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Chapter 3

IMMUNOMODULATION BY FOOD: ALLERGY MITIGATION BY DIETARY COMPONENTS

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ABSTRACT

Proper and well-balanced immune functioning is of pivotal importance to health and well-being. In the past decades, there has been an apparent decrease in the prevalence of classical infectious diseases, with a concomitant increase in immune-related disorders, including allergies. Causally, a relationship with changes in life-style-related factors, amongst which hygienic practice, is often laid.

Diet and nutrition can affect functioning of various immune parameters. This concept can be explored to prevent or mitigate allergic reactions via the development of targeted food products or ingredients. This review describes recent findings with respect to food products and ingredients that show potential in this respect, with special emphasis on pro- and prebiotics, ß-glucans, fungal immunomodulatory proteins and ω-3 polyunsaturated fatty acids. The micro-organism-related products appear to strengthen Th1-mediated immunity, thus possibly restoring defective immune maturation due to overly hygienic living conditions.

Also ω-3-PUFAs may hold promise to attenuate allergic symptoms, although this concept appears to function via mechanisms that are clearly distinct from the micro-organism derived compounds.

Keywords: Immunity, immunomodulation, allergy, probiotics, prebiotics, ß-glucans, fungal immunomodulatory proteins, ω-3 polyunsaturated fatty acids, diet, nutrition

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BACKGROUND

A properly functioning and balanced immune system is pivotal to maintain health and well-being. Roughly since world war II, the prevalence of 'traditional' infectious diseases, such as tuberculosis, hepatitis, or measles, has decreased, with a concomitant increase in immune-related disorders, such as multiple sclerosis, Crohn's disease, type 1 diabetes and various allergic diseases. Also, disease incidence decreased in North-South direction in the Northern hemisphere, and vice versa in the Southern hemisphere [1]. This suggests non-genetic factors playing a role, such as health care and medical practice (e.g. vaccination programmes), living environment and life style, welfare, dietary patterns, and the like. In particular for the strongly increased prevalence of asthma and allergic disease, a connection to hygienic practice and subsequent reduced exposure to microbes, which is supposed to result in a 'not fully ripened' immune system, was hypothesised [2]. This may include hygienic practice in food preparation, of which the virtue for public health is not to be disputed, but that may have contributed to impaired or delayed immune maturation. In addition, the development of food products that are composed of highly refined ingredients has probably led to decreased consumption of micronutrients that may be relevant for immune maturation [3]. It is interesting to note, that the incidence of tuberculosis appeared inversely related to the incidence of asthma and rhinoconjunctivitis. This could confirm the importance of a vigilant Th1-compartment in disease aetiology, as mycobacteria are known to elicit particularly strong Th1-responses [4], adding to the Th1/2-paradigm as being at the immunological basis of allergic disease [5]. In many other chronic diseases, for instance cardiovascular diseases, neoplastic malignancies, and metabolic syndrome, chronic inflammatory processes contribute to the development of associated health problems. Also in such clinical situations, an important role for immune interventions may be found [6-10]. The prophylactic and therapeutic relevance of immune modulation is illustrated by the possibility to develop antitumor strategies via vaccination e.g. as has been described for *Mycobacterium*-based vaccines and lung tumours, an effect supposedly based on selective enhancement of Th1-mediated immunity [11]. Another example is the claimed prophylactic effect of the Th1-stimulating mushroom-derived ß-glucan lentinan on malaria [12]. Altogether, immune modulation, e.g. via dietary strategies, is not only relevant for such specific clinical conditions, but holds promise for maintaining immune homeostasis in the healthy population as well, despite the heterogeneity of the concept of 'health'. Immune functioning strongly varies in distinguishable life stages. For instance, with increasing age, there is a decline in the functional capacity to elicit generalised and specific immune responses, accompanied by a decrease in production and functional response of regulatory cells. Overall, these developments result in impaired innate and adaptive immune responses, elicited self-antigen reactivity, a higher susceptibility towards infection and the development of malignant tissue. Overall implications of such senescence phenomena of immunity are that specific health parameters may vary with age, immune impairment eventually leading to increased risk of mortality [13-17].

IMMUNE FUNCTIONING AND DIETARY COMPONENTS

For quite a few dietary components, clear effects on immune functioning have been described. Protein energy malnutrition (PEM), for instance, has a profound effect on immune functioning, in particular in the elderly. The properties of T-cell and B-cell subsets and functions and of innate immunity are clearly linked to protein nutritional status. Immune responses can be restored by intervening in this protein shortage, where patients that suffer from inflammatory processes respond slower. The imbalance between normal macrophage functions and decreased T-cell functions is partly responsible for chronic inflammatory processes in vulnerable patients, because of which acute phase responses are more detrimental to nutritional status and nutrient reserves in elderly patients than in adults [18, 19]. Not only protein status, but also dietary protein-carbohydrate ratio is likely important in maintaining immune responsiveness: a low protein-high carbohydrate ratio for prolonged intervals seemed beneficial for immune status in a rat model [20].

Specific nutritional preparations have been developed, for clinical use in critically ill patients with a jeopardised immune functioning. Usually, such preparations contain at the least specific amino acids such as arginine and glutamine, nucleotides, and specific poly-unsaturated fatty acids, notably ω-3 PUFAs such as docosahexaenoic acid (22:6n-3; DHA) and eicosapentaenoic acid (20:5n-3; EPA) [21, 22]. Next to ω-3-PUFAs there is a clear interest in vitamins, such as A, C, D en E, and minerals as Zn and Se, which have been associated with modulating immune functions. A number of recent reviews and research papers addresses these concepts in detail [21-26]. For a number of other nutrients and products, research is still in a less advanced stage, e.g. at best in the stage of *in vitro* experimentation, animal models, and (limited) human trials. Some issues of research will be dealt with below.

Despite all knowledge and promising results on food and nutrition that can support and maintain immune homeostasis, research into the possible role of functional foods to mitigate (human) immune functioning is still in its infancy. Controversies around food health claims will remain as long as no improvement in e.g. identification of suitable biomarkers, and a more detailed comprehension of individual phenotypic responses in relation to underlying physiological and biochemical mechanisms to nutrients have emerged. In this context, immune modulating functional foods should be addressed as food products with health benefits for specific groups of consumers, for instance those with an unbalanced or skewed immune system or for those in which the immune system needs to be challenged similar to vaccination. As the balance and skewing can be in different directions, the immunomodulatory properties of products should match the specific needs of (groups of) individuals. This implies that products that are of benefit to e.g. allergic consumers do not necessarily have a beneficial effect on other consumers; requirements for neonates likely differ from those of the elderly, and so on. In research and intervention studies and positioning the eventual product, immunomodulatory properties of food products should therefore be approached and tuned with immunological specifications of target groups.

TARGETS AND PREREQUISITES

The objectives of the research area are to develop dietary or food products to support vital and balanced immune functioning in various life stages, for specific groups of consumers. In order to develop these products immunological read-outs are essential because:

1. Many chronic health threats, with enormous impact on quality of life as well as economic impact, is related to immune functioning, such as auto-immune diseases, allergies and asthma, or immune deficiencies [1].
2. The immune system plays a, in some cases (perhaps partial) causal, role in pathology of at the least quite a few chronic and age-related anomalies.
3. Food components can modulate immune responses, both positively as well as negatively (e.g. food allergy), as becomes increasingly evident from randomised double blind intervention studies as well as from clinical trials (see below).

Immune functioning as a paradigm offers advantages that may facilitate the translation from an experimental setting to possible application and implementation, as immune functioning may well serve as a theoretical framework for assessment of health impact. Already for a long time the pivotal role of immunity in health and well-being has been acknowledged resulting in:

- Many available immune parameters, diagnostically validated *in vitro* or *in vivo* test systems
- Many available animal models and cell lines for *in vitro* analysis
- Standard clinical practice and pathology of many chronic conditions in which the role of the immune system is relatively well documented
- An increased interest to link individual immune functionality to life style-and age-related parameters, where particularly food and nutrition show the best documented effects.

Further mechanistic underpinning of health effects of food intake appears a prerequisite to achieve further progress in the development of dietary tools for enhancing health homeostasis. Lack of sufficient scientific basis for such will result in contradictory claims and statements, confusion and loss of trust and confidence amongst consumers. Addressing this lacuna will contribute to a solid basis for food that can enhance maintenance of health and quality of life, including food that can successfully pass food health claims.

RESEARCH INTO IMMUNOMODULATION BY FOOD, WITH A FOCUS ON ALLERGY

Probiotics

According to the currently adopted definition by FAO/WHO, probiotics are: ‘Live microorganisms which, when administered in adequate amounts, confer a health benefit on

the host'. Some years ago, relationships between the composition of intestinal microbiota and the occurrence and incidence of allergic diseases were noted [27-29]. For Estonian and Swedish school children it was demonstrated that allergic children were less often colonised with *Lactobacilli* and more often with aerobic coliforms and *Staphylococcus aureus* [27, 28], or lower *Bifidobacterium*-counts and higher *Clostridium* colonisation numbers were found [29]. This was supposed to be relevant for proper maturation of intestinal immunity, and in particular to have impact on the balance between Th1 and Th2-cells [28, 29]. Such observations led to the hypothesis that nutritional and dietary intervention, via probiotics, were possible for this type of condition.

In a Finnish study, *Lactobacilli* (LGG), were given to 159 atopic pregnant women for 2 weeks and to their babies for 6 months. This resulted in 50% decreased incidence in atopic eczema, at 2 years of age, in infants in the LGG group compared to the placebo group [30]. Similarly, LGG and *Bifidobacterium lactis* Bb12 were shown to decrease severity of atopic eczema [31]. Suggesting a possible mechanism, another study illustrated that the tolerance-related cytokine IL-10 was elevated significantly in serum from patients in the LGG group [32]. Sometimes a combination of probiotics was beneficial in the management of atopic dermatitis [33] but sometimes this was not reproducible elsewhere [34].

In a group of teenagers and young adults, *Lactobacillus rhamnosis* did not lead to reduced birch-pollen allergy symptoms [35]. In addition, dietary LGG-supplementation a few weeks before delivery did not appear to have an effect on proliferation of PBMCs from atopic or control neonates. This suggested that there was no impact on sensitisation potential [36]. It however has to be kept in mind that specific probiotic strains can have different effects [37], that matrix and formulation can have effect on bioactivity of the product, that exposure and intervention may have been to short to reveal an effect and that there is a variation in the width of unbalances in natural skewing and therefore the composition of the test population is relevant. A good standardisation of strains, process and formulation, combined with (*in vitro*) product bioactivity quality control should be addressed before interventions are conducted. A more detailed genotyping and phenotyping practise of biological parameters in the study population is extremely relevant to analyse effects on the sub-group or individual level. This supports the idea that both product and population should be studied in depth before and during intervention studies.

Summarising, results of probiotics in allergy prevention are variable and heavily debated. *In vitro* observations suggest that a possible mechanism for positive effects on allergy-related immunity is the consequence of or leading to the restoration of imbalances between various T-cell subsets, possibly indirectly via TLR signalling in innate immune cells. *In vitro* effects are for certain, and *in vivo* results therefore likely are as well, strongly strain-dependent; *in vitro* screening of probiotics strains resulted in strain-dependent immune-effects, e.g. IL-10 inducing (tolerance enhancing?) or Th1-skewing [37]. Apart from this, effects on gut permeability or intestinal allergen processing cannot be ruled out as yet. In addition, responses may be dependent of individual patient characteristics as well, e.g. whether allergies have already fully developed or not and immune aberrancies are still prone to modulation. Recently, interactions between (inducible) *Lactobacillus*-S-layer protein and gut-DCs were described, which may contribute to the mechanism of action [38], in particular primarily on innate immunity.

The conclusion is that stringent requirements for experimental design are imperative to avoid contradictory data, and to further elaborate this promising area of research.

ß-Glucans

ß-(1→3)-(1→6)-Glucans are cell wall components that occur in a wide variety of organisms, such as bacteria, yeasts, algae, cereals (mainly containing (1→4)-cross-links [39]), fungi and lichens. It concerns polymers of glucose with a ß-(1→3)-backbone and ß-(1→6)-cross-links. Molecular weights are typically in the range of several hundreds of kDa, which indicates a polymerisation degree of several thousands of glucose units. ß-glucans gain particular research interest for their anticipated effects on immunity. A supposed mechanism for this is for instance via binding to cells from the innate immune compartment such as macrophages and to NK-cells, for which also receptors have been described (see below) [40, 41].

A number of studies into the effect of ß-glucans on allergic reactions has been published. The mitigating effect of a ß-glucan from *Aureobasidium pullulans* strain A1 on allergic reactions in ovalbumin-allergic Balb/C mice has been described by Kimura *et al*. [42]. It was found that feeding 0.5-1.0% of a ß-glucan from *Aureobasidium pullulans* strain A1 in the diet of ovalbumin-allergic Balb/C mice considerably reduced ova-specific IgE, and stimulated IL-4, IFN- and IL-12 production in ConA-stimulated splenocytes from the mice.

The ß-glucan (lentinan) from shiitake was evaluated in a human trial with cedar-pollen allergic patients. The ß-glucans, orally applied as superfine dispersed β-1, 3-glucans before symptom onset, exhibited significant alleviation of symptoms of Japanese cedar pollen–induced rhinitis, such as sneezing, nasal congestion, and conjunctivitis [43]. Allergic symptoms were not only relieved for seasonal allergy to cedar pollen but also for perennial allergy. Oral ingestion of ß-(1→3)-glucan in allergic individuals reduced the spontaneous increase in both allergen-specific and total IgE titres. Clinical responses to treatment correlated well with the capacity of monocytes to bind to ß-(1→3)-glucan [43]. Both the route of application (nasal application of ß-glucans resulting in the glucans acting as an allergen, [42]), and the degree of dispersion appeared important, as non-dispersed lentinan did not give the desired effects [43].

Similar allergy-mitigating effects have been observed for crude fungal extracts, such as from *Ganoderma lucidum* (Japanese lacquer mushroom), in a murine (C3H/HeJ) model for peanut allergy [44], and a model for house dust mite allergy [45].

ß-Glucans are able to activate innate pathways, e.g. in macrophages [40, 41] and were found to stimulate the production of TNF-α, IFN-γ and IL-12 when injected into ICR-mice [46]. Similar observations were made for models of human peripheral blood mononuclear cells in which various fungal extracts were screened [47]. A slightly different observation was made for the ß-glucan from *Aureobasidium pullulans*, that was found to particularly induce IL-8 production in PBMCs and in a monocyte cell line [48]. Activated innate immune cells may subsequently activate T-cells, also in PBMC-cultures, thus stimulating Th1-like responses, which is corroborated by the above cited studies. Characteristics of such stimulation may depend on typical characteristics of ß-glucans such as the molecular weight (the *Aureobasidium*-glucan being relatively small, ca. 100 kDa) and degree of branching (50-80%) [42], which may account for different observations. Various receptors for ß-glucans (dectin-1, complement receptor 3, TLR2 and TLR6, scavenger receptors and lactosylceramide) have been identified. Dectin-1 is particularly expressed on immune cells, such as dendritic cells, macrophages, monocytes, neutrophils, eosinophils, some T-cells and in humans also on B-cells. Possible expression of dectin-1 in intestinal cells is under debate

[40]. Interaction of dectin-1 and TLR2 after binding to ß-glucan-type ligands leads to synergistic effects on the production of cytokines such as TNF-α and IL12, a process in which NF-kB [49] and spleen tyrosine kinase are thought to be involved [50]. Dectin-1 preferentially binds to the ß-(1→3)-part of the glucan. Receptor binding studies, in which the Ig-labelled polysaccharide-binding domains of dectin-1 were used to detect glucan-binding, indicated that the minimally required length to allow binding to dectin-1 is 10-11 glucose residues [51]. It is important to realise that these observations were based on a rather abstracted experimental model, and that the possible physiological significance of the ß-(1→6)-branches is as yet not clarified. Binding of ß-glucans to dectin-1 and TLR-2 shows synergistic effects, which perhaps indicates a role for the ß-(1→6)-branched structure [49]. Single-chain ß-glucans appear to induce stronger responses (NF-kB, iNOS-production, TNF-α) in (murine) macrophages compared to oligomeric chains [41]. Increased solubility of ß-glucans enhances their effectiveness, as demonstrated for instance by the impact of sulphated, carboxymethylated, methylated, hydroxyethylated or hydroxypropylated derivatives on Sarcoma-180 tumour cells [52]. The relevance of increased solubility on allergy mitigation is as yet not clear, but the effect of degree of dispersion points in a similar direction [43].

The synthesis, modifications and breakdown of ß-glucans in food crops is a dynamic process. Genotypic variation, growing conditions, developmental stage at harvest, post harvest treatment and processing all can have effects on ß-glucan concentrations, bioavailability and bioactivity [53, 54 and own studies, in preparation]. This illustrates that product quality control, preferably based on bioactivity, should be included when conducting feeding or intervention trials.

In conclusion, there appear to be clear indications, from animal models as well as from a human study, that orally applied ß-glucans or glucan-containing preparations can be of help in relieving food and respiratory allergy-related symptoms. Taking this concept further, it might be promising to analyse possible effects also in other settings in which Th1-stimulation might be relevant, such as stimulation of cell-mediated immunity, vaccine adjuvant development, support of chemotherapy, and improved infection resistance. It appears as if ß-glucans activate, indirectly via innate activation, Th1-mediated immunity, in which TLR/CLR (dectin-)-signalling is involved.

A number of research issues is still open, such as e.g. how and where interactions between ß-glucans and immune cells take place (intestinally via microfold-(M-) cells? Via, into the intestinal lumen perturbing, dendritic cells? Through transport via tight junctions? And/or via para-cellular transport?). Also structure-function studies and research on application and delivery methods, for instance incorporation into food products or supplements, are required to develop this promising research direction.

Fungal Immunomodulatory Proteins (FIPs)

FIPs may be relevant for allergy mitigation, as these proteins were found to be able to inhibit food allergic, as well as respiratory allergic, reactions in mouse models, either applied orally or nasally.

When the FIP from *Flammulina velutipes* (golden needle mushroom; FIP-fve) was fed (at 200 µg/mouse, i.e. ca. 10 $mg.kg^{-1}$, every other day, for details see [55]) to ovalbumin-allergic Balb/c-mice, allergy symptoms, including symptom score, histamine release and intestinal

damage, were strongly suppressed. The Th2-dominant phenotype shifted towards a strongly elevated Th1-response (measured in splenocytes) [55]. This indicates potential application in allergy prophylaxis. Liu *et al*. reported that FIP-fve could be applied in local nasal immune therapy, suppressing allergic responses to house dust mite allergy in Balb/c mice [56]. Also in this study the cytokine profile shifted towards a stronger Th1-response.

FIPs are relatively small, ca. 15 kDa, proteins of fungal origin. FIP-fve, and FIPs from *Volvariella volvacea* ((paddy) straw mushroom; FIP-vvo), *Ganoderma lucidum* and *G. tsugae* (Japanese lacquer mushroom; LZ-8 and FIP-gts, resp.) have been described in some detail. These proteins are composed of resp. 114 (FIP-fve), 110 (LZ-8 and FIP-Gts) and 112 (FIP-vvo) amino acids, and share high sequence homology [57]. FIPs showed lectin-like properties, as they are able to agglutinate erythrocytes, and were proposed to be classified as such [59].

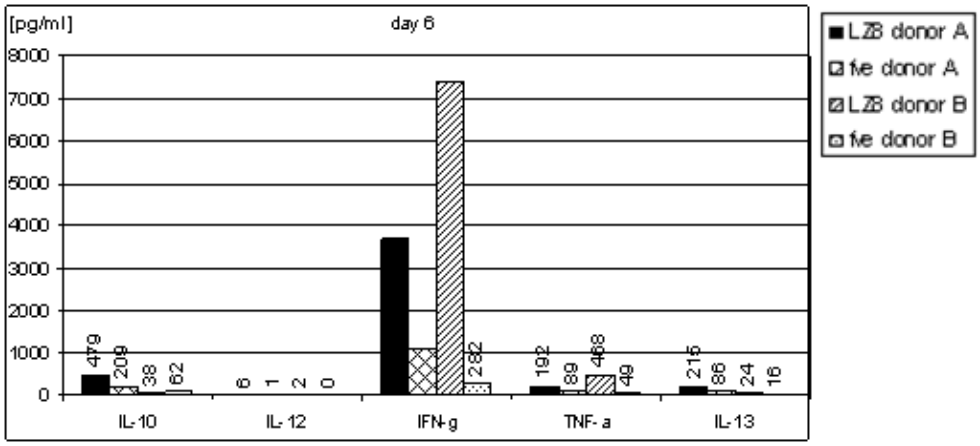

Figure 1. N-terminally His-tagged FIP-LZ8 and FIP-fve were cloned into the pET101/D-TOPO vector, with which E. coli was transfected. PBMC-cultures, prepared as in [62], were cultured in the presence of 25 μg/ml of rec-FIPs, and after 6 days, cytokine production was measured flow cytometrically.

The X-ray structure of FIP-fve indicated this protein to be a homodimer of which each subunit comprises a pair of N-terminal secondary structural elements, an α-helix followed by a β-strand, linked to a domain consisting almost exclusively of β-sheets adopting an Ig-like fold. Percentages of α-helix, ß-strand and loop were resp. 11.3, 42.6 and 46.1 [58, 59]. The α-helices H_A and H_B are amphipathic and the side chains of the amino acids on the hydrophobic face of one helix pack well against those of the other α-helix. This enables binding to each other via hydrophobic interactions, which may be important for immunomodulatory activity (see below). Lin *et al*. [60] predicted that the N-terminal residues 1-13 of FIP-Gts formed an α-helix. Recombinant mutants of FIP-Gts in which residues 1-13 (the N-terminal a-helix) were deleted, were incapable of dimerisation. Triple mutants in which Leu5, Phe7 and Leu9 were deleted no longer possessed the amphipathic character, the ability to form dimers with itself or with the wild type FIP-Gts protein and also lost the ability to induce INF-γ and IL-2 in hPBLs. This suggests that the dimerisation property is important for immunomodulatory activity [59, 60]. With respect to the putative mode of action of FIPs, stimulation of IFN-γ production in human PBMCs via p38-MAPK-activation has been suggested [61]. Own

observations [62, and unpublished results] indicated that natural FIPs or heterologously expressed FIPs (in *E. coli*) from *Flammulina* or *Ganoderma* have a moderate, questionably significant, effect on NO-production in RAW 264.7 cells (a murine monocyte cell line). A Th1-skewing effect in human PBMC-cultures from healthy volunteers could be observed (Figure 1).

In the heterologous expression system, the rec-LZ8 was produced as its monomer of approx. 15 kDa, whereas the FIP from *Flammulina* (rec-FIP-fve) occurred as a dimer of ca. 35 kDa in SDS-PAGE gels under denaturing conditions (Figure 2).

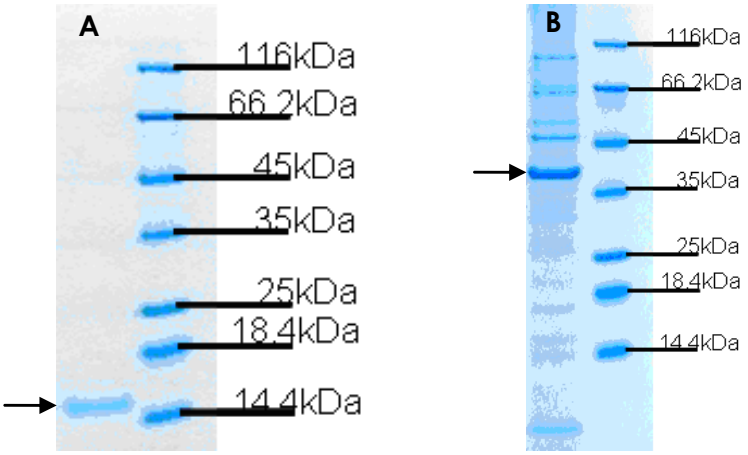

Figure 2.Isolation of FIP-LZ8 (panel A) and FIP-fve (panel B) (arrows) from inclusion bodies that were obtained from transfected E. coli. MW-markers are indicated.

Considering the apparent lower PBMC-stimulatory activity of rec-FIP-fve compared to that of rec-LZ8, it seems as if not only dimerisation is important for activity [60], but also the relative orientation towards each other of the units constituting the homodimer, as the α-helices appear to require a close contact to exert biological activity. The low activity towards NO-production in RAW 264.7 cells suggests that the primary site of action of FIPs may be towards cells from the adaptive immune compartment, particularly Th1-cells as IFN-γ production is most strongly upregulated (Figure 1).

Observations on an immunomodulatory protein from *Auricularia* (Jew's ear) mushroom, a protein that perhaps, but not (yet) confirmed, should also be considered as a FIP, partly corroborated the above findings for the Th1-skewing effect, but not for NO-production in RAW 264.7 cells [63].

FIPs, at the least their biological activities, were found to be relatively resistant towards common food processing conditions, such as freezing, thawing, dehydration, and acid/alkali-conditions [64, 65], which is interesting for their potential application as food or feed additive.

Questions as to their stability and bioactivity when incorporated in products or whether fresh mushroom can have the same effects as purified or recombinantly expressed proteins remain to be answered. Moreover the working mechanism should be further clarified. Recently, Ca^{2+}-transport and PKC-α-activation were proposed to be involved in subcellular activation mechanisms in PBMCs [66]. More animal model and human intervention studies will be required to study its effect *in vivo* and its benefit for specific groups or individuals.

Ω-3 POLYUNSATURATED FATTY ACIDS

A number of studies has used animal models to study the effect of ω-3-PUFAs on allergic reactions. Yin *et al*. [67] studied the effects of fish oil supplementation on airway inflammation, in a murine model of allergic inflammation produced by sensitization to ovalbumin. ω-3 Fatty acids were dose dependently built-in into mouse lung tissue after dietary supplementation. Oxidative stress was measured using isoprostane (IsoPs) levels (isomers of cyclooxygenase (COX)-derived prostaglandins). OVA challenge caused a significant increase of F2-IsoPs (from AA) in mouse lung, which suggests an increased level of oxidative stress. The fish oil supplementation group showed a significant decrease of F2-IsoPs and an increase of F3-IsoPs (from EPA) and of F4-IsoPs (from DHA). However, fish oil supplementation led to an increase in the production of proinflammatory cytokine IL-5 and IL-13. Furthermore, fish oil supplementation suppressed the production of PGE2 in the bronchoalveolar lavage [66].

Korotkova *et al*. [68] examined whether maternal intake of dietary fatty acids influences the induction of oral tolerance to OVA in neonatal rats. The results indicate that the dietary ratio of ω-6/ω-3 PUFA is important for the induction of neonatal oral tolerance. It was concluded that non-optimal feeding can have effects on the development of immunological tolerance to dietary antigen ingested by the mother, and that the ratio of ω-6/ω-3 FA in the diet may be relevant in the context of increased prevalence of allergy.

In human clinical trials, supplementing n-3 fatty FA to adults, with recognized allergies and bronchial asthma have so far been disappointing. This might indicate that once allergic immune responses are established, intervention is no longer able to mitigate these [69].

Woods *et al*. conducted a study to assess the influence of food and nutrient intake on asthma risk in young adults. Dietary intake was assessed by means of a validated semi quantitative food-frequency questionnaire. However, no association between fish intake and asthma was found [70].

Schnappinger *et al*. [71] conducted a study to assess the influence of fish consumption on allergic sensitisation and allergic diseases in adults. Allergic sensitisation was described as the presence of elevated serum levels of allergen-specific IgE antibodies, and is proposed to be fundamental to allergic diseases and is therefore considered a potential risk factor for the development of allergic diseases. The results of the study showed that fish and DHA intake are inversely related to allergic sensitisation in adult females, but not in males. No significant association was found between fish and DHA intake and allergic diseases. The gender-related differences in metabolism of PUFAs might be a possible explanation for the fact that a lower rate of allergic sensitisation was only found in women [72].

The immature immune system is still very susceptible to immunomodulatory environmental conditions, particularly in the pre- and postnatal period [73]. Therefore supplementation with anti-inflammatory ω-3 PUFAs during the pre-natal period might provide a non-invasive intervention window to modify immune development before any disease is established [69]. Dunstan et al. [69] performed a randomized double-blind controlled trial to determine whether maternal dietary supplementation with ω-3 PUFAs during pregnancy could alter the immune response in infants. Allergen-specific T-cell responses in cord-blood between fish-oil supplemented and control groups were compared. The fish oil supplementation group showed a significantly higher proportion of total ω-3

PUFAs in neonatal erythrocyte membranes as compared with the control group. At birth babies in the fish oil supplementation group generally had weaker cytokine (IL-5, IL-13, Il-10 and IFN-γ) responses to allergens (house dust mite, ovalbumin, and cat) and mitogens (phytohaemagglutinin) although this was not statistically significant [69].

Kull *et al*. [74] investigated the association between fish consumption during the first year of life and development of allergic diseases by four years of age. Data were obtained from a prospective birth cohort controlling for disease-related modification of exposure. Data on allergic heredity and various exposures were obtained when the infants were newborn by parental questionnaires. Subsequently information on diet, including consumption and time for introduction of fish was collected at 1 year of age. It was concluded that regular fish consumption before the age of 1 appears to be associated with a decreased risk of allergic disease and sensitization to food and inhalant allergens during the first four years of life.

In conclusion, there are some indications that ω-3-PUFA consumption has impacts on the development of allergy symptoms. Although important, it is not so easy to evaluate e.g. levels of ω-3-PUFA consumption in order to show some effect in the mitigation of rheumatic symptoms (3.5-4 g/day) [75], as exposure levels are not always reported unequivocally. A variety of mechanisms for the possible action of ω-3-PUFA on immune functioning has been proposed; for a review, see Kim *et al* [76].

PERSPECTIVES

The option of dietary immunomodulation appears to be feasible and realistic , at the least for the relatively well-defined immune responses such as in allergies. Considering the pivotal role of immune homeostasis in general health, and the very relevant and significant societal and economic perspectives, further pursuing this research avenue seems worthwhile. Some food components appear to exhibit effects on specific immune compartments This offers potential use in a variety of applications, depending on the specifications of underlying chronic processes and characteristics of consumers. Foods can thus play a significant role in fortifying and balancing immune responses.

As it becomes more and more clear that the aetiology and course of a given ‘disease’ various from person to person, the conclusion appears obvious that also treatments, or supporting dietary measures, should be matched with individual needs. This means that, for immunomodulatory foods, uniform approaches will not be maximally effective. This emphasises the need for more knowledge of individual consumers’ responses, and the metabolic parameters that are connected to first signs of derailed homeostasis in order to re-establish this via food based intervention. Together with in-depth knowledge on underlying mechanisms it will pave the way for product development in the near future.

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