

ORIGINAL ARTICLE

Dietary polyunsaturated fatty acids and non-digestible oligosaccharides reduce dermatitis in mice

Christin Weise¹, Dennis Ernst¹, Eric A. F. van Tol² & Margitta Worm¹

¹Allergy-Center-Charité, Department of Dermatology and Allergy, Charité–Universitätsmedizin Berlin, Berlin, Germany; ²Mead Johnson Nutrition, Evansville, USA

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Correspondence

Margitta Worm, Department of Dermatology and Allergology, Charité–Universitätsmedizin Berlin, Charitéplatz 1, Berlin 10117, Germany.
Tel.: +49 30 450 518 105
Fax: +49 30 450 518 919
E-mail: margitta.worm@charite.de

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Abstract

Background: Oral administration of specific food ingredients can modify mucosal and systemic inflammatory processes. Such food components are fatty acids or carbohydrates. Nevertheless, little is known about the impact of oral administration of polyunsaturated fatty acids (PUFA) and non-digestible oligosaccharides on allergen-induced dermatitis.

Method: In this pilot study, skin inflammation was induced by serial epicutaneous OVA applications in OVA-sensitized mice. In parallel, mice were fed with solid food containing arachidonic acid/docosahexaenoic acid (AA/DHA), galactooligosaccharide/polydextrose (GOS/PDX) or their combination. Skin lesions were assessed by clinical skin score, but also skin barrier parameters, immunohistochemical analyses, and local cytokine expression profile.

Results: Both dietary AA/DHA and GOS/PDX significantly ameliorated the severity of allergen-induced dermatitis. The clinical improvement upon oral AA/DHA and GOS/PDX supplementation was associated with a reduction in transepidermal water loss and reduced KI-67 expression in the skin. Lesional CD8⁺ and mast cells were reduced in all treatment groups, but appeared to be most pronounced in combined AA/DHA/GOS/PDX-treated mice. Moreover, in GOS/PDX-treated mice, IFN γ and TGF β expression was increased in skin lesions.

Conclusion: Dietary supplementation with DHA/AA and GOS/PDX ameliorates symptoms of allergen-induced dermatitis and may thus be beneficial in the dietary management of human atopic eczema.

The prevalence of atopic eczema (AE) is high in children affecting up to 20% aged under 6 yr (1). AE is often associated with other atopic conditions like asthma and allergic rhinitis. Its complex pathogenesis is linked to a genetic susceptibility, epidermal barrier defects, immune dysfunctions, but also environmental factors (2).

Epidemiological data indicate that environmental parameters can modify the immune function regardless the genetic background (3). Thereby, the beneficial role of breast-feeding on immune function (4) and many immune-related conditions (5) in infancy is well established. Importantly, current evidences support a strong protective effect of prolonged and exclusive nursing on AE.

Breast-feeding provides the best mixture of essential nutrients and bioactive factors (4). One important component of breast milk is polyunsaturated fatty acids (PUFA), especially for the development of the immune system. Arachidonic acid

(AA, C20:4n-6) and docosahexaenoic acid (DHA, C22:6n-3) are essential as they cannot be synthesized *de novo* by infants, and their synthesis from precursors is critically limited. Therefore, AA and DHA are regular components of many infant formulas throughout the world (6). In breast milk, AA levels are relatively constant on a worldwide basis with 0.4–0.7% of total fatty acids, whereas the DHA content is highly depended on maternal diet and lifestyle ranging from 0.2% to 1.0% (7).

Beside lactose and lipids, carbohydrates represent the third most abundant component in breast milk ranging between 1.0% and 1.2% (8, 9). These carbohydrates can be non-digestible, fermentable oligosaccharides. They have been reported to beneficially affect the host by selective stimulation of growth and activity of intestinal bacteria which can influence the postnatal development of the immune system. Infant formulas with galactooligosaccharides (GOS) and polydextrose

(PDX) may provide additional functional properties of human breast milk, such as promoting intestinal commensals and supporting immunomodulation. However, oligosaccharides are not currently used as supplements in infant formulas (10).

In this study, we investigated the impact of combined oral administration of PUFA and non-digestible oligosaccharides on the severity of allergen-induced dermatitis. The used doses and ratios were adopted from those of human breast milk. Our data show therapeutical effects in AA/DHA, but also GOS/PDX-treated mice. Interestingly, additive effects of PUFA and oligosaccharides are suggested because their combination in a master formula resulted in superior clinical improvement but also immunomodulation.

Materials and methods

Induction of allergen-induced dermatitis

Female 8–10 wk old BALB/c mice (Charles River, Sulzfeld, Germany) were kept within an accredited animal facility at the Charité-Universitätsmedizin Berlin and maintained under specific pathogen-free conditions. Procedures were performed in compliance with protocols approved by the local State Office of Health and Social Affairs.

For induction of allergen-induced dermatitis, mice were sensitized intraperitoneally (i.p.) with ovalbumin (OVA, Grade V, Sigma-Aldrich, Steinheim, Germany) followed by serial epicutaneous (e.c.) OVA applications as described previously (11, 12). Each mouse experienced three-one-week exposures to the same skin site separated by 2-wk intervals without application. For treatment evaluation, non-sensitized mice were used as negative control and PBS-patched mice as patch control. On day 64, the phenotypical outcome was evaluated, and transepidermal water loss (TEWL) was measured using DermaLab TEWL Modul (Cortex Technology™ Hadsund, Denmark).

Dietary intervention

During sensitization mice were fed with standard solid food (Ssniff®Spezialdiäten, Soest, Germany) containing either 0.32% AA (ARASCO®; Martek Biosciences, Columbia, MD, USA) together with 0.17% of total fat DHA (DHASCO®; Martek) and 1% GOS (Vivinal GOS; Friesland Foods Domo, Zwolle, the Netherlands) together with 1% PDX (Litesse Two Polydextrose; Danisco, Beaminster, UK) or the combination of these components. AA and DHA were provided through admixture of 0.067% ARASCO® and 0.035% DHASCO® that are AA- and DHA-enriched microbial-derived triglyceride oils. The standard solid chow did not contain these factors. Manufacturing of the experimental diets was performed by Ssniff®Spezialdiäten under cold and airtight conditions. During the whole experiment, body weight and chow consumption were daily monitored.

Assessment of skin inflammation

For evaluation of the dermatitis severity, a scoring system was used considering five hallmarks of human AE (erythema,

edema, crusting, dryness, extension) (13). For score calculation, parameters were graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe). Total skin score was taken as index of dermatitis severity and ranged from 0 (no skin lesion) to 15 (severe) (14).

Immunohistochemistry

Skin sections were immunohistochemically analyzed regarding KI-67 expression and CD4, CD8 and mast cell numbers as described before (11).

Immunoglobulin measurement

OVA-specific IgE, IgG1 (14), IgA (12), and total IgE (15) levels were measured in sera by ELISA as previously described.

Gene expression analysis

Total RNA and cDNA synthesis were prepared as described previously (11). The primers used were as follows: IFN γ : AACTATTTTAACTCAAGTGGCATAGAT, TGCTGTTGCTGAAGAAGGTAG; IL-4: GACTCTTTCGGGCTTTTCG, TGATGCTCTTTAGGCTTTCCA; IL-10: TTTAAGGTTACTTGGGTTC, AGGGTCTTCAGCTTCTCACC; TGF β : GCAACAACGCCATCTATGAG, AGACAGCCACTCAGGCGTAT. The relative mRNA expression levels were normalized with hypoxanthine phosphoribosyltransferase mRNA (CGTCGTGATTAGCGATGATG, AATCCAGCAGGTCAGCAAAG) as described elsewhere (11).

Statistical analysis

The data of this pilot study were analyzed by the Kruskal–Wallis test followed by a pairwise comparison of the groups using GraphPad Prism 5.0 (GraphPad Software Inc, La Jolla CA, USA). Correlations were assessed by Pearson's correlation test. A p -value ≤ 0.05 was considered to be statistically significant.

Results

Decreased severity of allergen-induced dermatitis upon dietary treatment

Our murine model of allergen-induced dermatitis (11, 12, 14) was used to investigate the therapeutical efficacy of dietary PUFA and oligosaccharides. Mice consumed daily dosages of 53 mg/kg body weight AA, 28 mg/kg DHA, 1.8 g/kg GOS and PDX. All mice showed comparable chow consumption, liver, spleen, and body weight. The tolerability was excellent, and no side effects occurred (data not shown).

The sensitization schedule induced local dermatitis with visible eczema symptoms, including dryness, erythema, edema, papules, oozing, and crusts (14). While non-sensitized mice fed with control diet showed no skin lesions, PBS-patched mice exhibited a skin score of 6.0 (4.0–9.0), which further increased to 9.0 (6.0–10.0) score points after e.c. OVA application

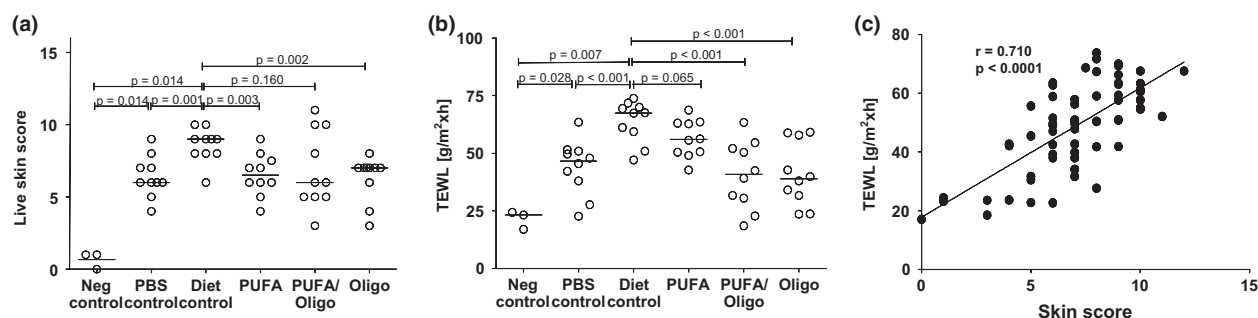


Figure 1 Reduced severity of allergen-induced dermatitis upon dietary treatment. (a) Clinical skin score was defined as sum of individual scores graded from 0 (none) to 3 (severe) for erythema, crusts, dryness, and extension. Data are shown as single values with median. (b) Improved skin barrier functions upon supplementation as measured by TEWL. Data are shown as single values with median. (c) Correlation between skin score and TEWL. Pearson's correlation coefficient (R) and p -value are given in the graph. $n = 53$. Oligo, oligosaccharides; PUFA, polyunsaturated fatty acids; TEWL, transepidermal water loss.

($p = 0.004$, Fig 1a). All supplemented groups showed less dermatitis symptoms in comparison with the diet control group. In the AA/DHA-fed group, skin score was reduced to 6.5 (4.0–9.0) ($p = 0.003$) and in GOS/PDX-fed mice to 7.0 (3.0–8.0) ($p = 0.002$). Interestingly, the lowest skin score was determined in the group receiving PUFA and oligosaccharides together (6.0 [3.0–11.0]). However, due to high interindividual variations, the improvement did not reach statistical significance. Thereby, the decreased symptom score upon

supplementation was mainly attributed to the reduction in crusting, dryness, and extension (data not shown).

The analysis of systemic humoral immune response revealed a significant increase in total IgE and OVA-specific IgG1 as well as a tendency toward an increase in OVA-specific IgE upon e.c. OVA application, whereas OVA-specific IgA remained unchanged. However, supplementation did not influence serum concentrations of these immunoglobulins (Fig 2).

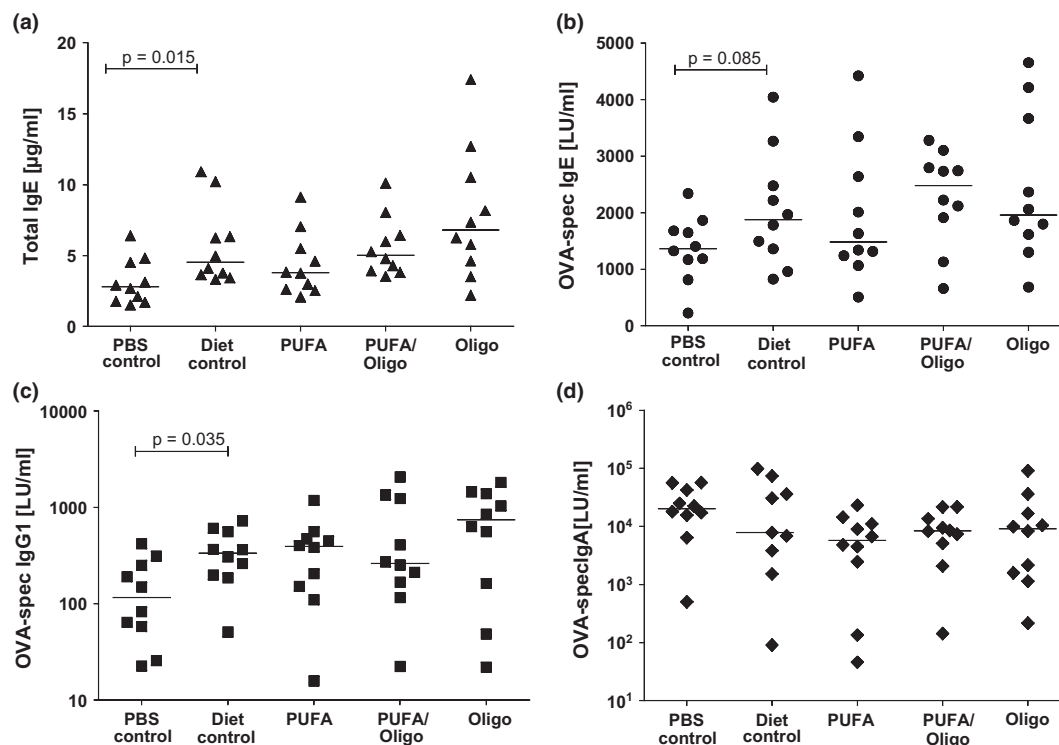


Figure 2 Serum concentrations of (a) total IgE, OVA-specific (b) IgE (c) IgG1 and (d) IgA. Data are shown as single values, bars represent median values; $n \geq 9$. Oligo, oligosaccharides; PUFA, polyunsaturated fatty acids.

Improved barrier function upon dietary treatment

The TEWL is an objective parameter for evaluating skin functions and is often impaired in human AE (16). In accordance with the skin score results, the lowest TEWL was determined in non-sensitized mice (22.6 ± 2.3 g/h/m²). Furthermore, e.c. OVA application increased TEWL from 44.0 ± 3.8 g/h/m² in PBS-treated mice to 63.9 ± 2.9 g/h/m² in OVA-patched diet control mice ($p < 0.001$, Fig 1b). More importantly, TEWL values were significantly reduced upon dietary supplementation with PUFA/oligosaccharides (40.6 ± 4.6 g/h/m², $p < 0.001$), but also in the GOS/PDX group (41.0 ± 4.3 g/h/m², $p < 0.001$). Although there was a trend toward a reduced TEWL in AA/DHA group as well (56.3 ± 2.6 g/h/m², $p = 0.065$), the data did not reach statistical significance. In this study, we observed a significant correlation between the skin score and the TEWL ($r = 0.710$, $p < 0.0001$, Fig 1c).

Decreased local inflammation upon dietary treatment

Next, we examined whether the reduced dermatitis severity was associated with changes of the local inflammatory response. Indeed, upon dietary supplementation, a significant decrease in epidermal KI-67 protein expression was determined by immunohistochemistry (Fig 3a). In the AA/DHA-fed group, a reduced KI-67 expression (3.0 [1.0 – 4.0]) was observed in comparison with control-fed mice (4.0 [3.0 – 5.0], $p = 0.013$). In line with the clinical results, GOS/PDX (2.3

[1.0 – 5.0]; $p = 0.029$) and PUFA/oligosaccharides- (2.3 [1.0 – 5.0]; $p = 0.041$) treated mice displayed the lowest KI-67 expression in the epidermis.

Additionally, a decrease in dermal CD8⁺ cells was detected in mice treated with PUFA and oligosaccharides (diet control: 237 ± 52 CD8⁺/mm²; PUFA/oligosaccharides: 61 ± 19 CD8⁺/mm², $p = 0.008$; Fig 3b). Moreover, the number of dermal CD8⁺ cells in the AA/DHA group (136 ± 37 CD8⁺/mm², $p = 0.140$) and in mice treated with GOS/PDX alone (109 ± 28 CD8⁺/mm², $p = 0.053$) tended to be reduced. We determined a significant correlation between the CD8⁺ T-cell numbers in the dermis and the skin score ($r = 0.315$, $p < 0.019$). This strengthens the clinical assessment of an ameliorated dermatitis upon PUFA/oligosaccharides treatment. In contrast, the number of dermal CD4⁺ T cells remained unchanged upon supplementation (Fig 3d).

As shown in Fig 3c, a trend to a reduced number of dermal mast cells was determined in mice receiving AA/DHA (diet control: 116 [37 – 169] mast cells/mm²; AA/DHA: 67 [28 – 108] mast cells/mm²; $p = 0.080$). Upon the combined supplementation of PUFA and oligosaccharides, a significant strong reduction in dermal mast cells was detected (50 [14 – 71] mast cells/mm²; $p = 0.008$). Although the decrease in mast cells in GOS/PDX-fed mice did not reach statistical significance (72 [47 – 139] mast cells/mm²; NS), a clear trend of reduction was present. Further mast cell analysis revealed a comparable degranulation status between diet control and the treatment groups (data not shown).

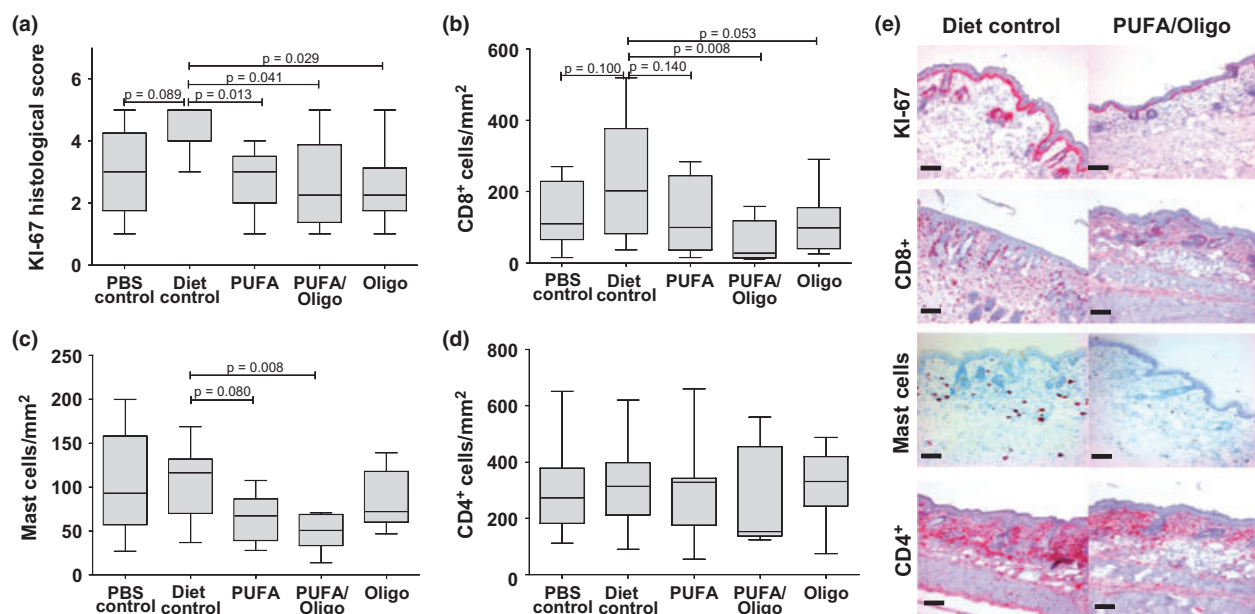


Figure 3 Immunohistochemical analysis of eczematous lesions upon dietary treatment. (a) KI-67 expression, (b) number of CD8⁺, (c) mast cells and (d) CD4⁺ cells with (e) representative histological illustrations of diet control (left panel) and PUFA/oligosaccharides group (right panel). Positively stained cells were analyzed per mm² in 5 μ m hematoxylin-counterstained skin sections at $\times 100$ magnification using digital measuring tools. Data are shown as box plots (25th and 75th percentile, median); $n \geq 9$, Bars, 100 μ m. Oligo, oligosaccharides; PUFA, polyunsaturated fatty acids.

Table 1 Relative mRNA expression in eczematous skin upon dietary treatment

mRNA expression	Sham	Diet control	PUFA	PUFA/Oligo	Oligo
IFN γ	179 (2–462)**	9 (0.04–337)	5 (0.09–350)	32 (0.08–282)	74 (19–272)*
IL-4	49 (9–132)	19 (4–120)	31 (3–182)	21 (7–83)	43 (22–65)
IFN γ /IL-4	2.5 (0.2–9.2)**	0.5 (0.01–2.2)	0.2 (0.02–5.0)	1.5 (0.01–9.6)	3.0 (0.5–4.7)**
IL-10	58 (0.3–219)	2 (0.01–36)	9 (0.01–109)	7 (0.2–278)	11 (1–200)
TGF β	1.3 (0.4–3.2)	0.9 (0.2–6.6)	0.6 (0.1–2.6)	0.9 (0.5–2.1)	2.0 (0.9–22.0)*

Expression of cytokine mRNA was determined by qRT-PCR. Relative expression of IFN γ , IL-4, IL-10, and TGF β in eczematous skin. Data are shown as median [minimum–maximum]; $n \geq 9$.

IFN, interferon; IL, interleukin; Oligo, oligosaccharides; PUFA, polyunsaturated fatty acids; TGF, transforming growth factor.

* $p < 0.05$, ** $p < 0.01$ statistically significant difference compared control-fed, OVA-sensitized mice.

Local inflammatory cytokine profile

The dermal cytokine expression is shaping the outcome of allergen-induced dermatitis and was therefore investigated next. Upon e.c. OVA application, IFN γ mRNA was significantly reduced. As the IL-4 expression remained stable, the IFN γ /IL-4 ratio was significantly decreased in OVA-patched diet control mice in comparison with PBS-patched mice (Table 1). While in AA/DHA and PUFA/oligosaccharides-fed mice, no significant alteration of local cytokine profile was measured. GOS/PDX supplementation resulted in a significant increase in TGF β and IFN γ expression leading to an enhanced IFN γ /IL-4 ratio.

Discussion

In the present study, dietary supplementation with AA/DHA and GOS/PDX in breast milk adopted doses was associated with an amelioration of allergen-induced dermatitis. In line with the clinical outcome data, the TEWL value was normalized upon oral supplementation with PUFA, but reaching a stronger efficacy in groups treated with oligosaccharides. This finding suggests positive effects of food ingredients like PUFA and carbohydrates on skin barrier functions.

A disturbed skin barrier is a hallmark of AE, thereby promoting inflammatory processes and directly influencing clinical outcome. TEWL is a clinically useful physical marker of epidermal barrier integrity and used as an objectively quantifying parameter of disease severity. Strategies improving skin barrier functions might prevent or slow down AE disease development, especially in children (16).

To our knowledge, this is the first report showing that oral supplementation of the PUFA AA and DHA together with the oligosaccharides GOS and PDX results in stabilization of skin barrier integrity in a mouse model of allergen-induced dermatitis.

PUFA and their metabolites are involved in AE pathogenesis (17). We have recently shown that dietary AA/DHA supplementation promotes the amelioration of allergen-induced dermatitis by directly targeting keratinocytes as crucial regulators of skin inflammation (11). As dietary PUFA ensure cutaneous membrane fluidity and sustain barrier integrity, AA/DHA supplementation may support regeneration of the disturbed skin barrier (18).

Supplementation of oligosaccharides in formula-fed infants reduces the incidence of AE in early childhood by stimulation of a bifidobacteria-dominated bowel flora (19). GOS and PDX were shown to exert bifidogenic effects in infants and adults as well (20). Their biological activity is related to microbial metabolism resulting in the generation of short chain fatty acids (SCFA) by fermentation. SCFA are basically responsible for the beneficial immunomodulation and intestinal barrier improvement mediated by prebiotics (21). The barrier dysfunction in atopic conditions allows the penetration of antigens supporting sensitization and inflammation. Thereby, an increased permeability enables dietary allergens to enter the body and to aggravate AE (22). In addition, in our mouse model of allergen-induced dermatitis, an enhanced intestinal permeability upon OVA sensitization was observed (own unpublished data). An improvement of barrier functions might be preventive and thus beneficially influence the disease outcome. Interestingly, PUFA in human breast milk progress the adhesion of probiotics to mucosal surfaces and facilitate prebiotic effects (5). Moreover, in GOS/PDX-fed mice, a significantly increased lesional IFN γ expression was detected, whereas IL-4 expression remained unchanged resulting in an increased IFN γ /IL-4 ratio upon dietary intervention. These results point to a shift toward Th1 during the local immune response. The data are supported by previous reports showing that prebiotic oligosaccharides modulate the Th1/Th2 balance by increasing Th1-related and dampening Th2-related parameters in an allergic mouse model (23). The epidermal barrier disruption in human AE has been related to decreased ceramide levels in the stratum corneum. Th2 cytokines promote the reduction in the ceramide concentrations in the epidermis and can thereby diminishing the skin barrier directly. On the other hand, Th1 cytokines, like IFN γ can increase epidermal ceramide production by up-regulation of their producing enzymes (24). Thus, a Th1 shift in lesional skin can support the barrier integrity as indicated by the decreased TEWL values upon oligosaccharide supplementation. However, in the PUFA/oligosaccharides group, the TEWL is significantly reduced although the increase in the IFN γ /IL-4 ratio did not reach statistical significance and therefore cannot be adequately explained by the discussed hypothesis. Although not evaluated in this study, we hypothesize that the detected Th1 shift reached a threshold value sufficient to induce

epidermal ceramide production resulting in an improved skin barrier function. In contrast, in AA/DHA-fed animals, a Th1 shift was not detectable. Consequently, the decrease in TEWL did not reach the level of the GOS/PDX-supplemented groups. Nevertheless, the clinical improvement in the PUFA group was comparable with that upon oligosaccharide supplementation indicating different but likewise efficient mechanisms, that is, direct action of DHA/AA or their bioactive metabolites on keratinocytes (11). Potentially, in PUFA/oligosaccharides-fed mice, these distinct mechanisms are overlapping, thereby causing a higher variance of the skin score values. However, this hypothesis requires to be proven by further investigations.

Abnormal keratinocyte proliferation is another important element of AE pathogenesis. KI-67 is exclusively expressed during the cell cycle and used as proliferation marker. A reduced expression of KI-67 in keratinocytes was associated with the clinical improvement of AE (25). The oral administration of experimental diets has led to a significantly reduced KI-67 expression by epidermal keratinocytes. This was most pronounced in mice fed with PUFA/oligosaccharides and GOS/PDX. In addition, a reduced number of CD8⁺ T cells in lesional skin were determined as well. While the reduction was seen as a trend in PUFA or oligosaccharides-treated animals, the decrease was significant in the groups receiving the combination. In the PUFA/oligosaccharides group, the CD8⁺ T-cell reduction was most prominent suggesting additive effects of both components. The importance of CD8⁺ T cells in development of murine skin inflammation was shown previously (26). Within this study, cytotoxic CD8⁺ T cells were early recruited to sites of allergen exposure, suggesting an involvement in the initiation of inflammation. Accordingly, their depletion abolished the inflammatory skin response. Furthermore, the role of CD8⁺ T cells in pathogenesis of human AE was recently demonstrated (26). CD8⁺ cells have been shown to induce keratinocyte apoptosis in a contact-dependent manner (27). The enhanced apoptosis of keratinocytes was associated with CD8⁺ cell infiltration and KI-67 accumulation (28). Accordingly, the improved skin status in the supplementation groups was associated with lower CD8⁺ numbers and a reduced KI-67 expression in skin lesions.

Mast cells are essentially involved in pathogenesis of human AE as well. They can control the recruitment, trafficking, and function of cells involved in skin inflammation like keratinocytes and T cells. Interestingly, mast cells can polarize T-cell response into the allergy-promoting Th2 direction. Like

professional antigen-presenting cells mast cells can present antigens to T cells directly. Consequently, AE skin shows increased mast cell numbers with an intensive degranulation status in lesions (29). Although the degranulation status was comparable between the experimental groups, we observed less dermal mast cells upon supplementation in our study. This was most prominent when mice received the combination of AA/DHA and GOS/PDX. PUFA are known to inhibit mast cell function (30), either by direct action on mast cells or indirectly as a result of less skin inflammation. In spite of a stable degranulation status, the local production of inflammatory mediators by mast cells may be beneficially modified by supplementation resulting in reduced skin inflammation. The same may be applied to the serum immunoglobulin concentrations which were not affected by supplementation in this study. Thereby, our data are in accordance with other publications showing that dietary intervention can improve *in vivo* allergic symptoms without significant modification of serum IgE levels (11, 12, 23).

In summary, dietary supplementation of AA/DHA and GOS/PDX, in ranges as found in human breast milk, ameliorated the disturbed epidermal skin barrier of allergen-induced dermatitis due to a reduction in the local inflammatory immune response. Our results suggest that the normalization of the skin status is most likely dependent on direct action of DHA/AA or their bioactive metabolites on keratinocytes and mast cells. In addition, alterations of the intestinal flora by prebiotics may promote immunomodulation through gut-derived mediators. Taken together, our observations support epidemiological findings indicating that the decline in nursing is promoting allergic pathogenesis and that supplementation of formula with PUFA and oligosaccharides can act in a preventive but also therapeutical manner in the context of AE through immunomodulatory mechanisms.

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References

1. Eller E, Kjaer HF, Host A, Andersen KE, Bindslev-Jensen C. Development of atopic dermatitis in the DARC birth cohort. *Pediatr Allergy Immunol* 2010; **21** (2 Pt 1): 307–14.
2. Spergel JM. From atopic dermatitis to asthma: the atopic march. *Ann Allergy Asthma Immunol* 2010; **105**: 99–106; quiz 7–9, 17.
3. Prescott S, Nowak-Węgrzyn A. Strategies to prevent or reduce allergic disease. *Ann Nutr Metab* 2011; **59** (Suppl. 1): 28–42.
4. Kramer MS. Breastfeeding and allergy: the evidence. *Ann Nutr Metab* 2011; **59** (Suppl. 1): 20–6.
5. Hosea Blewett HJ, Cicalo MC, Holland CD, Field CJ. The immunological components of human milk. *Adv Food Nutr Res* 2008; **54**: 45–80.
6. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LM. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. *Am J Clin Nutr* 2007; **85**: 1457–64.
7. Koletzko B, Lien E, Agostoni C, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *J Perinat Med* 2008; **36**: 5–14.
8. Chichlowski M, German JB, Lebrilla CB, Mills DA. The influence of milk oligosaccharides on microbiota of infants:

- opportunities for formulas. *Annu Rev Food Sci Technol* 2011; **2**: 331–51.
9. Veereman-Wauters G. Application of prebiotics in infant foods. *Br J Nutr* 2005; **93** (Suppl. 1): S57–60.
 10. Herfel TM, Jacobi SK, Lin X, et al. Polydextrose enrichment of infant formula demonstrates prebiotic characteristics by altering intestinal microbiota, organic acid concentrations, and cytokine expression in suckling piglets. *J Nutr* 2011; **141**: 2139–45.
 11. Weise C, Heunemann C, Loddenkemper C, Herz U, van Tol EA, Worm M. Dietary docosahexaenoic acid in combination with arachidonic acid ameliorates allergen-induced dermatitis in mice. *Pediatr Allergy Immunol* 2011; **22**: 437–504.
 12. Weise C, Zhu Y, Ernst D, Kuhl AA, Worm M. Oral administration of *Escherichia coli* Nissle 1917 prevents allergen-induced dermatitis in mice. *Exp Dermatol* 2011; **20**: 805–9.
 13. Hanifin JM, Rajka G. Diagnostic features of atopic-dermatitis. *Acta Dermato-Venereologica* 1980; **92**: 44–7.
 14. Dahten A, Koch C, Ernst D, Schnoller C, Hartmann S, Worm M. Systemic PPARgamma ligation inhibits allergic immune response in the skin. *J Invest Dermatol* 2008; **128**: 2211–8.
 15. Rühl R, Koch C, Marosvolgyi T, et al. Fatty acid composition of serum lipid classes in mice following allergic sensitisation with or without dietary docosahexaenoic acid-enriched fish oil substitution. *Br J Nutr* 2008; **99**: 1239–46.
 16. Gupta J, Grube E, Ericksen MB, et al. Intrinsically defective skin barrier function in children with atopic dermatitis correlates with disease severity. *J Allergy Clin Immunol* 2008; **121**: 725–30, e2.
 17. Sala-Vila A, Miles EA, Calder PC. Fatty acid composition abnormalities in atopic disease: evidence explored and role in the disease process examined. *Clin Exp Allergy* 2008; **38**: 1432–50.
 18. Feingold KR. Thematic review series: skin lipids. The role of epidermal lipids in cutaneous permeability barrier homeostasis. *J Lipid Res* 2007; **48**: 2531–46.
 19. Moro G, Arslanoglu S, Stahl B, Jelinek J, Wahn U, Boehm G. A mixture of prebiotic oligosaccharides reduces the incidence of atopic dermatitis during the first six months of age. *Arch Dis Child* 2006; **91**: 814–9.
 20. Scalabrin DM, Mitmesser SH, Welling GW, et al. New prebiotic blend of polydextrose and galacto-oligosaccharides has a bifidogenic effect in young infants. *J Pediatr Gastroenterol Nutr* 2012; **54**: 343–52.
 21. Gourbeyre P, Denery S, Bodinier M. Probiotics, prebiotics, and synbiotics: impact on the gut immune system and allergic reactions. *J Leukoc Biol* 2011; **89**: 685–95.
 22. Proksch E, Folster-Holst R, Jensen JM. Skin barrier function, epidermal proliferation and differentiation in eczema. *J Dermatol Sci* 2006; **43**: 159–69.
 23. Vos AP, van Esch BC, Stahl B, et al. Dietary supplementation with specific oligosaccharide mixtures decreases parameters of allergic asthma in mice. *Int Immunopharmacol* 2007; **7**: 1582–7.
 24. Sawada E, Yoshida N, Sugiura A, Imokawa G. Th1 cytokines accentuate but Th2 cytokines attenuate ceramide production in the stratum corneum of human epidermal equivalents: an implication for the disrupted barrier mechanism in atopic dermatitis. *J Dermatol Sci* 2012; **68**: 25–35.
 25. Sapuntsova SG, Mel'nikova NP, Deigin VI, Kozulin EA, Timoshin SS. Proliferative processes in the epidermis of patients with atopic dermatitis treated with thymodepressin. *Bull Exp Biol Med* 2002; **133**: 488–90.
 26. Hijnen D, Knol EF, Gent YY, et al. CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-gamma, IL-13, IL-17, and IL-22. *J Invest Dermatol* 2012: Advanced online publication at 2012/12/06.
 27. Werfel T. The role of leukocytes, keratinocytes, and allergen-specific IgE in the development of atopic dermatitis. *J Invest Dermatol* 2009; **129**: 1878–91.
 28. Klimczak A, Lange A. Apoptosis of keratinocytes is associated with infiltration of CD8 + lymphocytes and accumulation of Ki67 antigen. *Bone Marrow Transplant* 2000; **26**: 1077–82.
 29. Liu FT, Goodarzi H, Chen HY. IgE, mast cells, and eosinophils in atopic dermatitis. *Clin Rev Allergy Immunol* 2011; **41**: 298–310.
 30. Ishihara K, Murata M, Kaneniwa M, et al. Effect of tetracosahexaenoic acid on the content and release of histamine, and eicosanoid production in MC/9 mouse mast cell. *Lipids* 1998; **33**: 1107–14.