively (Fig. 2(c) and (d)).

**Effect of reactive oxygen species inhibition on IL-4 and IL-13 secretion**

Since AA, EPA and DHA were able to suppress ROS and IL-4 and IL-13 secretion, it was assessed whether these allergy-related cytokines were under the regulation of ROS in HMC-1. Pre-incubation with 1,3-dimethyl-2-thiourea at the highest concentrations (40–60 mM) resulted in a decrease in IL-4 secretion of ionomycin plus PMA-stimulated HMC-1 (35±1 (SEM 68±8) pg/ml, 100 U/ml (35 ± g/ml), \( P<0.01 \)) but not of IL-13 (Fig. 3(c) and (d)). Incubation with ROS inhibitors did not reduce mitochondrial activity or increase cell death as shown by the WST-1 assay and trypan blue exclusion, respectively (data not shown).

**Effects of mitogen-activated protein kinases and NF-κB inhibitors on IL-13 release**

ROS have been described to operate upstream in the signalling cascade. MAPK and NF-κB are known to contribute to cytokine secretion by MC. To determine the involvement of MAPK and NF-κB signalling in the secretion of IL-13 and the possible effects of LC-PUFA on these pathways, we examined the effects of the ERK inhibitor PD98059, the p38 inhibitor...
SB203580, the JNK inhibitor SP600125 and the NF-κB inhibitor Bay117082 on IL-13 secretion by HMC-1 cells in the absence or presence of pre-incubation with 25 μM LC-PUFA since at this concentration, the differences between AA v. EPA or DHA on the suppression of IL-13 secretion were most pronounced. The solvents did not have an effect on mediator release (data not shown).

At the concentration of 25 μM, DHA reduced ionomycin/phorbol 12-myristate 13-acetate (Iono/PMA) induced IL-13 secretion by HMC-1 by 12.7% (SEM 61.9) pg/ml, respectively). Mean values were significantly different between the LC-PUFA: † SB203580, 50 μM, DHA reduced ionomycin/phorbol 12-myristate 13-acetate (Iono/PMA) as compared with unstimulated HMC-1 cells (left). Long-chain PUFA (LC-PUFA) reduced ROS generation in stimulated HMC-1 in a dose-dependent manner (shift to left) as shown in this example for DHA (25 and 100 μM) (right). Unlabelled – iono/PMA; –, unlabelled + iono/PMA; †, 0 + iono/PMA; –, 25 μM + iono/PMA; –, 100 μM + iono/PMA. (a) ROS generation (n 3) by iono/PMA-stimulated HMC-1 after LC-PUFA incubation in mean fluorescence intensity (MFI). Values are means, with standard errors represented by vertical bars. AA; EPA; DHA. ROS (mean of n 3 per data point) were found to correlate positively with (c) IL-4 (mean of n 6; P<0.006, Pearson’s correlation coefficient (r) 0.897) and (d) IL-13 (mean of n 6; P<0.005, r 0.907) secretion in HMC-1. Mean values were significantly different from those of the control group (0+): *P<0.05, **P<0.01, ***P<0.001 (one-way ANOVA followed by Dunnett’s test for multiple comparisons). Mean values were significantly different between the LC-PUFA: † P<0.05 (one-way ANOVA followed by Bonferroni’s multiple comparison test).

Discussion

Upon activation, MC initiate and maintain allergic inflammation due to the release of various inflammatory mediators. MC-derived mediators such as PGD2, TNF-α, IL-4 and IL-13 increase the susceptibility to develop allergic disease and enhance allergic symptoms(6–10). The present study shows the differential effects of the n-6 LC-PUFA AA v. the n-3 LC-PUFA EPA or DHA on mast cell phenotype.

IgE-mediated MC degranulation was enhanced upon 24 h pre-incubation with AA. The LAD2 cell line is the only human analogue that can degranulate in an IgE-dependent manner. Teshima et al.(34) reported that AA significantly increased degranulation after AA incubation.

Discussion

Upon activation, MC initiate and maintain allergic inflammation due to the release of various inflammatory mediators. MC-derived mediators such as PGD2, TNF-α, IL-4 and IL-13 increase the susceptibility to develop allergic disease and enhance allergic symptoms(6–10). The present study shows the differential effects of the n-6 LC-PUFA AA v. the n-3 LC-PUFA EPA or DHA on mast cell phenotype.

IgE-mediated MC degranulation was enhanced upon 24 h pre-incubation with AA. The LAD2 cell line is the only human analogue that can degranulate in an IgE-dependent manner. Teshima et al.(34) reported that AA significantly increased degranulation after AA incubation.
The same trend was shown for EPA and DHA. In addition to membrane fluidity, LC-PUFA may affect events in signal transduction and MC mediator release. IgE-mediated MC activation involves recruitment of tyrosine kinase, linker for activation of T cells and Syk, as well as Ca mobilisation\(^\text{(34)}\). Nakano et al.\(^\text{(34)}\) have shown that supplementation of RBL-2H3 cells with AA or EPA augmented the activation of linker for activation of T cells and Syk when compared with control cells. In addition, AA-supplemented cells had increased intracellular Ca concentration\(^\text{(34,35)}\).

Besides LAD2 cells, HMC-1 is often used as a human MC line to circumvent costly isolation procedures for human tissue MC. They lack a functional IgE receptor but can be cultured in large quantities and produce sufficient amounts of mediators for analysis. To simulate IgE receptor signalling, the cells are stimulated by ionomycin (Ca ionophore) and PMA (activating protein kinase C). FcεRI signalling in MC also leads to the simultaneous activation of Ca and protein kinase C, by inositol triphosphate and diacylglycerol, respectively\(^\text{(36)}\). Hence, similar downstream signalling pathways are activated and these pathways act synergistically to provide exocytosis.

Supplementation of LC-PUFA to HMC-1 or LAD2 cells readily resulted in effective AA, EPA or DHA membrane incorporation in a dose-dependent manner. LC-PUFA incorporation seems to be slightly less efficient in LAD2 cells when compared with HMC-1, which may be the result of serum-free culturing of these cells. In both MC lines, EPA and DHA incorporate at the cost of AA and vice versa, which results in changes in membrane \(n\)-6 to \(n\)-3 LC-PUFA and EPA:DHA ratios due to alterations in membrane composition. In addition, 18:1\(\text{n-9}\) followed by 18:0 and 16:0, were exchanged for the supplemented AA, EPA or DHA (data not shown), enabling efficient incorporation of high amounts of LC-PUFA having implications for the biological function of MC.

The differential effects of \(n\)-3 v. \(n\)-6 LC-PUFA on cytokine secretion by HMC-1 were demonstrated in the present study. The \(n\)-6 LC-PUFA AA increased TNF-\(\alpha\) and PGD\(_2\) secretion by HMC-1, while the \(n\)-3 LC-PUFA EPA and DHA dose-dependent reduced PGD\(_2\) release and were most effective in suppressing allergy-driving IL-4 and IL-13 secretion.
These MC mediators are important in the initiation and persistence of the allergic response(6–10). Besides their role in allergic disease, MC and their products can regulate the adaptive (acquired) immune response via the effects on the maturation, function and migration of B cells, T cells and dendritic cells(3,40).

The pro-inflammatory effect of AA is clearly demonstrated by the dramatic induction of PGD$_2$ release. In contrast to AA, EPA and DHA reduced PGD$_2$ secretion by activated HMC-1. EPA has been shown to reduce IgE-mediated PGD$_2$ generation by cultured human MC as well(41). PGD$_2$ is the main prostanoid secreted by activated MC and associated with allergic diseases. It can decrease IL-12 secretion by dendritic cells and promote Th2 polarisation(42,43). Furthermore, PGD$_2$ is important in the MC-dependent activation of Th2 lymphocytes, eosinophils and basophils via chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2)(77). Recently, CRTH2 antagonists have shown to be promising in the treatment of asthma and related disorders. A randomised, double-blind comparison of a CRTH2 antagonist and placebo in a population with moderate persistent asthma showed beneficial effects of the CRTH2 antagonist, including an improvement in lung function and asthma symptoms(44). These results provide evidence for an important role for PGD$_2$ in asthma and other allergic disorders, which implicate a beneficial effect of EPA and DHA by reducing PGD$_2$ generation in these patients.

Nakano et al.(54) reported that AA dose-dependently augmented TNF-α release using RBL-2H3 cells, similar to what was shown in these experiments in human MC. MC-derived TNF-α has recently been incriminated to worsen allergic symptoms via the induction of adhesion molecules, enabling influx of inflammatory cells resulting in, for example, airway inflammation and the development of airway hyperresponsiveness(60). However, studies using anti-TNF-α treatment have not been consistent, showing marked heterogeneity in responses(45,46). This makes the involvement of TNF-α-dependent pathways in LC-PUFA effects less likely.

The present study showed that EPA and DHA, in particular DHA, most effectively inhibit IL-4 and IL-13 secretion from human MC. This has not been reported previously. AA was also able to reduce IL-13 secretion but less effectively than EPA or DHA, while enhancing TNF-α and PGD$_2$ secretion. IL-13 is produced by Th2 cells, MC, eosinophils and basophils, and is critical in the induction and persistence of allergic disease. In allergic asthma, IL-13 is required for the induction of clinical symptoms(8–10). Furthermore, cytokines including IL-4 and IL-13 affect B-cell development and induce IgE isotype switching(47). Recent studies reporting about fish oil supplementation during pregnancy have shown inhibition of IL-13 release by neonatal mononuclear cells in response to allergens as well as reduced levels of IL-13 in cord blood plasma(15,48).

LC-PUFA are known to affect signal transduction cascades, leading to the transcription and production of cytokines. A variety of tissues and cells, including MC, produce ROS such as superoxide and H$_2$O$_2$ upon stimulation which are upstream regulators of signal transduction pathways(27,30). Although the effect of ionomycin/PMA stimulation on ROS production by HMC-1 was small in the present experiments, the reduction in ROS generation after LC-PUFA supplementation appeared to be specific for activated MC. Furthermore, within these experiments, IL-4 and IL-13 secretion by HMC-1 after stimulation was found to correlate positively with the amount of ROS. This is in agreement with a study in bone marrow-derived MC in which IL-4 and IL-13 secretion after IgE-mediated activation was associated with increased ROS generation(49). Unfortunately, no sensitive method for measuring intracellular ROS generation upon cell activation is currently available, while only minor changes in ROS upon activation are required to activate intracellular signalling cascades(28). Similar to the present in vitro results, fish oil has been shown to decrease ROS production in several animal studies and in healthy human volunteers(50–54).

By contrast, other animal studies have reported contradictory results for ROS production in macrophages after fish oil supplementation(55,56). Studies assessing the impact of LC-PUFA on ROS production in MC are rare. Nakano et al.(54) found that AA and EPA enhance ROS production in stimulated RBL-2H3 cells. The canine mastocytoma cell line C2 was incubated with 18:2n-6 (linoleic acid), AA, 18:3n-3 (ω-linolenic acid) and EPA, which all increased ROS production. However, this was possibly due to lipid peroxidation since antioxidant supplementation resulted in a lower increase in ROS production(57). LC-PUFA, in general, are oxidised easily because of their high degree of unsaturation, and thereby form oxygen radicals in many cell types(27,58). In the present experiments in HMC-1, the antioxidants L(-)-ascorbic acid and α-tocopherol were used, which act as free radical scavengers and protect LC-PUFA from harmful lipid peroxidation and neutralise the free radicals formed. This may explain some of the discrepancies observed in the effects of LC-PUFA on ROS generation in in vitro studies. As suggested by others, the number of double bonds present may be important in the anti-inflammatory effects generated by LC-PUFA. It may explain the most potent inhibition of ROS generation by the fatty acid with the highest degree of unsaturation, namely DHA. This possibly results in the inhibition of IL-4 and IL-13 secretion by AA < EPA < DHA(59). The present results imply that EPA and DHA act via similar mechanisms in the suppression of IL-4 and IL-13 secretion from MC, DHA just being slightly more effective than EPA. Probably the anti-allergic effects of AA are overruled since AA also enhances pro-inflammatory PGD$_2$ and TNF-α secretion. Indeed, it has been shown that high maternal intake of margarine and vegetable oils rich in n-6 PUFA during the last 4 weeks of pregnancy is associated with enhanced occurrence of atopic eczema in offspring(60), by contrast, fish oil supplementation during pregnancy and lactation reduces the susceptibility of developing allergic disease in the neonates(18–23).

Use of ROS inhibitors confirmed the involvement of ROS in the IL-4 and IL-13 secretion pathway in MC. ROS consist of a number of different mediators and although both IL-4 and IL-13 secretion could be blocked using the general ROS inhibitor 1,3-dimethyl-2-thiourea, use of superoxide dismutase showed that IL-4 but not IL-13 secretion is under the regulation of superoxide. LC-PUFA may be less able to affect this.
superoxide cascade in relation to other ROS mediators since IL-13 was suppressed more effectively by n-3 LC-PUFA than IL-4. ROS generation by MC can contribute to the secretion of inflammatory cytokines via NF-κB and/or MAP kinase signalling. Previous studies have shown that IL-13 secretion by RBL-2H3 cells is regulated by JNK and p38. However, as revealed using inhibitors of these pathways, the MAPK ERK, p38 and JNK were all involved in IL-13 secretion by activated HMC-1, while the NF-κB inhibitor Bay117082 did not reduce IL-13 release. This suggests ROS generation to be upstream of the MAPK signalling cascade.

Recently, a study with anti-IL-4/IL-13 demonstrated an improvement in asthma endpoints in patients with severe, uncontrolled asthma, suggesting a possible role for dietary supplementation with the n-3 LC-PUFA EPA and DHA in allergic disease. However, even though some studies have reported modest improvement of atopic dermatitis, there is no convincing evidence yet for dietary n-3 LC-PUFA supplementation alone for the treatment of those with established atopic disease. Thus, although AA, EPA and DHA suppress allergy-related mediator release by MC, the effects are moderate and may not be strong enough for treatment purposes. However, when MC were treated with suboptimal doses of DHA and MAPK inhibitors, the suppression of IL-13 secretion by the p38 inhibitor SB203580 (20 μM) and the JNK inhibitor SP600125 (10 μM), but not the ERK inhibitor PD98059 nor the NF-κB inhibitor Bay117082, was further supported by DHA. The addition of DHA to p38 and JNK inhibitors was as effective as the higher inhibitor doses tested (50 and 20 μM, respectively). In addition to DHA, EPA was able to support SB203580 in the suppression of IL-13 secretion, while AA did not enhance the efficacy of any of the inhibitors. Hence, the combination of n-3 LC-PUFA with other drugs seems to be promising in reducing allergic type mediator release of MC. EPA and more prominently DHA, but not AA, added to the inhibitory effect of MAPK inhibitors on IL-13 secretion. Dietary n-3 LC-PUFA may therefore be able to optimise the efficacy and/or safety of novel strategies to treat allergies using drugs aiming to suppress IL-4, IL-13 and/or PGD2.

In conclusion, the n-6 LC-PUFA AA promotes the allergic cascade by enhancing degranulation and TNF-α and PGD2 secretion by activated MC. In contrast, the n-3 LC-PUFA EPA and DHA suppress PGD2, IL-13 and IL-4 secretion as well as ROS generation most effectively. Hence, LC-PUFA differentially modulate the MC phenotype. MC are involved in the initiation and perpetuation of allergic disease, and the suppression of allergy-related mediators by dietary n-3 LC-PUFA may contribute to reduced susceptibility to develop or sustain allergic disease.

Supplementary material
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