



DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

Mouse Chow Composition Influences Immune Responses and Food Allergy Development in a Mouse Model

verfasst von / submitted by

Eleonore Weidmann

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of
Magistra der Pharmazie (Mag.pharm.)

Wien, 2020 / Vienna, 2020

Studienkennzahl lt. Studienblatt /
degree programme code as it appears on
the student record sheet:

A 449

Studienrichtung lt. Studienblatt /
degree programme as it appears on
the student record sheet:

Diplomstudium Pharmazie

Betreut von / Supervisor:

Assoz. Prof. Priv.-Doz. DDr. Eva Untersmayr-Elsenhuber

Danksagung

Zuallererst möchte ich mich bei meiner Betreuerin Prof. DDr. Eva Untersmayr-Elsenhuber bedanken, die es mir ermöglicht hat, nicht nur meine Diplomarbeit unter ihrer Anleitung zu machen, sondern auch ein Paper zu veröffentlichen.

Danke an Nazanin Samadi und Martina Klems, die mich eingeschult und mich mit ihrem Rat zu einer ganz passablen Mitarbeiterin gemacht haben. Danke für eure Ohren, die ihr mir immer wieder geliehen habt, den Spaß und die gute Zusammenarbeit.

Danke an alle KollegInnen, die gekommen und gegangen sind und einen wesentlichen Beitrag zu meiner Diplomarbeit und zur Erhaltung meines Verstandes und meiner Laune geliefert haben.

Danke an alle meine Freunde, die mir - jeder auf seine Art - meine gesamte Studienzeit verschönert haben und auch nicht so schöne Momente erträglich gemacht haben.

Danke an Florian Mayer, der mich im langen langen Endspurt ausgehalten, unterhalten, beruhigt, bekocht, mich aus irrationalen Sorgen geholt und mein Selbstvertrauen gestärkt hat.

Und das größte „Danke“ geht an meine Eltern, die sich mit mir gefreut und mit mir gelitten haben, die mich durch meine Studienzeit finanziell sowie seelisch getragen und unterstützt haben und die wesentlich geholfen haben, den (Alb-)Traum Pharmazie wahr werden zu lassen.

Inhaltsverzeichnis

1. Einleitung	6
2. Beitrag des Autors der Diplomarbeit zur Publikation	9
3. Publikation: Mouse Chow Composition Influences Immune Responses and Food Allergy Development in a Mouse Model	10
3.1 Introduction.....	11
3.2 Materials and Methods	13
3.2.1. Animals and Diet.....	13
3.2.2 Immunization with OVA	15
3.2.3 Oral Challenge with OVA.....	16
3.2.4 Measurement of OVA-Specific Antibody Levels	16
3.2.5 Splenocyte Stimulation and Cytokine Read-Out.....	17
3.2.6 Statistic and Data Analysis	18
3.3 Results.....	19
3.3.1 Mouse Feed Influences Systemic and Local Intestinal Antibody Production	19
3.3.2 Cytokine Production Reveal a Tolerogenic Milieu Based in Mouse Feed.....	20
3.3.3 Temperature Changes after Oral OVA Challenge Indicate Food Allergy Development	21
3.4 Discussion	23
3.5 Conclusions	26
3.6 References	27
4. Abstracts	32
4.1 Deutsch	32
4.2 Englisch.....	32
5. Zusammenfassung	33
6. Referenzen	35

1. Einleitung

Nahrung ist fester Bestandteil unseres Lebens. Ohne Nahrung können wir nicht überleben. Jedoch gibt es Situationen, in denen uns Nahrungsbestandteile nicht nur stärken und gesund erhalten, sondern uns auch schädigen, wenn beispielsweise eine Nahrungsmittelallergie besteht. Bei einer Nahrungsmittelallergie spricht man meistens von einer durch Antikörper vom Typ Immunglobulin E (IgE) ausgelösten Überempfindlichkeit gegen ein Allergen, die mit der Freisetzung von Mediatoren wie Histamin aus Mastzellen und andere Entzündungszellen einhergeht. Das ist die sogenannte Sofortreaktion, die schon nach wenigen Minuten bis Stunden eintreten kann und sich in Schwellung und Rötung von Haut und Schleimhäuten, tränenden Augen, Durchfall, Ausschlag, Juckreiz und Asthmaanfällen mit Atemnot zeigen kann und im schlimmsten Fall mit Atem- und Kreislaufstillstand enden kann. Bei der Spätreaktion – die erst nach Stunden bis Tagen eintritt – ist die Aktivierung von Entzündungszellen wie Makrophagen, basophilen, eosinophilen und neutrophilen Granulozyten sowie T-Zellen entscheidend, die auch zu chronischen Entzündungen im Gastrointestinaltrakt führen können.

Allergene sind Antigene, die sich chemisch nicht auf eine Struktur vereinheitlichen lassen, meistens sind es jedoch Glykoproteine. Zu den häufigsten gehören glutenhaltiges Getreide, Krebstiere, Eier, Fische, Sojabohnen, Milch, Sellerie, Erdnüsse und Schalenfrüchte, welche alle seit 2011 von der Europäischen Union auf Lebensmitteln und seit 2014 auch auf unverpackten Lebensmitteln verpflichtend gekennzeichnet werden müssen, auch wenn sie nur in geringen Mengen enthalten sind.

Eines der in Hühnerei enthaltenen Hauptallergene ist Ovalbumin, ein Glykoprotein, das gerne als Modellallergen in der Forschung verwendet wird und auch im Rahmen dieser Diplomarbeit eingesetzt wurde.

Um Mäuse allergisch gegenüber Ovalbumin zu machen, gibt es verschiedene Möglichkeiten. Oft werden Cholera-Toxine oder Staphylococcus-Enterotoxine eingesetzt. [1] Im Zuge dieser Diplomarbeit wurden Magensäuresekretionshemmer eingesetzt, die in unserer Arbeitsgruppe nach einem lang bewährten Protokoll verwendet werden. [2] Der niedrige pH-Wert im Magen ist entscheidend für die Funktion von gastrischen und pankreatischen Proteasen, die verdauungslabile Proteine in kurze Peptide und Peptone zerlegen, die vom Immunsystem nicht mehr als Antigene erkannt werden können. Weiters hilft der saure pH-Wert gegenüber intestinalen Infektionen und schützt die Mukosa vor Autodigestion. [3] Durch Hemmung der Magensäuresekretion steigt der pH-Wert an, Proteasen können nicht aktiviert werden, um Proteine zu zerlegen und diese werden vom Immunsystem als Antigene erkannt.

Warum immer mehr Menschen, vor allem Kinder, auf eigentlich harmlose Nahrungsmittel reagieren, ist noch immer nicht im Detail geklärt, eine Rolle spielen auf jeden Fall Genetik, Epigenetik und Geschlecht. Eine weitere Hypothese, warum es zu Allergien kommt, ist die Hygiene-Hypothese. Diese besagt, dass Kinder, deren Immunsystem von klein auf vielfältigen mikrobiellen Antigenen ausgesetzt wird, mit geringerer Wahrscheinlichkeit Allergien entwickeln, da ihr Immunsystem viele verschiedene Faktoren kennen lernt und somit harmlose Strukturen – sprich Allergene – als nicht gefährlich erkennt. [4] Sei es durch Haustiere, ältere Geschwister oder dem Spielen in der Natur - die individuelle Darmflora jedes Menschen kann sich so möglichst umfangreich entfalten und bietet die Grundlage für ein ausgereiftes mukosales Immunsystem. [1] Durch übertriebene Hygiene nehmen wir unserem Immunsystem die Chance, mit möglichst vielen Mikroorganismen in Berührung zu kommen.

Die bisher einzige Methode eine Reaktion bei bestehender Nahrungsmittelallergie zu vermeiden, ist das strikte Weglassen des Allergens aus der Ernährung durch mühsames Studieren von Lebensmitteletiketten und der Aneignung eines umfangreichen Wissens über Vorkommen des betreffenden Allergens. Für den Notfall sollte jeder Allergiker, der zu schweren Reaktionen neigt, auch ein Antihistaminikum, ein Glukokortikoid und ein Adrenalinpräparat mit sich führen, um im Notfall selbst rasch eingreifen zu können und die Entwicklung eines anaphylaktischen Schocks zu vermeiden. Diese Maßnahmen sind jedoch oft sehr einschränkend für den Allergiker und sein soziales Umfeld und führen zu einer stark reduzierten Lebensqualität.

Um dies zu vermeiden, arbeitet man an spezifischen Immuntherapien, um bei bestehender Allergie eine anhaltende Unempfindlichkeit zu erreichen. Bei der oralen Immuntherapie beginnt man mit einer äußerst geringen Dosis des Allergens, auf die der Körper noch mit keiner allergischen Reaktion antwortet, um danach die Dosis schrittweise in den ersten Wochen zu steigern. Nachteile dieser Methode sind die lange Dauer der Therapie (Jahre!) und die häufig vorkommenden lokalen und systemischen Nebenwirkungen, wie schwere gastrointestinale Beschwerden. [1,5] Weniger Nebenwirkungen werden bei der epikutanen Immuntherapie (von einem speziellen Pflaster wird das Allergen durch die Epidermis aufgenommen) beobachtet, da die systemische Route umgangen wird. Diese Methode ist vielversprechend, doch am Menschen noch nicht ausführlich genug erforscht, um kommerziell zugänglich zu sein. [5,6]

Das Ziel wäre natürlich, eine Allergieentwicklung generell zu verhindern. Mit diesem Thema hat sich unter anderem die große Learning Early About Peanut Screening Studie (LEAP) befasst und bestätigen können, dass die Einführung von Allergenen – in diesem Fall Erdnüsse – in die Ernährung von hochgradig allergiegefährdeten und ekzematösen Kleinkin-

dern im Alter von 4 bis 10 Monaten zu einer verringerten Prävalenz von Erdnussallergie geführt hat. [7,8] Weiters konnte dieselbe Arbeitsgruppe mit denselben Probanden über 5 Jahre hinaus in der Persistence of Oral Tolerance to Peanut Studie (LEAP-ON) beweisen, dass ein Jahr Allergenvermeidung nach 4 Jahren Allergenkontakt keine Allergieentwicklung hervorruft. [9] Ein ähnlicher Studienaufbau findet sich in der Enquiring About Tolerance Studie (EAT), die mehrere Allergene gleichzeitig bei ausschließlich gestillten und nicht allergievorbelasteten Kleinkindern verabreicht hat. Die Ergebnisse dieser Studie zeigten jedoch, dass die frühe Einführung in die Ernährung keinen Einfluss auf eine Allergievermeidung hat, was in dieser Studie wahrscheinlich dosisabhängig war. [10,11] Generell gehen ÄrztInnen und DiätologInnen aber weg von der Vermeidung und hin zu einer frühen Einführung von Allergenen in die Ernährung eines Kleinkindes. [12]

Diese Arbeit befasst sich mit dem Zusammenspiel von Ernährung und dem Immunsystem. Es werden bestimmte Zusammensetzungen unserer Nahrung diskutiert, die sich positiv auf unser Immunsystem aus- und einer Allergieentwicklung entgegenwirken. Dazu wurde ein Mausmodell durchgeführt, bei dem zwei verschiedene Mäusefutter – die sich in Makro- und Mikronährstoffen, Vitamin- und Lipidgehalt unterscheiden – bei gleichzeitiger Magensäuresuppression verglichen wurden, um deren Auswirkung auf die Entwicklung einer Allergie zu untersuchen.

2. Beitrag des Autors der Diplomarbeit zur Publikation

Die Publikation "Mouse Chow Composition Influences Immune Responses and Food Allergy Development in a Mouse Model" wurde im Journal "Nutrients" im Zuge der Sonderausgabe "Contributions of Diet and Gastrointestinal Digestion to Food Allergy" veröffentlicht. Die Rohfassung der Publikation sowie alle Grafiken wurden unter Anleitung von Assoz. Prof. DDr. Eva Untersmayr-Eisenhuber von mir selbst verfasst und erstellt.

Unter Aufsicht und Anleitung durch die Arbeitsgruppenleitung war ich in die Immunisierungs- sowie Abschluss-Experimente eines Teils der in der Publikation zusammengefassten Daten involviert. Die in der Publikation zusammengefassten, in Laborexperimenten erhobenen Daten wurden (mit Ausnahme eines Teils der Zytokin-ELISAs) von mir durchgeführt und zur Gänze statistisch ausgewertet.

3. Publikation: Mouse Chow Composition Influences Immune Responses and Food Allergy Development in a Mouse Model

Eleonore Weidmann ¹, Nazanin Samadi ¹, Martina Klems ¹, Denise Heiden ¹, Klara Seppova ¹, Davide Ret ^{1,2} and Eva Untersmayr ^{1,*}

¹ Institute of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, 1090 Vienna, Austria

² Division of Macromolecular Chemistry, Institute of Applied Synthetic Chemistry, Vienna University of Technology, 1060 Vienna, Austria

* Correspondence: eva.untersmayr@meduniwien.ac.at; Tel.: +43-1-40400-51210

Received: 28 September 2018; Accepted: 7 November 2018; Published: 13 November 2018

Abstract: Our diet is known to substantially influence the immune response not only by support of mucosal barriers but also via direct impact on immune cells. Thus, it was of great interest to compare the immunological effect of two mouse chows with substantial differences regarding micro-, macronutrient, lipid and vitamin content on the food allergic response in our previously established mouse model. As the two mouse chows of interest, we used a soy containing feed with lower fatty acid (FA) amount (soy-containing feed) and compared it to a soy free mouse chow (soy-free feed) in an established protocol of oral immunizations with Ovalbumin (OVA) under gastric acid suppression. In the animals receiving soy-containing feed, OVA-specific IgE, IgG1, IgG2a antibody levels were significantly elevated and food allergy was evidenced by a drop of body temperature after oral immunizations. In contrast, mice on soy-free diet had significantly higher levels of IL-10 and were protected from food allergy development. In conclusion, soy-containing feed was auxiliary during sensitizations, while soy-free feed supported oral tolerance development and food allergy prevention.

Keywords: food allergy; diet; mouse chow; experimental mouse model; oral immunizations; polyunsaturated fatty acids; vitamin D; soy; linseed oil

3.1 Introduction

It is well established that composition of the diet plays a paramount role in establishing an adequate immune response in humans with impact on allergic diseases [1]. To give some examples, essential fatty acids (FA) such as polyunsaturated FAs are vital for an adequate body function but have to be incorporated with food, like linseed oil or fish due to the fact that mammals lack the double bonds introducing enzymes [2,3]. Having the capacity to regulate inflammation, polyunsaturated FA metabolites derive either from omega-6 (*n*-6) or omega-3 (*n*-3) FAs. While *n*-6 polyunsaturated FAs such as arachidonic acid, linoleic acid or γ -linoleic acid give rise to pro-inflammatory eicosanoid mediators such as prostaglandins, leukotrienes and lipoxins, *n*-3 polyunsaturated FAs such as α -linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are described to be associated with immunomodulation [4–7]. To give an example, EPA, a metabolite of α -linolenic acid is a precursor of 17-Hydroxyeicosapentaenoic acid (series E resolvin precursor) [8] or 17,18-epoxyeicosatetraenoic acid, which was repeatedly reported to possess anti-inflammatory and anti-allergic properties [9,10]. Recent studies show that especially the ratio of *n*-3 to *n*-6 polyunsaturated FAs is of great importance with regards to allergy development or allergy prevention. A higher amount of *n*-3 versus a lower amount of *n*-6 polyunsaturated FAs seems to be the ideal ratio to ensure a balanced healthy immune response [4,11,12]. However, within the “Western” high fat diet the content of *n*-6 polyunsaturated FAs is much higher compared to *n*-3 polyunsaturated FAs. This type of diet was recently revealed to contribute to allergy and asthma development in a large meta-analysis [13].

Other essential dietary components influencing the immune response are vitamins. Taking vitamin D as an example, controversial findings regarding impact on allergy development have been published. Poole and colleagues studied the epigenetic, genetic and cellular modulations of vitamin D concluding that numerous mechanisms might contribute to the positive impact of Vitamin D on allergy prevention [14–17]. In contrast, high levels of vitamin D by extensive maternal supplementation during pregnancy, birth, lactation or due to supplementation in early childhood was reported to be associated with enhanced allergen sensitizations [18–20]. Thus, there seems to be an U-shaped association between 25-hydroxyvitamin D (the active component of vitamin D) and IgE levels with either too low or too high levels of vitamin D being associated with allergy susceptibility [14,21,22].

With increasing knowledge regarding the influence of dietary components such as lipids, vitamins and other micronutrients on the appearance of inflammatory processes and the potential subsequent allergic development, control of immune-active dietary components influencing disease outcome is also essential for experimental models investigating mechanisms of type 2 immune responses [23]. Thus, food associated immune changes might be a crucial bias impeding comparability and reproducibility of results generated from experimental mod-

els. When aiming at establishing an oral sensitization protocol mimicking disease in food allergic patients, it is essential to prevent pre-exposure to the food protein of interest or any potentially cross-reactive proteins, as allergy induction might be impaired due to prior oral tolerance induction [24]. However, adequate diet control was reported to be not only essential in the animals involved in the experiments but also in parental generations or while suckling [24,25]. Besides control of food allergen exposure, there is a long list of dietary factors that can possibly change allergen-specific immune responses. Thus, in our present study we aimed to analyse the impact of two regular mouse chows used in animal housing for experimental research with different nutritional composition to evaluate the influence on the immune response in a previously established model of food allergy [26].

3.2 Materials and Methods

3.2.1. *Animals and Diet*

32 female Balb/c mice (Core Unit for Biomedical Research, Division for Laboratory Animal Science and Genetics, Himberg, Austria) were randomly divided in 4 groups of 8 and were kept under conventional condition (12 h light/dark cycle at 22 °C). 2 out of the 4 groups (a and n) were fed with LasQCdiet[®]Rod16-A (Soest, Germany), composed of a higher amount of long-chain polyunsaturated FAs due to the added linseed oil and lack of soybean product (soy-free feed; Table 1). The other 2 groups (A and N) were fed with ssniff fortified V1534-300 (Soest, Germany) consisting of a lower percentage of long-chain polyunsaturated FAs and soybean products (soy-containing feed; Table 1). All groups had unlimited access to water and food.

Animals were treated according to European Union guidelines of animal care and the protocol has been approved by the local Ethics committee of the Medical University of Vienna and by the Austrian Federal Ministry of Science and Research with the approval number BMWFV-66.009/0182-WF/V/3b/2017.

Our model allergen of interest was Ovalbumin (OVA; Albumin from chicken egg white, Sigma Aldrich, Vienna, Austria, #A5503). As a control the mice were kept naive. For all treatments OVA was freshly dissolved in water to gain requested concentrations.

Table 1. Dietary composition of different mouse chow according to manufacturers.

Soy-Containing Feed (Ssniff Rat/Mouse Maintenance Fortified V1534-300)	Soy-Free Feed (Lasvendi LasQCdiet Rod16-A)
Dietary Ingredients (in Descending Amount)	Dietary Ingredients (in Descending Amount)
Wheat soybean products barley minerals oat hulls molasses vitamins & trace elements L-lysine HCl DL-methionine	Wheat corn gluten oats corn barley linseed oil molasses brewer's yeast minerals vitamins/trace elements-mix
Energy per kg	Energy per kg
Gross Energy (GE) 16.2 MJ Metabolizable Energy (ME) 13.5 MJ	Gross Energy (GE) 15.9 MJ Metabolizable Energy (ME) 13.1 MJ calc. formula §14 FMVO
Minerals [%]	Minerals [%]
Calcium 1.0 Phosphorus 0.7 Sodium 0.24 Magnesium 0.22 Potassium 0.92 Ca/P = 1.43: 1	Calcium 1.0 Phosphorus 0.65 Sodium 0.3 Magnesium 0.25
Amino acids [%]	Amino acids [%]
Arginine 1.19 Cysteine 0.35 Histidine 0.49 Isoleucine 0.79 Leucine 1.39 Lysine 1.1 Methionine 0.38 Phenylalanine 0.89 Threonine 0.72 Tryptophan 0.25 Alanine 0.87 Aspartic acid 1.84 Glutamic acid 4.22 Glycine 0.89 Proline 1.31 Serine 1.01 Valine 0.92 Met+Cys 0.73 Phe+Tyr 1.5	Arginine 0.8 Cysteine 0.35 Histidine 0.4 Isoleucine 0.65 Leucine 1.7 Lysine 0.9 Methionine 0.45 Phenylalanine 0.85 Threonine 0.6 Tryptophan 0.2 Tyrosine 0.6

Vitamins per kg	Vitamins per kg
Vitamin A 25,000 IU	Vitamin A 25,000 IU
Vitamin D ₃ 1500 IU	Vitamin D ₃ 1800 IU
Vitamin E 135 mg	Vitamin E 120 mg
Vitamin K (as MNB) 20 mg	Vitamin K 80 mg
Thiamine (B1) 86 mg	Thiamine (B1) 100 mg
Riboflavin (B2) 32 mg	Riboflavin (B2) 30 mg
Pyridoxine (B6) 31 mg	Pyridoxine (B6) 25 mg
Cobalamin (B12) 150 µg	Cobalamin (B12) 120 mg
Biotin 710 µg	Biotin 400 µg
Choline 1370 mg	Choline 1500 mg
Folate 10 mg	Folate 6 mg
Pantothenate 59 mg	Pantothenate 35 mg
Niacin 153 mg	Niacin 80 mg
Trace Elements per kg	Trace Elements per kg
Copper 15 mg	Copper 15 mg
Iodine 2.1 mg	Iodine 4 mg
Iron 186 mg	Iron 200 mg
Manganese 68 mg	Manganese 120 mg
Selenium 0.3 mg	Selenium 0.2 mg
Zinc 91 mg	Zinc 75 mg
	Cobalt 1.5 mg
Fatty Acids [%]	Fatty Acids [%]
C 14:0 0.01	
C 16:0 0.45	C 16:0 0.5
C 18:0 0.09	C 18:0 0.2
C 20:0 0.01	C 20:0 0.01
C 16:1 0.01	C 18:1 0.9
C 18:1 0.62	C 18:2 1.9
C 18:2 1.76	C 18:3 0.75
C 18:3 0.23	

¹ Physiological fuel value (Atwater), corresponds to 3230 kcal/kg. Abbreviations: L = levo; HCl = hydrochloride; DL = dextrolevo; FMVO = Futtermittelverordnung (animal feed regulations).

3.2.2 Immunization with OVA

On the first day of an immunization cycle each mouse of groups a and A received an intravenous injection of 116 µg proton pump inhibitor (PPI, Omep 40 mg[®], Hexal AG, Holzkirchen, Germany) diluted in 100 µL 0.9% sterile sodium chloride solution. On the second and third day the mice were treated with another intravenous injection followed by an intramuscular injection one hour later with the same solution. 15 min after the intramuscular injection each mouse was fed with 0.2 mg OVA in combination with 2 mg sucralfate (Sucralan[®], Gerot-Lannach, Vienna, Austria) in 100 µL of distilled water. 2 Groups (a and A) were exposed to the OVA immunization, 2 groups (n and N) stayed naive as negative controls (Table 2). The immunizations took place on the first three days of a two-week cycle. After 6 rounds of immunization mice were orally challenged and sacrificed.

Table 2. General overview.

Group	Sensitization	Oral Challenge	Number of Mice	Mouse Chow
a	200 µg OVA oral + acid suppression	2 mg/mL OVA oral	8	soy-free feed
n	naive	2 mg/mL OVA oral	8	soy-free feed
A	200 µg OVA oral + acid suppression	2 mg/mL OVA oral	8	soy-containing feed
N	naive	2 mg/mL OVA oral	8	soy-containing feed

Abbreviations: OVA = Ovalbumin.

3.2.3 Oral Challenge with OVA

All 32 mice were orally challenged with 2 mg OVA in 100 µL double distilled water per mouse to provoke a systemic allergic reaction before sacrifice. Temperature was measured before as well as 10 min, 30 min and 1 h after oral gavage. The temperature was measured either with a rectal thermometer (Thermalert TH-5, Physitemp, Clifton, NJ, USA) or with a wireless temperature reader (DAS-7007S, PLEXX, Elst, Netherlands).

3.2.4 Measurement of OVA-Specific Antibody Levels

Blood was collected by heart puncture on sacrifice day to perform an enzyme-linked immunosorbent assay (ELISA) for evaluation of OVA-specific IgE, IgG1 and IgG2a levels. Purified mouse IgE Isotype control (0.1 µg/mL, BD Biosciences, Franklin Lakes, NJ, USA) and purified mouse IgG1 and IgG2a (0.4 µg/mL, SouthernBiotech, Birmingham, AL, USA) in 0.1 M sodium carbonate buffer, pH 9.6 (coating buffer) were used to prepare serial dilutions as standard curves. 96-well plates (Thermo Fisher Scientific, Waltham, MA, USA) were coated with 10 µg/mL OVA antigen in coating buffer and stored overnight at 4 °C. Plates were blocked with 1% dry milk powder (DMP) dissolved in tris-buffered saline containing 0.05% Tween (TBS-T) for 2 h at room temperature (RT). After blocking, samples were diluted in 0.1% DMP/TBS-T (1:20 dilution for IgE, 1:200 dilution for IgG1 and IgG2a) and left for soaking overnight at 4 °C. On the third day purified Rat Anti-Mouse IgE, IgG1 and IgG2a (1:500 dilution for all antibodies, BD Pharmingen, Heidelberg, Germany) in 0.1% DMP/TBS-T were added and left for 2 h at RT for detection of the OVA bound antibodies. ECLTM anti-rat IgE horseradish peroxidase linked with whole antibody from goat (1:1000, GE Healthcare, Little Chalfont, UK) followed as an enzyme substrate.

For detection of total and OVA-specific IgA intestinal levels, intestines were harvested after sacrifice and rinsed with 2 mL of extraction buffer (complete Mini, Roche, Basel, Switzerland). The intestinal content was extracted (4 h at 4 °C), centrifuged (5 min at 10 621g-

force) and supernatants were collected and stored at $-20\text{ }^{\circ}\text{C}$ until further use. For total IgA detection, plates were coated with rat anti-mouse IgA ($2\text{ }\mu\text{g/mL}$, $100\text{ }\mu\text{L/well}$, BD Biosciences) diluted in TBS overnight at $4\text{ }^{\circ}\text{C}$. TBS-T with 1% bovine serum albumin (BSA) was used for blocking (2 h at RT). Samples were diluted (1:1000 in 0.1% BSA/TBS-T) and the standard curve was prepared with purified mouse IgA isotype control ($1.6\text{ }\mu\text{g/mL}$ in 0.1% BSA/TBS-T, BD Biosciences). Samples and standards were added to the wells and incubated for 30 min at RT. As detection antibody, biotin anti-mouse IgA ($2\text{ }\mu\text{g/mL}$ in 0.1% BSA/TBS-T, 2 h at RT, BD Biosciences) was used and streptavidin horseradish peroxidase (1:5000 in 0.1% BSA/TBS-T, 1 h at RT, Vector Laboratories, Burlingame, CA, USA) as an enzyme substrate. For detection of OVA-specific IgA antibodies, plates were coated with OVA in coating buffer ($10\text{ }\mu\text{g/mL}$) as well as purified mouse IgA isotype control (BD Biosciences) for the standard curve (serial dilution from $0.1\text{ }\mu\text{g/mL}$) overnight at $4\text{ }^{\circ}\text{C}$. After blocking (2 h at RT), undiluted samples ($100\text{ }\mu\text{L/well}$) were added to incubate overnight at $4\text{ }^{\circ}\text{C}$. Biotin anti-mouse IgA (1:250 in 0.1% BSA/TBS-T, BD Biosciences) was used as a detection antibody (2 h, RT) and streptavidin horseradish peroxidase (1:5000 in 1% BSA/TBS-T, 1 h at RT, Vector Laboratories) as an enzyme substrate afterwards.

For all ELISAs, the plates were washed manually at least 3 times with TBS-T between incubation steps. Tetramethylbenzidine (BD OptEIA TMB Substrate Reagent Set; BD Bioscience) was used as substrate and reaction was stopped with $1.8\text{ mol/L H}_2\text{SO}_4$ 15 min at the latest. Read-out was made in the range of 450–630 nm using Infinite M200 Microplate reader (Tecan, Männedorf, Switzerland).

3.2.5 Splenocyte Stimulation and Cytokine Read-Out

During sacrifice, spleen was removed and further processed under sterile conditions. Pre-lysis medium (RPMI medium 1640 with 1% Penicillin-Streptomycin (PenStrep) and 1% Glutamine (gibco, Thermo Fisher Scientific)) was added, centrifuged and the supernatant was removed. In total 5 mL of lysis buffer (ACK Lysing Buffer, Lonza, Gampel, Switzerland) was added and lysis was stopped with 5 mL complete medium (RPMI medium 1640 with 1% PenStrep, 1% L-glutamine and 10% foetal bovine serum (FBS)) after 5 min. Splenocytes were washed 2 times with 5 mL of complete medium, filtered and kept on ice until further use. After cell counting and adjusting splenocyte concentrations to 4×10^6 cells/mL, cells were incubated with 3 stimulants: medium as a negative control, Concanavalin A (ConA) as a positive control and OVA (all $5\text{ }\mu\text{g/mL}$). After a 72 h incubation period, stimulated splenocytes were centrifuged, supernatants collected and stored at $-20\text{ }^{\circ}\text{C}$ for later detection of cytokines.

For cytokine detection ready-to-use ELISA kits were utilized. Mouse IL-4 ELISA Ready-Set-Go! by eBioscience was deployed for detecting Interleukin 4 (IL-4) level and Mouse IL-10

Uncoated ELISA Kit by Invitrogen by Thermo Fisher Scientific for Interleukin 10 (IL-10) level. Instructions included were followed to achieve results.

3.2.6 Statistic and Data Analysis

All data was statistically analysed with GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, California, USA) [27]. The samples were tested for Gaussian distribution by Kolmogorov-Smirnov normality test. Groups among themselves were compared with Kruskal-Wallis non-parametric test and Dunn's multiple comparison test as a post-hoc test.

3.3 Results

3.3.1 Mouse Feed Influences Systemic and Local Intestinal Antibody Production

Out of the 32 mice, two groups (each $n = 8$ mice) (a and n) were fed with soy-free feed whereas the two groups of A and N were fed with soy-containing feed. Groups a and A were immunized with OVA under gastric acid suppression for 6 times while groups n and N remained naive. Only in group A receiving soy-containing feed, IgE serum levels were significantly elevated in comparison to both naive groups (n and N; Figure 1). Comparable findings were observed when measuring IgG1 serum level. Group A showed significantly higher level than groups n and N (Figure 2a). For IgG2a serum levels, a significant difference was observed additionally between group A and a (Figure 2b).

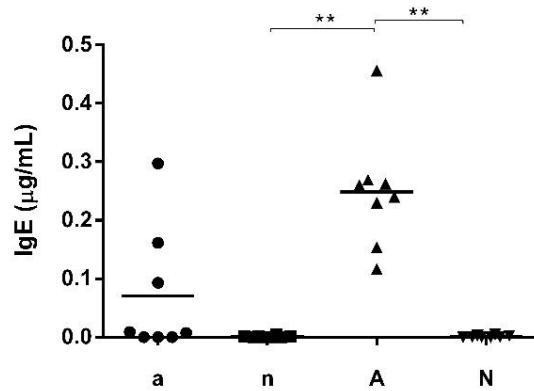


Figure 1. IgE levels in serum after immunization. Group a and n were fed with soy-free feed and group A and N with soy-containing feed. Groups A and a were immunized with 200 µg Ovalbumin (OVA) under gastric acid suppression. Mice of groups N and n were kept naive. Only in group A significantly higher IgE levels were measured than in the naive groups (n and N) (** $p < 0.01$).

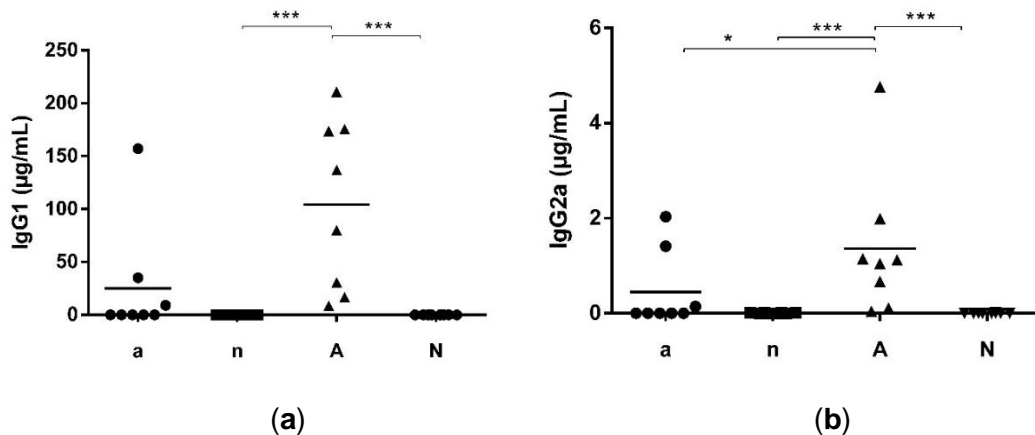


Figure 2. IgG1 and IgG2a serum levels after immunization. **(a)** IgG1 levels of group A were significantly increased in comparison to groups n and N; **(b)** IgG2a level of group A were significantly elevated compared to all other groups (* $p < 0.05$, *** $p < 0.001$).

To analyse the local immune response, we measured the total (Figure 3a) and OVA-specific (Figure 3b) IgA levels in intestinal lavages collected from the intestine directly after final experiments. Total IgA intestinal level (Figure 3a) were comparable between the four groups. OVA-specific IgA (Figure 3b) however, was significantly elevated in group a, compared to the naive mice of groups n and N.

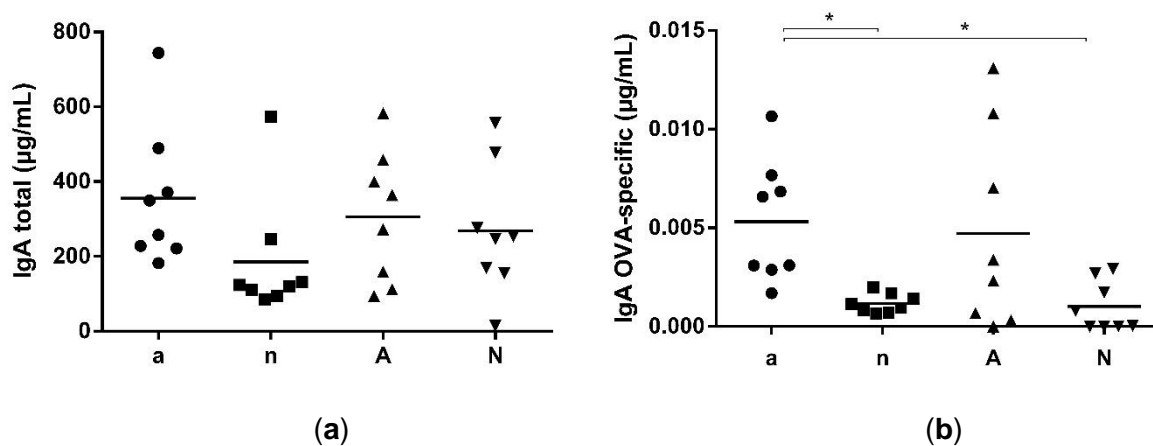


Figure 3. Total and OVA-specific IgA levels in intestinal lavages. After sacrifice, intestinal content was collected and screened for total and OVA-specific IgA levels. **(a)** No differences were revealed for the groups regarding total IgA levels; **(b)** Significantly higher OVA-specific IgA was detected in sera of group a, compared to the naive animals (groups n and N) (* $p < 0.05$).

3.3.2 Cytokine Production Reveal a Tolerogenic Milieu Based in Mouse Feed

Next, we evaluated the production of IL-4 and IL-10 in OVA stimulated spleen cells. All four groups showed no significant difference regarding the IL-4 concentration (Figure 4a). However, concentrations of IL-10 (Figure 4b) was significantly higher in group a, compared to all other groups.

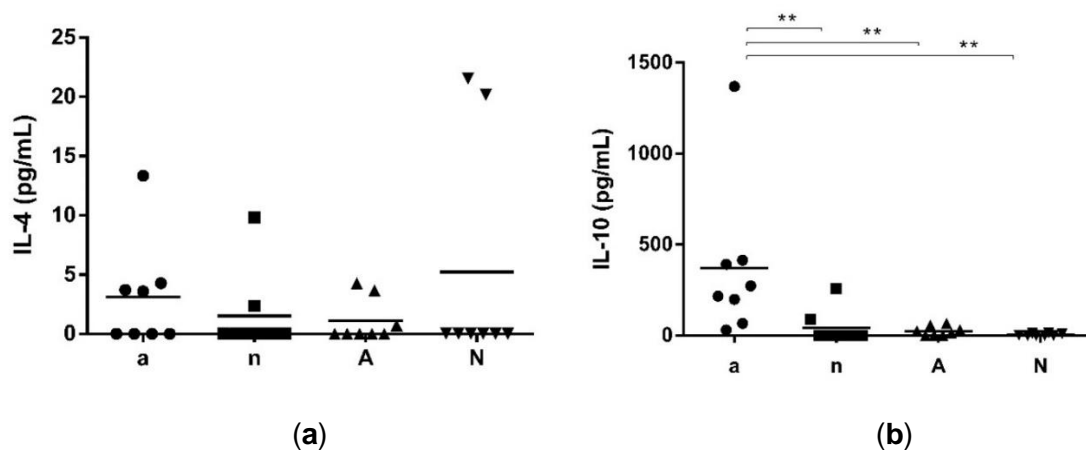


Figure 4. IL-4 and IL-10 levels in spleen cell supernatants after stimulation with OVA. (a) No differences regarding IL-4 levels were found between the groups. (b) IL-10 was significantly increased in group a, in comparison to the naive groups n and N (** $p < 0.01$).

3.3.3 Temperature Changes after Oral OVA Challenge Indicate Food Allergy Development

Before sacrifice, mice were orally challenged with 2 mg OVA and their core body temperature was measured. Mice of groups a and n revealed no changes in body temperature. In group A, a significant drop of temperature was measured 10 min (Figure 5a) and 30 min (Figure 5b) after oral challenge indicating food allergy. In the naive group N receiving soy-containing feed, a marginal decrease of body temperature was observed 10 min and 30 min after oral challenge (Figure 5a, b).

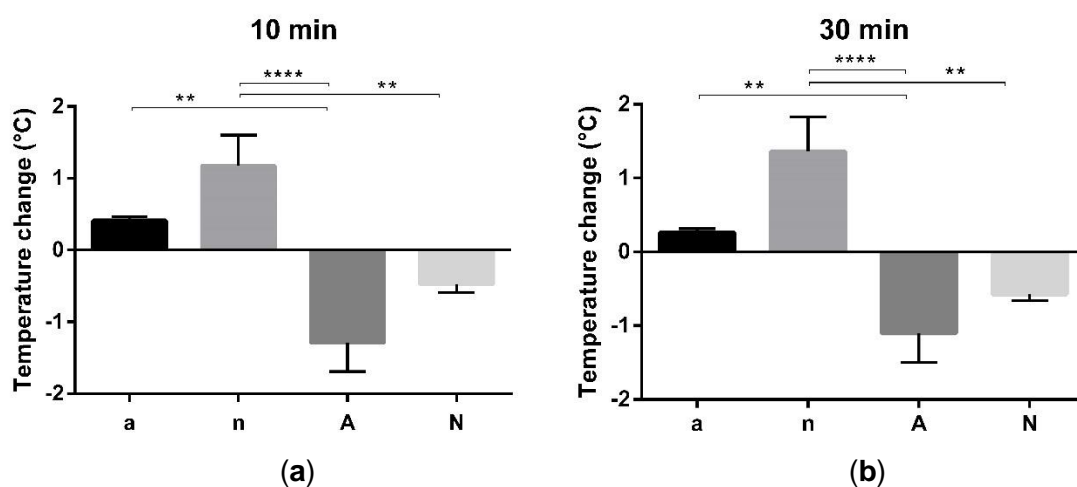


Figure 5. Temperature changes after oral OVA challenges. Temperature was measured before and 10 min, 30 min and 1 h after oral challenge. The drop of body temperature was calculated. (a) Group A showed a significant drop of temperature

compared to groups a and n 10 min after oral challenge, while core body temperature of group N was also significantly lower compared to group n. **(b)** The same findings were observed 30 min after oral challenge. (** $p < 0.01$, **** $p < 0.0001$).

3.4 Discussion

In our current study, we could detect a clear elevation of IgE, IgG1 and IgG2a level in serum of the immunized group fed with soy proteins and low FA amounts (soy-containing feed; group A) in comparison to the naive groups associated with a drop of core body temperature as a sign of anaphylaxis after oral challenge, indicating a clinically manifest IgE-mediated food allergy. Group a receiving the linseed oil based diet (soy-free feed) showed high levels of IL-10, which is known to be associated with regulatory T cell induction [28]. Furthermore, no elevated allergen specific IgE was observed, indicating a protection from food allergy development. In groups a and n, elevated levels of core body temperature have to be seen in context of enhanced activity of mice showing no clinical reaction upon oral allergen challenge.

Composition of food substantially influences immune response and might contribute to development of food allergies. Due to westernization of the diet associated with elevated amounts of saturated and *n*-6 polyunsaturated FAs, lower levels of *n*-3 polyunsaturated FAs and an overuse of salt and refined sugar, negative effects on the immune system are observed [29]. With focus on the innate immune response, three major active components could be defined: low antioxidant intake, high fat intake and a chronic metabolic surplus associated with obesity and chronic inflammation [30]. Representing a risk factor for airway inflammation, high fat intake was discussed to enhance development of asthma via interference with the immune system [31,32]. A recent systematic review even concluded that fast food is associated with asthma and wheeze and other allergic diseases in a dose-response correlation [13].

Thus, for the prevention of food allergy development a healthy diet is of paramount importance and even influences the immune response before birth as the maternal diet provides the basis for developing the innate immune response in infants [33,34].

Without any doubt there are several dietary components of major importance with regards to immunity. Trace elements from dietary sources like iron are a direct target in the body's defence against infection, as they are essential nutrients for pathogens. Recent studies concluded that also manganese and zinc are vital for bacterial survival [35]. By sequestration of zinc and manganese, bacteria are starved and dietary manganese levels were suggested to substantially influence intestinal microbiota composition with direct impact on the immune response [23,36]. Of interest, in our study manganese levels in the allergy protective soy-free feed were nearly double the amount as in the soy-containing feed. With regards to the influence of microbial composition on food allergy, our group has previously reported that protection from food allergy development is associated with the presence of distinct bacterial strains in faecal samples [37].

Another major difference of the two mouse chows was FA levels. A higher amount of *n*-6 polyunsaturated FAs than *n*-3 polyunsaturated FAs was proven to support allergy development in Balb/c mice [12] and is, thus, in line with our findings regarding the allergy development in group A. A higher ratio of *n*-6 to *n*-3 polyunsaturated FAs in human pregnancy was associated with an increased risk of allergic rhinitis in infants by the age of five years [38]. Increasing *n*-3 polyunsaturated FAs cannot eliminate or decrease the negative impact of westernized diet [39], although the anti-inflammatory metabolites of *n*-3 polyunsaturated FAs DHA and EPA can oppose the actions of *n*-6 polyunsaturated FAs especially concerning eicosanoid synthesis [4,40]. The ratio of *n*-6 to *n*-3 polyunsaturated FAs therefore plays an important role in balancing the immune response. As a healthy ratio, 4:1 *n*-6 to *n*-3 polyunsaturated FAs is suggested, while in western diet a ratio of 15–17:1 is detected [4,11,12]. The ratio of *n*-6 polyunsaturated FAs to *n*-3 polyunsaturated FAs in soy-containing feed was 8:1, while it is 3:1 in soy-free feed.

Another difference in chow composition concerns vitamin D levels. In our study higher vitamin D levels were positively associated with protection from food allergy development. An U-shaped association between 25-hydroxyvitamin D and IgE level has been suggested [14,21,22]. Vitamin D has an essential impact on the immune system and allergy development via many mechanistic pathways [41]. Despite the fact that differences regarding vitamin D levels in the two mouse chows were small, it is known that also limited changes of dietary vitamin D levels impact on serum levels in rodents [42]. Moreover, in both chows vitamin D content is above national research council recommendations for rodents requirement [43]. Thus, due to the complex nutrient composition of both chows with high calcium levels an additional impact of vitamin D on the immune response cannot be excluded in this study. The observed elevated IL-10 levels could be ascribed to chow differences, as both vitamin D and *n*-3 polyunsaturated FAs are proven to have a positive impact on regulatory T cell induction [44,45].

Another main difference in mouse feed composition was the presence of soy proteins in the chow supporting allergy development. This is in contrast to findings from Chang and colleagues reporting that elimination of soy from the rodents feed resulted in sensitization to peanut [46]. Phytoestrogens or more specific isoflavones like daidzein and genistein in soy are well known to possess anti-inflammatory, antioxidative and chemo-preventive properties [47]. Consumed through the diet, they can suppress allergic sensitization in humans and supplementation could prevent a food allergy development [48]. However, in our study we observed the opposing effect as only mice receiving soy-containing feed were successfully sensitized to OVA. This might be due to mast cell activating function of oestrogens [49] which should be addressed in future research.

One limitation in the current study is the fact that we did not measure plasma levels of the different immune modulating nutrients. However, due to the complexity of interactions, we expect that the observed impact on the immune response is due to an interplay of different components present in the mouse chow.

3.5 Conclusions

We found a significant correlation of different mouse chow composition pattern with allergy development in our experimental food allergy model. As has been underlined recently [50], diet composition and dietary control is of high relevance for rodent models and ignorance of dietary impact in experimental models might result in false conclusions. Moreover, our study supports the concept of allergy prevention via dietary components, which warrants extensive further research.

Author Contributions: Conceptualization, E.U.; data curation, E.W; formal analysis, E.W.; funding acquisition, E.U.; methodology, E.W, N.S., M.K., D.H., K.S. and D.R.; project administration, E.U.; resources, E.U.; supervision, E.U.; validation, E.W., N.S., M.K. and D.H.; visualization, E.W.; writing-original draft preparation, E.W. and E.U.; writing-review and editing, N.S., M.K., D.H., K.S. and D.R.

Funding: This research was funded in part by a research grant of Nordmark Arzneimittel GmbH & Co. KG, Uetersen, Germany, by the Austrian Science fund project KLI 248 and by the research budget of the Medical University of Vienna (all to EU).

Conflicts of Interest: The authors declare no conflict of interest.

3.6 References

1. Julia, V.; Macia, L.; Dombrowicz, D. The impact of diet on asthma and allergic diseases. *Nat. Rev. Immunol.* **2015**, *15*, 308–322, doi:10.1038/nri3830.
2. Hillyer, L.M.; Woodward, B. A comparison of the capacity of six cold-pressed plant oils to support development of acquired immune competence in the weanling mouse: Superiority of low-linoleic-acid oils. *Br. J. Nutr.* **2002**, *88*, 171, doi:10.1079/BJN2002602.
3. Park, H.J.; Park, J.S.; Hayek, M.G.; Reinhart, G.A.; Chew, B.P. Dietary fish oil and flaxseed oil suppress inflammation and immunity in cats. *Vet. Immunol. Immunopathol.* **2011**, *141*, 301–306, doi:10.1016/j.vetimm.2011.02.024.
4. Wall, R.; Ross, R.P.; Fitzgerald, G.F.; Stanton, C. Fatty acids from fish: The anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr. Rev.* **2010**, *68*, 280–289.
5. Das, U.N. Biological significance of essential fatty acids. *J. Assoc. Physicians India* **2006**, *54*, 309–319.
6. Hogenkamp, A.; van Vlies, N.; Fear, A.L.; van Esch, B.C.; Hofman, G.A.; Garssen, J.; Calder, P.C. Dietary fatty acids affect the immune system in male mice sensitized to ovalbumin or vaccinated with influenza. *J. Nutr.* **2011**, *141*, 698–702, doi:10.3945/jn.110.135863.
7. Wang, X.; Ma, D.W.L.; Kang, J.X.; Kulka, M. *n*-3 polyunsaturated fatty acids inhibit Fc ϵ receptor I-mediated mast cell activation. *J. Nutr. Biochem.* **2015**, *26*, 1580–1588, doi:10.1016/j.jnutbio.2015.07.027.
8. Arita, M. Mediator lipidomics in acute inflammation and resolution. *J. Biochem.* **2012**, *152*, 313–319, doi:10.1093/jb/mvs092.
9. Kunisawa, J.; Arita, M.; Hayasaka, T.; Harada, T.; Iwamoto, R.; Nagasawa, R.; Shikata, S.; Nagatake, T.; Suzuki, H.; Hashimoto, E.; et al. Dietary ω 3 fatty acid exerts anti-allergic effect through the conversion to 17,18-epoxyeicosatetraenoic acid in the gut. *Sci. Rep.* **2015**, *5*, 9750, doi:10.1038/srep09750.
10. Kubota, T.; Arita, M.; Isobe, Y.; Iwamoto, R.; Goto, T.; Yoshioka, T.; Urabe, D.; Inoue, M.; Arai, H. Eicosapentaenoic acid is converted via ω -3 epoxygenation to the anti-inflammatory metabolite 12-hydroxy-17,18-epoxyeicosatetraenoic acid. *FASEB J.* **2014**, *28*, 586–593, doi:10.1096/fj.13-236224.
11. Willemsen, L.E.M. Dietary *n*-3 long chain polyunsaturated fatty acids in allergy prevention and asthma treatment. *Eur. J. Pharmacol.* **2016**, *785*, 174–186, doi:10.1016/J.EJPHAR.2016.03.062.
12. Thang, C.L.; Boye, J.I.; Shi, H.N.; Zhao, X. Effects of supplementing different ratios of omega-3 and omega-6 fatty acids in western-style diets on cow's milk protein allergy in a mouse model. *Mol. Nutr. Food Res.* **2013**, *57*, 2029–2038, doi:10.1002/mnfr.201300061.

13. Wang, C.S.; Wang, J.; Zhang, X.; Zhang, L.; Zhang, H.P.; Wang, L.; Wood, L.G.; Wang, G. Is the consumption of fast foods associated with asthma or other allergic diseases? *Respirology* **2018**, doi:10.1111/resp.13339.
14. Poole, A.; Song, Y.; Brown, H.; Hart, P.H.; Zhang, G.B. Cellular and molecular mechanisms of vitamin D in food allergy. *J. Cell. Mol. Med.* **2018**, *22*, 3270–3277, doi:10.1111/jcmm.13607.
15. Baek, J.H.; Shin, Y.H.; Chung, I.H.; Kim, H.J.; Yoo, E.-G.; Yoon, J.W.; Jee, H.M.; Chang, Y.E.; Han, M.Y. The link between serum vitamin D. level, sensitization to food allergens and the severity of atopic dermatitis in infancy. *J. Pediatr.* **2014**, *165*, 849–854, doi:10.1016/j.jpeds.2014.06.058.
16. Wu, J.; Zhong, Y.; Shen, X.; Yang, K.; Cai, W. Maternal and early-life vitamin D deficiency enhances allergic reaction in an ovalbumin-sensitized BALB/c mouse model. *Food Nutr. Res.* **2018**, *62*, doi:10.29219/fnr.v62.1401.
17. Allen, K.J.; Koplin, J.J.; Ponsonby, A.-L.; Gurrin, L.C.; Wake, M.; Vuillermin, P.; Martin, P.; Matheson, M.; Lowe, A.; Robinson, M.; et al. Vitamin D insufficiency is associated with challenge-proven food allergy in infants. *J. Allergy Clin. Immunol.* **2013**, *131*, 1109–1116, doi:10.1016/J.JACI.2013.01.017.
18. Norizoe, C.; Akiyama, N.; Segawa, T.; Tachimoto, H.; Mezawa, H.; Ida, H.; Urashima, M. Increased food allergy and vitamin D: Randomized, double-blind, placebo-controlled trial. *Pediatr. Int.* **2014**, *56*, 6–12, doi:10.1111/ped.12207.
19. Nwaru, B.I.; Hadkhale, K.; Hämäläinen, N.; Takkinen, H.-M.; Ahonen, S.; Ilonen, J.; Toppari, J.; Niemelä, O.; Haapala, A.-M.; Veijola, R.; et al. Vitamin D intake during the first 4 years and onset of asthma by age 5: A nested case-control study. *Pediatr. Allergy Immunol.* **2017**, *28*, 641–648, doi:10.1111/pai.12773.
20. Weisse, K.; Winkler, S.; Hirche, F.; Herberth, G.; Hinz, D.; Bauer, M.; Röder, S.; Rolle-Kampczyk, U.; von Bergen, M.; Olek, S.; et al. Maternal and newborn vitamin D status and its impact on food allergy development in the German LINA cohort study. *Allergy* **2013**, *68*, 220–228, doi:10.1111/all.12081.
21. Hyppönen, E.; Berry, D.J.; Wjst, M.; Power, C. Serum 25-hydroxyvitamin D and IgE—A significant but nonlinear relationship. *Allergy* **2009**, *64*, 613–620, doi:10.1111/j.1398-9995.2008.01865.x.
22. Rothers, J.; Wright, A.L.; Stern, D.A.; Halonen, M.; Camargo, C.A., Jr. Cord blood 25-hydroxyvitamin D levels are associated with aeroallergen sensitization in children from Tucson, Arizona. *J. Allergy Clin. Immunol.* **2011**, *128*, 1093–1099, doi:10.1016/j.jaci.2011.07.015.

23. Barcik, W.; Untersmayr, E.; Pali-Schöll, I.; O'Mahony, L.; Frei, R. Influence of microbiome and diet on immune responses in food allergy models. *Drug. Discov. Today. Dis. Models* **2015**, *17*, 71–80, doi:10.1016/j.ddmod.2016.06.003.
24. Bernard, H.; Ah-Leung, S.; Drumare, M.-F.; Feraudet-Tarisse, C.; Verhasselt, V.; Wal, J.-M.; Créminon, C.; Adel-Patient, K. Peanut allergens are rapidly transferred in human breast milk and can prevent sensitization in mice. *Allergy* **2014**, *69*, 888–897, doi:10.1111/all.12411.
25. Knippels, L.M.; Penninks, A.H.; Houben, G.F. Continued expression of anti-soy protein antibodies in rats bred on a soy protein-free diet for one generation: The importance of dietary control in oral sensitization research. *J. Allergy Clin. Immunol.* **1998**, *101*, 815–820, doi:10.1016/S0091-6749(98)70309-4.
26. Diesner, S.C.; Knittelfelder, R.; Krishnamurthy, D.; Pali-Schöll, I.; Gajdzik, L.; Jensen-Jarolim, E.; Untersmayr, E. Dose-dependent food allergy induction against ovalbumin under acid-suppression: A murine food allergy model. *Immunol. Lett.* **2008**, doi:10.1016/j.imlet.2008.08.006.
27. Available online: <http://www.graphpad.com> (accessed on 11 June 2018).
28. Tordesillas, L.; Berin, M.C. Mechanisms of oral tolerance. *Clin. Rev. Allergy Immunol.* **2018**, *55*, 107–117, doi:10.1007/s12016-018-8680-5. URL
29. Myles, I.A. Fast food fever: Reviewing the impacts of the Western diet on immunity. *Nutr. J.* **2014**, *13*, 61, doi:10.1186/1475-2891-13-61.
30. Wood, L.G.; Gibson, P.G. Dietary factors lead to innate immune activation in asthma. *Pharmacol. Ther.* **2009**, *123*, 37–53, doi:10.1016/j.pharmthera.2009.03.015.
31. Lawson, J.A.; Rennie, D.C.; Dosman, J.A.; Cammer, A.L.; Senthilselvan, A. Obesity, diet and activity in relation to asthma and wheeze among rural dwelling children and adolescents. *J. Obes.* **2013**, *2013*, 315096, doi:10.1155/2013/315096.
32. Wood, L.G.; Garg, M.L.; Gibson, P.G. A high-fat challenge increases airway inflammation and impairs bronchodilator recovery in asthma. *J. Allergy Clin. Immunol.* **2011**, *127*, 1133–1140, doi:10.1016/j.jaci.2011.01.036.
33. Skypala, I.J.; McKenzie, R. Nutritional issues in food allergy. *Clin. Rev. Allergy Immunol.* **2018**, 1–13, doi:10.1007/s12016-018-8688-x.
34. Mulligan, C.M.; Friedman, J.E. Maternal modifiers of the infant gut microbiota: Metabolic consequences. *J. Endocrinol.* **2017**, *235*, R1–R12, doi:10.1530/JOE-17-0303.
35. Kehl-Fie, T.E.; Skaar, E.P. Nutritional immunity beyond iron: A role for manganese and zinc. *Curr. Opin. Chem. Biol.* **2010**, *14*, 218–224, doi:10.1016/j.cbpa.2009.11.008.
36. Damo, S.M.; Kehl-Fie, T.E.; Sugitani, N.; Holt, M.E.; Rathi, S.; Murphy, W.J.; Zhang, Y.; Betz, C.; Hench, L.; Fritz, G.; et al. Molecular basis for manganese sequestration by cal-

- protectin and roles in the innate immune response to invading bacterial pathogens. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 3841–3846, doi:10.1073/pnas.1220341110.
37. Diesner, S.C.; Bergmayr, C.; Pfitzner, B.; Assmann, V.; Krishnamurthy, D.; Starkl, P.; Endesfelder, D.; Rothballer, M.; Welzl, G.; Rattei, T.; et al. A distinct microbiota composition is associated with protection from food allergy in an oral mouse immunization model. *Clin. Immunol.* **2016**, *173*, 10–18, doi:10.1016/j.clim.2016.10.009.
 38. Nwaru, B.I.; Erkkola, M.; Lumia, M.; Kronberg-Kippilä, C.; Ahonen, S.; Kaila, M.; Ilonen, J.; Simell, O.; Knip, M.; Veijola, R.; et al. Maternal intake of fatty acids during pregnancy and allergies in the offspring. *Br. J. Nutr.* **2012**, *108*, 720–732, doi:10.1017/S0007114511005940.
 39. Calder, P.C. *n*-3 Polyunsaturated fatty acids, inflammation and inflammatory diseases. *Am. J. Clin. Nutr.* **2006**, *83*, S1505–S1519, doi:10.1093/ajcn/83.6.1505S.
 40. Miles, E.A.; Calder, P.C. Can early omega-3 fatty acid exposure reduce risk of childhood allergic disease? *Nutrients* **2017**, *9*, doi:10.3390/nu9070784.
 41. Mirzakhani, H.; Al-Garawi, A.; Weiss, S.T.; Litonjua, A.A. Vitamin D and the development of allergic disease: How important is it? *Clin. Exp. Allergy* **2015**, *45*, 114–125, doi:10.1111/cea.12430.
 42. Fleet, J.C.; Gliniak, C.; Zhang, Z.; Xue, Y.; Smith, K.B.; McCreedy, R.; Adedokun, S.A. Serum metabolite profiles and target tissue gene expression define the effect of cholecalciferol intake on calcium metabolism in rats and mice. *J. Nutr.* **2008**, *138*, 1114–1120, doi:10.1093/jn/138.6.1114.
 43. Council, N.R. *Nutrient Requirements of Laboratory Animals: Fourth Revised Edition*; The National Academies Press: Washington, DC, USA, 1995.
 44. Cantorna, M.T.; Snyder, L.; Lin, Y.-D.; Yang, L.; Vitamin, D. and 1,25(OH)₂D regulation of T. cells. *Nutrients* **2015**, *7*, 3011–3021, doi:10.3390/nu7043011.
 45. Lian, M.; Luo, W.; Sui, Y.; Li, Z.; Hua, J. Dietary *n*-3 PUFA protects mice from con a induced liver injury by modulating regulatory T cells and ppar- γ expression. *PLoS ONE* **2015**, *10*, e0132741, doi:10.1371/journal.pone.0132741.
 46. Chang, L.M.; Song, Y.; Li, X.-M.; Sampson, H.A.; Masilamani, M. Dietary elimination of soybean components enhances allergic immune response to peanuts in BALB/c mice. *Int. Arch. Allergy Immunol.* **2015**, *166*, 304–310, doi:10.1159/000430497.
 47. Masilamani, M.; Wei, J.; Sampson, H.A. Regulation of the immune response by soybean isoflavones. *Immunol. Res.* **2012**, *54*, 95–110, doi:10.1007/s12026-012-8331-5.
 48. Masilamani, M.; Wei, J.; Bhatt, S.; Paul, M.; Yakir, S.; Sampson, H.A. Soybean isoflavones regulate dendritic cell function and suppress allergic sensitization to peanut. *J. Allergy Clin. Immunol.* **2011**, *128*, 1242–1250, doi:10.1016/j.jaci.2011.05.009.

49. Jensen-Jarolim, E.; Untersmayr, E. Gender-medicine aspects in allergology. *Allergy* **2008**, *63*, 610–615, doi:10.1111/j.1398-9995.2008.01645.x.
50. Nielsen, F.H. 90th Anniversary commentary: The AIN-93 purified diets for laboratory rodents—The development of a landmark article in the journal of nutrition and its impact on health and disease research using rodent models. *J. Nutr.* **2018**, *148*, 1667–1670, doi:10.1093/jn/nxy121.

4. Abstracts

4.1 Deutsch

Durch die Zusammensetzung unserer Ernährung kann unser Immunsystem wesentlich beeinflusst werden. Dieser Mausversuch untersuchte den Einfluss von zwei verschiedenen Mausefuttern mit unterschiedlicher Zusammensetzung an Mikro- und Makronährstoffen, Vitaminen und Fetten. Ein Futter enthielt Leinöl und einen höheren Gehalt an mehrfach ungesättigten Fettsäuren (Soja-freies Futter) im Gegensatz zum anderen Futter, das Sojaprodukte und einen geringeren Gehalt an mehrfach ungesättigten Fettsäuren (Soja-enthaltendes Futter) enthielt. Nach oraler Immunisierung mit Ovalbumin und gleichzeitiger Magensäuresuppression nach einem etablierten Protokoll konnten beim Soja-enthaltenden Futter signifikant erhöhte Ovalbumin-spezifische IgE-, IgG1- und IgG2a-Antikörperspiegel sowie einen Abfall der Körpertemperatur festgestellt werden, während beim Soja-freien Futter ein erhöhter IL-10-Spiegel verzeichnet wurden. Es konnte also bewiesen werden, dass das Soja-enthaltende Futter zu einer Sensibilisierung und einer Nahrungsmittelallergie geführt hat, während das Soja-freie Futter wesentlich zum Schutz vor einer Allergieentwicklung beigetragen hat.

4.2 Englisch

Our immune system can be substantially influenced by composition of our diet. This mouse experiment investigated the impact of two different mouse chows with different composition of micro- and macronutrients, vitamins and lipids. One chow contained linseed oil and a high amount of polyunsaturated fatty acids (soy-free feed) in contrast to the other chow which contained soy products and a lower amount of polyunsaturated fatty acids (soy-containing feed). After oral immunization with Ovalbumin under concomitant gastric acid suppression based on an established protocol there were significant elevated Ovalbumin-specific IgE-, IgG1- and IgG2a-antibody levels in the soy-containing feed, whereas the soy-free feed had elevated IL-10 levels. It is therefore proven that soy-containing feed lead to sensitization and food allergy, while soy-free feed contributed protection against allergy development.

5. Zusammenfassung

Dass verschiedene Aspekte der Ernährung auf unser Immunsystem wirken, wurde schon früher bewiesen. Wir verwenden viel mehr Salz und raffinierten Zucker als nötig und unsere Lipidaufnahme hat sich stark erhöht und sich von ungesättigten Omega-3-Fettsäuren zu vermehrt gesättigten Omega-6-Fettsäuren verschoben, was einer westlichen Ernährung entspricht.

Die zwei verwendeten Mausfutter unterscheiden sich vorrangig in Mangan-, Vitamin D-, Omega-3-Fettsäuren- und Omega-6-Fettsäuregehalt, die im Soja-freien Futter in wesentlich größeren Mengen enthalten sind als im Soja-enthaltenden Futter. So ist Mangan wichtig für die richtige mikrobielle Zusammensetzung im Gastrointestinaltrakt zum Schutz vor einer Nahrungsmittelallergieentwicklung. [13,14] Vitamin D ist in Zusammenhang mit der komplexen Zusammensetzung des Futters – obwohl nur geringe Mengenunterschiede zwischen den zwei Mausfutter aufzuweisen sind – ein nicht zu vernachlässigender Faktor zum Schutz vor einer Nahrungsmittelallergie. [15]

Der größte Unterschied zwischen den zwei Mausfutter ist das Vorhandensein von Leinöl im protektiven Soja-freien Futter. Dadurch ergeben sich Unterschiede hinsichtlich des Gehalts an Omega-3- und Omega-6-Fettsäuren verglichen mit dem Soja-haltigen Futter. Omega-6-Fettsäuren sollten in möglichst niedrigen Mengen vorhanden sein, um die Entwicklung einer Allergie zu verhindern, doch kommt es auch auf das Verhältnis von Omega-6- zu Omega-3-Fettsäuren an, das im Idealfall 4:1 sein sollte, in der westlichen Ernährung jedoch ein Verhältnis von 15-17:1 aufzeigt. [16–18] Das Soja-freie Futter hat ein gesundes Verhältnis von 3:1, während das Soja-enthaltene Futter ein Verhältnis von 8:1 aufweist und somit der westlichen Ernährung nahekommt.

Die 32 Mäuse waren in vier gleiche Gruppen aufgeteilt, wobei jeweils zwei Gruppen (A und N) das Soja-enthaltende Futter und die anderen zwei Gruppen (a und n) das Soja-freie Futter erhalten haben. N und n blieben naiv, während Gruppen A und a sechs Immunisierungszyklen durchmachten.

Um die zwei Mausfutter auf Auswirkungen auf das Immunsystem zu untersuchen, wurden ELISAs (Enzyme-linked Immunosorbent Assay) durchgeführt, um Antikörperspezifische IgE-, IgG1-, IgG2a-Spiegel im Serum sowie Gesamt- und Ovalbumin-spezifische IgA-Antikörper Titer in intestinalen Lavagen zu messen. Zytokin-ELISA (IL-4 und IL-10) von mit Ovalbumin-stimulierten Milzzellen wurden ebenfalls durchgeführt. Temperaturmessungen wurden direkt nach der oralen Provokation mit Ovalbumin und vor dem Sacrifice vorgenom-

men.

Gruppe A zeigte hohe IgE-, IgG1- und IgG2a-Serumlevel im Vergleich zu allen anderen Gruppen und außerdem einen starken Abfall der Körperkerntemperatur als Zeichen einer Anaphylaxie nach oraler Provokation mit Ovalbumin. Diese Mäuse waren vom Futter nicht geschützt und hatten eine klinisch manifeste IgE-vermittelte Nahrungsmittelallergie entwickelt. Gruppe a zeigte dagegen erhöhte IL-10-Level, dafür weder erhöhte IgE-Level noch einen Abfall der Körperkerntemperatur und war somit geschützt vor einer Sensibilisierung.

Insgesamt wurde bestätigt, dass die Ernährung die Entstehung einer Nahrungsmittelallergie beeinflussen kann und nicht außer Acht gelassen werden sollte. Vor allem in murinen Modellen sollte auf diesen Aspekt geachtet werden, da die Immunantwort entscheidend beeinflusst wird. [19]

6. Referenzen

1. Tordesillas, L.; Berin, M. C.; Sampson, H. A. Immunology of Food Allergy. *Immunity* **2017**, *47*, 32–50, doi:10.1016/j.immuni.2017.07.004.
2. Diesner, S. C.; Knittelfelder, R.; Krishnamurthy, D.; Pali-Schöll, I.; Gajdzik, L.; Jensen-Jarolim, E.; Untersmayr, E. Dose-dependent food allergy induction against ovalbumin under acid-suppression: A murine food allergy model. *Immunol. Lett.* **2008**, doi:10.1016/j.imlet.2008.08.006.
3. Pali-Schöll, I.; Jensen-Jarolim, E. Anti-acid medication as a risk factor for food allergy. *Allergy Eur. J. Allergy Clin. Immunol.* 2011.
4. Liu, A. H. Revisiting the hygiene hypothesis for allergy and asthma. *J. Allergy Clin. Immunol.* **2015**, *136*, 860–865, doi:10.1016/J.JACI.2015.08.012.
5. Senti, G.; von Moos, S.; Kündig, T. M. Epicutaneous Immunotherapy for Aeroallergen and Food Allergy. *Curr. Treat. options allergy* **2014**, *1*, 68–78, doi:10.1007/s40521-013-0003-8.
6. Lanser, B. J.; Leung, D. Y. M. The Current State of Epicutaneous Immunotherapy for Food Allergy: a Comprehensive Review. *Clin. Rev. Allergy Immunol.* **2018**, *55*, 153–161, doi:10.1007/s12016-017-8650-3.
7. Du Toit, G.; Roberts, G.; Sayre, P. H.; Plaut, M.; Bahnson, H. T.; Mitchell, H.; Radulovic, S.; Chan, S.; Fox, A.; Turcanu, V.; Lack, G.; Learning Early About Peanut Allergy (LEAP) Study Team Identifying infants at high risk of peanut allergy: the Learning Early About Peanut Allergy (LEAP) screening study. *J. Allergy Clin. Immunol.* **2013**, *131*, 135-43.e1–12, doi:10.1016/j.jaci.2012.09.015.
8. Du Toit, G.; Roberts, G.; Sayre, P. H.; Bahnson, H. T.; Radulovic, S.; Santos, A. F.; Brough, H. A.; Phippard, D.; Basting, M.; Feeney, M.; Turcanu, V.; Sever, M. L.; Gomez Lorenzo, M.; Plaut, M.; Lack, G. Randomized Trial of Peanut Consumption in Infants at Risk for Peanut Allergy. *N. Engl. J. Med.* **2015**, *372*, 803–813, doi:10.1056/NEJMoa1414850.
9. Du Toit, G.; Sayre, P. H.; Roberts, G.; Sever, M. L.; Lawson, K.; Bahnson, H. T.; Brough, H. A.; Santos, A. F.; Harris, K. M.; Radulovic, S.; Basting, M.; Turcanu, V.; Plaut, M.; Lack, G. Effect of Avoidance on Peanut Allergy after Early Peanut Consumption. *N. Engl. J. Med.* **2016**, *374*, 1435–1443, doi:10.1056/NEJMoa1514209.
10. Perkin, M. R.; Logan, K.; Marrs, T.; Radulovic, S.; Craven, J.; Flohr, C.; Lack, G.; Team, E. S. Enquiring About Tolerance (EAT) study: Feasibility of an early allergenic food introduction regimen. *J. Allergy Clin. Immunol.* **2016**, *137*, 1477, doi:10.1016/J.JACI.2015.12.1322.
11. Perkin, M. R.; Logan, K.; Tseng, A.; Raji, B.; Ayis, S.; Peacock, J.; Brough, H.; Marrs, T.; Radulovic, S.; Craven, J.; Flohr, C.; Lack, G. Randomized Trial of Introduction of Allergenic Foods in Breast-Fed Infants. *N. Engl. J. Med.* **2016**, *374*, 1733–1743, doi:10.1056/NEJMoa1514210.
12. Fewtrell, M.; Bronsky, J.; Campoy, C.; Domellöf, M.; Embleton, N.; Fidler Mis, N.; Hojsak, I.; Hulst, J. M.; Indrio, F.; Lapillonne, A.; Molgaard, C. Complementary Feeding: A Position Paper by the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN) Committee on Nutrition. *J. Pediatr. Gastroenterol. Nutr.* **2017**, *64*, 119–132, doi:10.1097/MPG.0000000000001454.

13. Barcik, W.; Untersmayr, E.; Pali-Schöll, I.; O'Mahony, L.; Frei, R. Influence of microbiome and diet on immune responses in food allergy models. *Drug Discov. Today. Dis. Models* **2015**, *17–18*, 71–80, doi:10.1016/j.ddmod.2016.06.003.
14. Damo, S. M.; Kehl-Fie, T. E.; Sugitani, N.; Holt, M. E.; Rathi, S.; Murphy, W. J.; Zhang, Y.; Betz, C.; Hench, L.; Fritz, G.; Skaar, E. P.; Chazin, W. J. Molecular basis for manganese sequestration by calprotectin and roles in the innate immune response to invading bacterial pathogens. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 3841–6, doi:10.1073/pnas.1220341110.
15. Mirzakhani, H.; Al-Garawi, A.; Weiss, S. T.; Litonjua, A. A. Vitamin D and the development of allergic disease: how important is it? *Clin. Exp. Allergy* **2015**, *45*, 114–25, doi:10.1111/cea.12430.
16. Wall, R.; Ross, R. P.; Fitzgerald, G. F.; Stanton, C. Fatty acids from fish: The anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutr. Rev.* 2010, *68*, 280–289.
17. Willemsen, L. E. M. Dietary n-3 long chain polyunsaturated fatty acids in allergy prevention and asthma treatment. *Eur. J. Pharmacol.* **2016**, *785*, 174–186, doi:10.1016/J.EJPHAR.2016.03.062.
18. Thang, C. L.; Boye, J. I.; Shi, H. N.; Zhao, X. Effects of supplementing different ratios of omega-3 and omega-6 fatty acids in western-style diets on cow's milk protein allergy in a mouse model. *Mol. Nutr. Food Res.* **2013**, *57*, 2029–2038, doi:10.1002/mnfr.201300061.
19. Bøgh, K. L.; van Bilsen, J.; Głogowski, R.; López-Expósito, I.; Bouchaud, G.; Blanchard, C.; Bodinier, M.; Smit, J.; Pieters, R.; Bastiaan-Net, S.; de Wit, N.; Untersmayr, E.; Adel-Patient, K.; Knippels, L.; Epstein, M. M.; Noti, M.; Nygaard, U. C.; Kimber, I.; Verhoeckx, K.; O'Mahony, L. Current challenges facing the assessment of the allergenic capacity of food allergens in animal models. *Clin. Transl. Allergy* **2016**, *6*, 21, doi:10.1186/s13601-016-0110-2.