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Docosahexaenoic Acid Alleviates Atopic Dermatitis in Mice by Generating T Regulatory Cells and M2 Macrophages

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Han *et al.* (this issue) describe a novel mechanism by which docosahexaenoic acid (DHA) may suppress atopic dermatitis symptoms in mice. They find that DHA induces FoxP3 T regulatory cells *in vivo*, M2 macrophages drive transforming growth factor- β and IL-10 conversion of CD4 T cells to CD4 FoxP3 T regulatory cells *in vitro*, and DHA-treated M2 macrophages suppress atopic dermatitis in mice.

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In 1929, George and Mildred Burr reported that a deficiency of essential fatty acids in the diet of rats led to increased transepidermal water loss and skin abnormalities, including scaly skin, inflamed tails, dandruff, loss of fur, and sores (Burr and Burr, 1929,1930; Smith and Mukhopadhyay, 2012). The animals could be cured by administration of complex unsaturated oils but not saturated fatty acids. In humans, essential fatty acid deficiency leads to dermatitis and increased transepidermal water loss, which can be reversed by the oral or topical administration of essential fatty acids (Hansen *et al.*, 1958; Prottey *et al.*, 1975). These early studies paved the way to the use of fatty acid supplementation to treat a variety of inflammatory disorders, including the use of fish oil containing docosahexaenoic acid (DHA), an omega-3 ($n=3$) long chained polyunsaturated fatty acid (PUFA). As atopic dermatitis rates have tripled in the past 30 years, affecting 10–20% of children and 1–3% of adults, altered lipid composition of the diet has been investigated (Calder, 2006, 2013). Additional associations with increased body hygiene (hygiene

hypothesis) and genetics (Boguniewicz and Leung, 2011) have been identified.

Several clinical trials have addressed the beneficial effects of DHA supplementation on atopic dermatitis (Koch *et al.*, 2008), allergy (Birch *et al.*, 2010), and atopic outcomes for infants whose mothers took DHA supplementation during pregnancy and lactation (Furuhjelm *et al.*, 2011). The outcome of fatty acid supplementation during pregnancy and during early childhood for atopy and allergy is an area of active investigation. Noakes *et al.* (2012) reported decreased cord blood cytokine responses to immune agonists after salmon supplementation, including 1160 mg/day DHA, during the last 20 weeks of pregnancy. However, they observed no differences in IgE at birth, total IgE at 6 months of age, incidence and severity of atopic dermatitis at 6 months of age, and skin-prick-test positivity at 6 months of age. A randomized double-blinded trial demonstrated that infants given DHA- and arachidonic acid (ARA)-supplemented formula during the first year of life had lower allergic outcomes at 3 years of age (Birch *et al.*,

2010). The DHA/ARA group ($n=38/89$ infants) had significantly lower odds for developing upper respiratory infections, wheezing/asthma, wheezing/asthma/atopic dermatitis, or any other allergy, whereas controls had significantly shorter times to first diagnosis of upper respiratory infections, wheezing/asthma, or any other allergy. In another study, 145 pregnant women at risk for having an allergic infant were randomized to daily supplementation with eicosapentaenoic acid (EPA) and DHA or placebo from the 25th gestational week, continuing through 3.5 months of breastfeeding (Furuhjelm *et al.*, 2011). Although no differences in the prevalence of allergic symptoms were found between groups, the decreased cumulative incidence of IgE-associated disease observed during the first year persisted until 2 years of age. High proportions of DHA and EPA in maternal and infant plasma phospholipids correlated with reduced severity of the allergic phenotype.

In a randomized controlled trial of 53 adult patients with atopic dermatitis who received 5400 mg per day of DHA over 8 weeks, the DHA-treated individuals had fewer atopic symptoms, on the basis of the SCORAD, a severity scoring scale (Koch *et al.*, 2008). *Ex vivo* peripheral blood mononuclear cells from patients with atopic dermatitis who were given DHA had significantly reduced IgE secretion levels when stimulated with anti-CD40 and IL-4, compared with control subjects (Koch *et al.*, 2008). This IgE difference may not have been seen in earlier studies because of lower amounts of DHA supplementation, indirect DHA supplementation through the mothers, or differing DHA effects in infants vs. adults.

Cumulatively, these studies are promising, and they suggest DHA supplementation as a means of improving the symptoms of atopic dermatitis. Clinical trials continue to investigate the outcomes of allergic disease and atopy, as related to omega-3 fatty acid, and specifically DHA supplementation. In parallel, the underlying immune-mediated mechanisms by which atopic dermatitis is mediated, particularly in barrier tissues such as the lung and the skin, are in progress. Normally, when

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Clinical Implications

- High amounts of supplemental docosahexaenoic acid (DHA; 100 mg kg⁻¹ per day) may improve the symptoms of atopic dermatitis in mouse models.
- DHA stimulates FoxP3 T regulatory cell generation, which suppresses inflammation.
- DHA may alleviate symptoms of patients with atopic dermatitis *via* M2 macrophage induction of FoxP3 T regulatory cells.

the stratum corneum barrier is dehydrated, breakdown products of filaggrin act as osmolytes to hydrate its corneocytes (Boguniewicz and Leung, 2011). The most significant genetic predisposition to atopic dermatitis is loss of function mutations in the *FLG* gene coding filaggrin, which alters the skin barrier of patients with atopic disease by decreasing stratum corneum hydration (Cork *et al.*, 2009). Disruption of the epidermal barrier can also lead to atopy through environmental means, such as the excessive use of soap and detergents, which raises the pH, increasing protease activity and decreasing synthesis of the lipid lamellae which, in turn, encases the corneocytes to prevent water loss (Cork *et al.*, 2009).

In addition to the role of barrier defect, innate and adaptive immunity has been studied in atopic disease. Ma *et al.* (2014) found that there were

decreased levels of T regulatory cells, FoxP3, and TGF- β (transforming growth factor- β) mRNA and increased levels of Th17 (T helper type 17), IL-17, and IL-23 in the peripheral blood of patients with atopic dermatitis compared with healthy control patients, with the severity of atopic dermatitis correlating positively with Th17 levels and negatively with T regulatory cell levels. Macrophages have also been implicated; macrophage numbers are increased in atopic dermatitis compared with normal skin, with a mixture of M1 and M2 macrophages, but the roles of these macrophage subtypes in the different inflammatory phases of atopic dermatitis have not yet been determined (Kasraie and Werfel, 2013). Dendritic cell numbers do not change in atopic dermatitis, but there is increased overlap between the macrophage and dendritic cell phenotypes, suggesting

the possibility of heterogeneous dendritic cell/macrophage populations in the skin of patients with atopic dermatitis (Kiekens *et al.*, 2001).

Now Han *et al.* (this issue, 2015) report a potential cellular immune mechanism for DHA in suppressing allergy and atopic dermatitis in pre-clinical mouse models (Figure 1). They observed that DHA supplementation suppresses the development of atopic dermatitis in mice, with consonant reductions in Serum IgE (a major activator of mast cells), histamine production, ear thickness, and a significant reduction in lymph node size. Several possible potential mechanisms are presented. DHA was observed to induce a 2-fold greater T regulatory cell expression of CTLA4, a T-cell inhibitory receptor associated with tolerance. DHA induced TGF- β , which could then induce FoxP3 T regulatory cells, and DHA enhanced M2 macrophage function, which in turn could enhance TGF- β production. Indeed, transfusion of DHA-M2 macrophages protected against atopic dermatitis and induced T regulatory cells in sites of inflammation. However, the transfusion of DHA-M2 macrophages should be compared with transfusion of M2 macrophages alone to detect whether there is an additional benefit.

This study finds optimal benefits of DHA supplementation in mice at 100 mg kg⁻¹ per day, with 50 mg kg⁻¹ per day being the minimal effective dose (Han *et al.*, 2015). This corresponds to 3,500–7,000 mg in a standard 70 kg human, which correlates with the range administered in clinical trials in which benefit was seen (Koch *et al.*, 2008). Of note, the therapeutic dose needed in both of these studies exceeds the 90–450 mg DHA in standard open-label omega-3 fatty acid supplements (Laidlaw *et al.*, 2014). However, patients in the former study had excellent DHA tolerability with only three participants reporting slight GI discomfort and no other reported side effects, suggesting 5400 mg per day as an acceptable level of DHA (Koch *et al.*, 2008). Because this dosing corresponds to the DHA levels given to the mice, changes in cytokine profiles and T regulatory cell upregulation with DHA

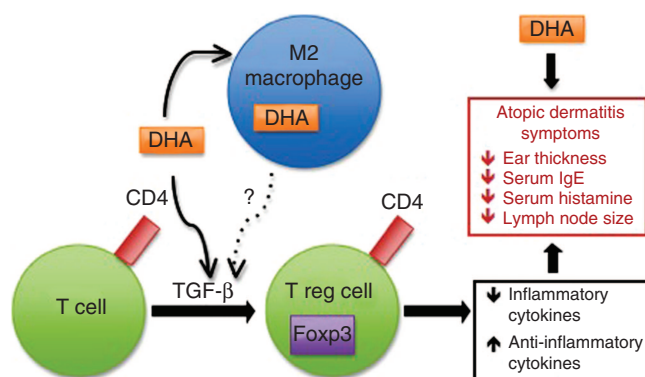


Figure 1. DHA suppression of atopic dermatitis symptoms potentially occurs through T regulatory cells and M2 macrophages. Han *et al.* (2015) found that DHA decreases atopic dermatitis symptoms and increases FoxP3 T regulatory cells *in vivo*. In this model, DHA directly increases TGF- β mediated conversion of CD4 T cells to CD4 FoxP3 T regulatory cells and induces M2 macrophages to drive TGF- β and IL-10 conversion of CD4 T cells to CD4 FoxP3 T regulatory cells *in vitro*. DHA-M2 macrophage injection leads to better control of atopic dermatitis *in vivo* compared with no treatment. These findings suggest that there may be a role for M2 macrophages in preferentially mediating the conversion of CD4 T cells to CD4 FoxP3 T regulatory cells in atopic dermatitis. DHA, docosahexaenoic acid; TGF- β , transforming growth factor- β ; T reg cell, T regulatory cell.

supplementation in this mouse model of atopic dermatitis may correlate with the changes that occur in humans with atopic dermatitis who are given equivalent DHA doses (Han *et al.*, 2015).

There is little data in the literature related to changes in cytokine signaling in humans after taking supplemental DHA. One review addressed the effects of omega-3 fatty acid (DHA and EPA) in various inflammatory conditions (Sijben and Calder, 2007), and suggested that omega-3 fatty acids may decrease inflammatory markers in certain inflammatory conditions such as inflammatory bowel disease. Other notable findings were that local rather than systemic inflammatory cytokines correlated with clinical outcomes in inflammatory lung diseases. In certain conditions like rheumatoid arthritis clinical improvement was more significant compared with inflammatory cytokine profile changes after omega-3 fatty acid supplementation (Sijben and Calder, 2007).

In neurobiology, DHA has been found to be one of the few substances that reduces the risk of Alzheimer's disease, with patients consuming fish once or more per week having 60% less risk of Alzheimer's disease compared with those who never or rarely eat fish (Morris *et al.*, 2003). Furthermore, in this study, total intake of n-3 PUFAs and DHA, but not EPA, correlated with a reduced risk of Alzheimer's disease. DHA was found to benefit patients with Alzheimer's disease by enhancing A β ₄₂ phagocytosis by microglia with predominantly M2 markers and by shifting away from pro-inflammatory M1 macrophage activation and pro-inflammatory cytokines (Hjorth *et al.*, 2013).

A randomized controlled trial in which DHA and EPA supplementation was given to Alzheimer's disease patients showed that IL-6, IL-1 β , and granulocyte colony-stimulating factor secretion are decreased when peripheral blood mononuclear cells are stimulated with lipopolysaccharide (LPS) after 6 months of 1,700 mg per day DHA and 600 mg per day EPA (Vedin *et al.*, 2008). Similarly Han *et al.* have shown that secretion of IL-6 is

decreased in LPS-stimulated macrophages and that IL-1 β , IL-6, and TNF- α expression are decreased in M1 macrophages that are co-cultured with M2 macrophages for 24 hours (Han *et al.*, 2015). The decrease in IgE in DHA-fed mice parallels the *ex vivo* IgE secretion of stimulated peripheral blood mononuclear cells from DHA-supplemented patients (Koch *et al.*, 2008).

The use of DHA for autoimmunity and inflammation is increasingly common, but little has been done to rigorously study the effects of DHA on immunity outside of a neurobiology context or outside of the lipid literature. Future clinical trials will likely look at DHA effects in a larger population of patients with atopic dermatitis, measuring both clinical outcomes and changes in inflammatory cell and cytokine profiles. Future studies may address the use of high DHA doses to treat atopic individuals, and they may also address the additional mechanistic clues concerning how DHA can modulate the immune system to improve symptoms in atopic dermatitis and other autoimmune and inflammatory conditions.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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