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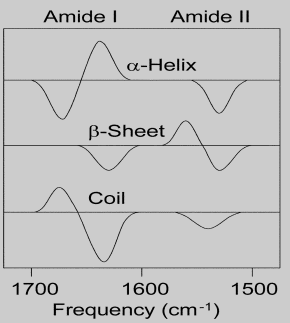

Reviews

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Effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins

Fumonisins are *Fusarium* mycotoxins that occur in corn and corn-based foods. They are toxic to animals and at least one analogue, fumonisin B_1, is carcinogenic to rodents. Their effect on human health is unclear, however, fumonisins are considered to be risk factors for cancer and possibly neural tube defects in some heavily exposed populations. It is therefore important to minimize exposures in these populations. Cleaning corn to remove damaged or moldy kernels reduces fumonisins in foods while milling increases their concentration in some and reduces their concentration in other products. Fumonisins are water-soluble and nixtamalization (cooking in alkaline water) lowers the fumonisin content of food products if the cooking liquid is discarded. Baking, frying, and extrusion cooking of corn at high temperatures ($\geq 190^{\circ}C$) also reduces fumonisin concentrations in foods, with the amount of reduction achieved depending on cooking time, temperature, recipe, and other factors. However, the chemical fate of fumonisins in baked, fried, and extruded foods is not well understood and it is not known if the reduced concentrations result from thermal decomposition of fumonisins or from their binding to proteins, sugars or other compounds in food matrices. These possibilities might or might not be beneficial depending upon the bioavailability and inherent toxicity of decomposition products or the degree to which bound fumonisins are released in the gastrointestinal tract. In this review the affects of cooking and processing on the concentration and chemical structure of fumonisins as well as the toxicological consequences of known and likely fumonisin reaction products are discussed.

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Diagnosis and therapy of food allergy

According to the recently revised nomenclature for allergy [1] the term “Food Hypersensitivity” is proposed to define a reaction on food exposure causing objectively reproducible symptoms or signs at a dose tolerated by normal subjects. Those reactions to food in which immunologic mechanisms are demonstrated comprise the term “Food Allergy”. Immunologic reactions to food in which an immunoglobulin E (IgE)-mediated mechanism is established are defined as IgE-mediated food allergy. This review focuses on IgE-mediated allergic reactions to foods.

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Clinical role of lipid transfer proteins in food allergy

Lipid transfer proteins are widespread plant food allergens, highly resistant to food processing and to the gastrointestinal environment, which have recently been described as true food allergens in the Mediterranean area, where they have been associated with severe allergic reactions to foods in patients without pollen allergy. In this review we analyze their molecular structure, biological function, and clinical relevance in food allergy.

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Allergy assessment of foods or ingredients derived from biotechnology, gene-modified organisms, or novel foods

The introduction of novel proteins into foods carries a risk of eliciting allergic reactions in individuals sensitive to the introduced protein and a risk of sensitizing susceptible individuals. No single predictive test exists to perform a hazard assessment in relation to allergenic properties of newly expressed proteins in gene-modified organisms (GMOs). Instead, performance of a weighted risk analysis based on the decision tree approach has been suggested. The individual steps of this analysis comprise sequence homology to known allergens, specific or targeted serum screens for immunoglobulin E (IgE) cross-reactions to known allergens, digestability studies of the proteins in simulated gastric and/or intestinal fluids, and animal studies. These steps are discussed and five examples of risk evaluation of GMOs or novel foods are presented. These include ice-structuring protein derived from fish, microbial transglutaminase, GMO-soybeans, amylase and the Nangai nut.

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T lymphocytes and food allergy

Food allergy is a hypersensitivity reaction to normally harmless substances and involves humoral immune responses, mediated by immunoglobulin (IgE) synthesized by B lymphocytes, and cellular immune responses mediated by T lymphocytes. An IgE-mediated mechanism leads to clinical symptoms occurring immediately after food ingestion, *e.g.*, "the oral allergy syndrome". For delayed reactions involving the gastrointestinal tract or the skin, the underlying immune mechanisms are less clear. In order to elucidate the cellular response to food allergens, human allergen-specific T cell cultures generated *in vitro* represent helpful tools. The majority of food allergen-specific $CD4^+$ T lymphocytes isolated from food-allergic individuals was found to synthesize high levels of IL-4 and IL-13, two cytokines required for initiation of IgE synthesis. Due to selective homing profiles, food-specific T cells seem also to be involved in defining the target organ of the allergic inflammation. Recent data provide evidence that in addition to IgE-mediated inflammation, food allergen-specific T lymphocytes may also cause inflammatory responses independently of IgE-mediated mechanisms.

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***Bacillus cereus*, the causative agent of an emetic type of food-borne illness**

Bacillus cereus is the causative agent of two distinct forms of gastroenteritic disease connected to food-poisoning. It produces one emesis-causing toxin and three enterotoxins that elicit diarrhea. Due to changing lifestyles and eating habits, *B. cereus* is responsible for an increasing number of food-borne diseases in the industrial world. In the past, most studies concentrated on the diarrhoeal type of food-borne disease, while less attention has been given to the emetic type of the disease. The toxins involved in the diarrhoeal syndrome are well-known and detection methods are commercially available, whereas diagnostic methods for the emetic type of disease have been limited. Only recently, progress has been made in developing identification methods for emetic *B. cereus* and its corresponding toxin. We will summarize the data available for the emetic type of the disease and discuss some new insights in emetic strain characteristics, diagnosis, and toxin synthesis.

Research Articles

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Systematic studies of structure and physiological activity of alapyridaine. A novel food-born taste enhancer

By application of taste dilution analysis (+)-(*S*)-1-(1-carboxyethyl)-5-hydroxy-2-(hydroxymethyl)-pyridinium inner salt was recently successfully identified as a multimodal taste enhancer in beef bouillon. While being taste-less on its own, this so-called alapyridaine was found to intensify the human perception of sweet, salty, and umami taste. To gain information on the molecular requirements of this novel class of taste enhancer, a range of structurally related pyridinium betaines were synthesized, purified, and their physiological activities sensorially evaluated. Removal or modification of the hydroxyl and the hydroxymethyl group, respectively, induced a loss in bioactivity, thus indicating the 2-(hydroxymethyl)-5-hydroxypyridinium moiety as an essential structural element for taste enhancement. Regarding the amino substituent, neither the prolongation or removal of the alkyl chain or the carboxy function in the 1-(1-carboxy-2-ethyl)-moiety, nor the incorporation of an additional carboxy function led to any active derivative, thus demonstrating that also the structure of the nitrogen substituent is rather conserved for taste enhancement. But substitution of the methyl group by a benzyl group yielded a compound showing similar taste enhancing activities as found for alapyridaine. Interestingly, additional insertion of glycine between the 1-(1-carboxy-2-phenylethyl)-moiety and the pyridinium ring resulted in a compound eliciting comparable taste enhancing effects as shown for the compound lacking the glycine spacer. In contrast to these multimodal taste enhancers, substitution of the alanine moiety in alapyridaine by an arginine moiety revealed an one-dimensional taste enhancer exclusively increasing the human sensitivity for salty taste.

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Sensitive *in vitro* test systems to determine androgenic/antiandrogenic activity

We report on the establishment of one transgenic and two endogenous reporter gene assays to determine androgenic/antiandrogenic activity. A transient transactivation assay was developed in COS-7 African green monkey kidney cells. Three plasmids were co-transfected by electroporation: the human androgen receptor expression vector pSG5AR, the reporter gene vector pMamneoLuc, expressing luciferase under the control of the mouse mammary tumor virus (MMTV) promotor containing 4 hormone responsive elements (HREs), and the control plasmid pSVβ. Transcriptional activation was measured by luciferase-mediated chemoluminescence. In T47D human breast cancer cells two endogenous reporter gene systems were established: one based on the androgen-induced expression of prostate-specific antigen (PSA), the other based on the androgen-repressed expression of testosterone repressed message 2 (TRPM-2). PSA protein was measured by enzyme-linked immunosorbent assay (ELISA), TRPM-2 m-RNA by reverse transcriptase polymerase chain reaction (RT-PCR). All three test systems were validated using the physiological androgen dihydrotestosterone (DHT) as agonist and the established antiandrogens hydroxyflutamide and p,p′-dichlorodiphenylethene (p,p′-DDE) as antagonists. The PSA assay was the most sensitive test system with an EC_{50} of 0.7 nM for DHT-induced response. The transient transactivation assay in COS-7 cells was less sensitive with an EC_{50} of 9.7 nM DHT. In the PSA assay hydroxyflutamide was a more potent antagonist (IC_{30} = 0.02 μM) than p,p′-DDE (IC_{30} = 0.9 μM). In the transient transactivation assay in COS-7 cells, both compounds elicited about 30% of the agonistic response induced by 100 nM DHT, thus showing partial agonistic properties. In summary, three highly sensitive and complementary *in vitro* test systems, together achieving enhanced specificity for detection and assessment of androgenic/antiandrogenic activity have been established and validated.

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Heterocyclic amines in some Swedish cooked foods industrially prepared or from fast food outlets and restaurants

Accurate assessment of human intake of mutagenic/carcinogenic heterocyclic amines (HAs) is necessary for epidemiological studies and future risk assessment. Using questionnaires, the frequency of consumption of specific dishes can be obtained at an individual level and linked to analyzed concentrations of different compounds in corresponding dishes. Some typical Swedish cooked meat dishes, hamburgers and kebab, industrially prepared or from fast food outlets and restaurants, were analyzed regarding their content of 11 different HAs. The amount of each of these compounds was below 0.1 ng/g cooked weight in most of the industrially prepared products. The total amount of HAs was highest in the kebab samples. The intake of HAs from 200 g of the dishes was estimated to range from not detectable levels to 0.6 μg. The results of the present study indicate that the content of HAs in a specific dish may vary with origin, and that the concentrations of HAs in commercial fried meat products are generally low, although some of these food items may contain elevated amounts.

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Levels of mycotoxins and sample cytotoxicity of selected organic and conventional grain-based products purchased from Finnish and Italian markets

The contamination levels of 16 different *Fusarium*- and *Aspergillus*-mycotoxins were chemically determined from randomly selected organic and conventional grain-based products purchased from Finnish and Italian markets. The cytotoxicity of the samples was analyzed with an *in vitro* test using feline fetal lung cells. Overall, the concentrations of the mycotoxins studied were low in all of the samples. Enniatins B and B1 as well as deoxynivalenol were the most predominant mycotoxins in the samples, being present in 97%, 97%, and 90% of the samples, respectively. The geographical origin or the agricultural practice had no influence on the mycotoxin concentrations of the samples. The babyfoods included in the samples had significantly lower concentrations of mycotoxins than the other products with a mean total mycotoxin content of 47 μg/kg compared with 99 μg/kg for the other kinds of food. All the samples evoked toxicity in the *in vitro* test, but no correlation between cytotoxicity and the mycotoxin concentrations was observed.

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Antioxidant activity of plant extracts on the inhibition of citral off-odor formation

Grape seed, pomegranate seed, green tea, and black tea extracts were used to inhibit the off-odor from citral degradation. A 0.1 M citrate buffer (pH 3), containing 100 ppm citral and 200 ppm gallic acid equivalent plant extract, was incubated at 40°C. The reaction mixtures were analyzed by high-performance liquid chromatography (HPLC) at days 0, 6, 10, 13, and 16 to monitor degradation of citral and formation of α, *p*-dimethylstyrene, *p*-cymene-8-ol, and *p*-methylacetophenone. The addition of plant phenolic extracts could not inhibit citral degradation, however, all four plant extracts significantly inhibited *p*-methylacetophenone formation. The samples, with the addition of plant extracts, exhibited higher concentrations of α, *p*-dimethylstyrene and *p*-cymene-8-ol than the control. This is presumed to be due to the oxygen-scavenging effect of plant extracts blocking the pathway from *p*-cymene-8-ol to *p*-methylacetophenone. Our results suggest that these plant extracts act as general antioxidants inhibiting the generation of *p*-methylacetophenone regardless of the types of water-soluble phenolic compounds existing in the plant extracts.

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The substitution pattern of anthocyanidins affects different cellular signaling cascades regulating cell proliferation

The aglycons of the most abundant anthocyanins in food, cyanidin (cy) and delphinidin (del), represent potent inhibitors of the epidermal growth factor receptor (EGFR). Structure-activity studies show that the presence of vicinal hydroxy substituents at the phenyl ring at the 2-position (B-ring) is crucial for target interaction. The presence of a single hydroxy group or introduction of methoxy substituents at the B-ring results in a substantial loss of inhibitory properties. However, biological activity is not exclusively limited to compounds bearing vicinal hydroxy groups. A contradictory structure-activity relationship is observed for the inhibition of cAMP-specific phosphodiesterases (PDEs). Of the anthocyanidins tested, malvidin, bearing methoxy substituents in the 3′- and 5′-positions, most effectively inhibited cAMP hydrolysis. The absence of methoxy groups and/or replacement by hydroxy substituents was found to strongly diminish PDE-inhibitory properties. We found that either effective EGFR inhibition or effective PDE inhibition is required to achieve a shut-down of the central mitogen-activated protein kinase (MAPK) pathway, a signaling cascade crucial for the regulation of cell growth. This is consistent with the finding that efficient reduction of cell growth is limited to anthocyanidins that are potent EGFR- or PDE-inhibitors including cy and del or malvidin (mv), respectively. In summary, depending on the substitution pattern at the B-ring, anthocyanidins interfere with different signaling cascades involved in the regulation of cell growth.

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The pig caecum model: A suitable tool to study the intestinal metabolism of flavonoids

Pig caecum was used under anaerobic conditions to metabolize flavonoids from several classes, *i. e.*, chrysin **1**, naringenin **2,** quercetin **3**, and hesperetin **4**. Whereas chrysin **1** was not converted by the pig intestinal flora under the experimental conditions used, naringenin **2** was transformed to 3-(4-hydroxyphenyl)-propionic acid and 3-phenylpropionic acid. Quercetin **3** was metabolized to phloroglucinol, 3,4-dihydroxyphenylacetic acid, and 3,4-dihydroxytoluene. Hesperetin **4** was degraded *via* eriodictyol to 3-(3-hydroxyphenyl)-propionic acid and phloroglucinol. Structural elucidation of the formed metabolites was performed by high-performance liquid chromatography – diode array detection (HPLC – DAD) as well as HPLC-electrospray ionization – mass spectrometry (ESI-MS (MS)) and high resolution gas chromatography-mass spectrometry (HRGC-MS) analyses. The time course of microbial conversion of **2** – **4** was determined by HPLC-DAD analysis, revealing slow degradation of **2** and rapid transformation of **3** and **4**. The results lead to the conclusion that the pig caecum model is a suitable *ex vivo* model for studying the intestinal degradation of flavonoids.

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Cow's milk allergens identification by two-dimensional immunoblotting and mass spectrometry

Cow's milk allergy (CMA) has become a common disease in early childhood, its prevalence ranging from 1.6% to 2.8% among children younger than 2 years of age. The role of different cow's milk protein (CMP) in the pathogenesis of CMA is still controversial. Even if the proteins most frequently and most intensively recognized by immunoglobulin E (IgE) seem to be the most abundant in milk (caseins and β-lactoglobulin), with an although great variability all milk proteins appear to be potential allergens, even those that are present in trace amounts (*i. e.*, lactoferrin, IgG, and BSA). In this work proteomics techniques have been applied for CMP allergens analysis. Allergens have been identified by immunoblotting following resolution of CMP components by two-dimensional electrophoresis. Sera from 20 milk-allergic subjects, as proven by oral provocation test, CAP-RAST and skin prick test, have been used for cow's milk major allergen identification. Cow's milk proteins and their isoforms were identified by matrix assisted laser desorption/ionization-time of flight (MALDI-TOF)-mass spectrometry. In our group of patients, the prevalence of CMP allergens, *i. e.*, the total number of subjects sensitized to CMP divided by the total number of the subjects enrolled in the study, was: 55% α_{s1}-casein, 90% α_{s2}-casein, 15% β-casein, 50% κ-casein, 45% β-lactoglobulin, 45% BSA, 95% IgG-heavy chain, 50% lactoferrin, and 0% α-lactalbumin.

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Recombinant tropomyosin from *Penaeus aztecus* (rPen a 1) for measurement of specific immunoglobulin E antibodies relevant in food allergy to crustaceans and other invertebrates

Immunoglobulin E (IgE)-mediated food allergy to crustaceans and mollusks is relatively common and affected individuals typically react to a range of different species. The only known major allergen of shrimp was first described over 20 years ago and later identified as the muscle protein tropomyosin. This protein may be useful as a defined and relevant diagnostic marker for allergic sensitization to invertebrate foods. In order to generate an assay reagent suitable for this purpose, tropomyosin from the shrimp *Penaeus aztecus* (Pen a 1) was produced as a recombinant protein in *Escherichia coli* and characterized with respect to IgE antibody binding properties in comparison to natural shrimp tropomyosin. Hexahistidine-tagged rPen a 1 accumulated as a predominantly soluble protein in the *E. coli* expression host and a two-step chromatographic procedure provided a high yield of pure and homogeneous protein. rPen a 1 displayed chromatographic and folding characteristics similar to those of purified natural shrimp tropomyosin. Serum preincubation with serial protein dilutions revealed similar capacity of recombinant and natural tropomyosin to compete with immobilized shrimp extract for IgE binding. rPen a 1 was further shown to extensively and specifically compete for IgE binding to extracts of other crustacean species, house dust mite and German cockroach.

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Immunoglobulin E-reactivity of wheat-allergic subjects (baker's asthma, food allergy, wheat-dependent, exercise-induced anaphylaxis) to wheat protein fractions with different solubility and digestibility

Baker's asthma, food allergy to wheat, and wheat-dependent, exercise-induced anaphylaxis (WDEIA) are different clinical forms of wheat allergy. We investigated the correlation of solubility and digestion stability of wheat allergens with the IgE-reactivity patterns of different patient groups. Three wheat protein fractions were extracted according to their solubility: salt-soluble albumins and globulins, ethanol-soluble gliadins, and glutenins soluble only after treatment with detergents and reducing reagents. Sera from subjects with history of each variant of wheat allergy were characterized by CAP FEIA and immunoblotting. There was a high degree of heterogeneity of recognized allergens between the different subject groups as well as within these groups. However, subjects with WDEIA showed similar immunoglobulin E (IgE)-reactivity patterns to gliadins and especially to a 65 kDa protein. Subjects with baker's asthma as well as the food-allergic subjects had the most intense IgE-reactivity to the albumin/globulin fraction. The latter group additionally showed IgE-reactivity to the other fractions. Divergent results of immunoblotting and CAP-FEIA demonstrated that the detection of wheat-specific IgE highly depends on the applied method, thus the diagnostic tool must be carefully chosen. Most wheat allergens were rapidly digested as analyzed by determination of IgE-reactivity on immunoblots to wheat extracts after simulation of gastric and duodenal digestion. However, ethanol-soluble gliadins were stable to gastric enzymes and exhibit low solubility in gastric and duodenal fluids. Therefore, they are likely to be important in food allergy to wheat.

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Isolation and characterization of natural Ara h 6: Evidence for a further peanut allergen with putative clinical relevance based on resistance to pepsin digestion and heat

Peanut allergy is a significant health problem because of its prevalence and the potential severity of the allergic reaction. The characterization of peanut allergens is crucial to the understanding of the mechanism of peanut allergy. Recently, we described cloning of the peanut allergen Ara h 6. The aim of this study was isolation and further characterization of nAra h 6. We purified nAra h 6 from crude peanut extract using gel filtration and anion exchange chromatography. The preparation was further characterized by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) with subsequent immunoblotting. Stability of nAra h 6 was studied by an *in vitro* digestibility assay as well as by resistance against thermal processing. Sequencing of nAra h 6 identified the *N*-terminal amino acid sequence as MRRERGRQGDSSS. Further results clearly demonstrated stability of nAra h 6 against pepsin digestion and heating. Immunoglobulin G (IgE) binding analysis and its biological activity shown by RBL 25/30-test of natural Ara h 6 supported the importance of this peanut allergen. Investigation of nAra h 6 revealed evidence for a further peanut allergen with putative clinical relevance based on resistance to pepsin digestion and heat.

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Evaluation of the potential allergenicity of the enzyme microbial transglutaminase using the 2001 FAO/WHO Decision Tree

All novel proteins must be assessed for their potential allergenicity before they are introduced into the food market. One method to achieve this is the 2001 FAO/WHO Decision Tree recommended for evaluation of proteins from genetically modified organisms (GMOs). It was the aim of this study to investigate the allergenicity of microbial transglutaminase (m-TG) from *Streptoverticillium mobaraense*. Amino acid sequence similarity to known allergens, pepsin resistance, and detection of protein binding to specific serum immunoglobulin E (IgE) (RAST) have been evaluated as recommended by the decision tree. Allergenicity in the source material was thought unlikely, since no IgE-mediated allergy to any bacteria has been reported. m-TG is fully degraded after 5 min of pepsin treatment. A database search showed that the enzyme has no homology with known allergens, down to a match of six contiguous amino acids, which meets the requirements of the decision tree. However, there is a match at the five contiguous amino acid level to the major codfish allergen Gad c1. The potential cross reactivity between m-TG and Gad c1 was investigated in RAST using sera from 25 documented cod-allergic patients and an extract of raw codfish. No binding between patient IgE and m-TG was observed. It can be concluded that no safety concerns with regard to the allergenic potential of m-TG were identified.

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Food allergy to apple and specific immunotherapy with birch pollen

Conflicting results concerning the effect of specific pollen immunotherapy (SIT) on allergy to plant foods have been reported. The aim of this study was to investigate the effect of SIT using a birch pollen extract on food allergy with focus on allergy to apple. Seventy-four birch pollen-allergic patients were included in a double-blind, double-dummy, and placebo-controlled comparison of sublingual-swallow (SLIT) and subcutaneous (SCIT) administration of a birch pollen extract. Sixty-nine percent of these patients reported allergy to apple. The clinical reactivity to apple was evaluated by open oral challenges with fresh apple and a questionnaire. The immunoglobulin E (IgE)-reactivity was assessed by skin prick test (SPT), specific IgE, and leukocyte histamine release (HR). Forty patients were included in the final evaluation of the effect of SIT. The challenges were positive in 9 (SCIT), 6 (SLIT), and 8 (placebo) patients after treatment compared to 10, 4, and 10 patients, respectively, before SIT. The symptom scores to apple during challenges decreased in all groups, but only significantly in the placebo group ($p = 0.03$). As evaluated by the questionnaire, the severity of food allergy in general did not change and there were no differences between the groups. In spite of a significant effect on seasonal hay fever symptoms and use of medication and decrease in IgE-reactivity, SIT was not accompanied by a significant decrease in the severity of allergy to apple compared to placebo. Therefore, oral allergy syndrome (OAS) to apple should not be considered as a main criterion for selecting patients for birch pollen immunotherapy at present.

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Development of an epitope-specific analytical tool for the major peanut allergen Ara h 2 using a high-density multiple-antigenic peptide strategy

Using the major peanut allergen Ara h 2 as an example, an analytical tool enabling the determination of immunoglobulin E (IgE)-epitopes in processed food allergens was developed. We synthesized a multiple-antigenic peptide (MAP) of the IgE-reactive linear epitope 3 (amino acid positions 27 – 36) of Ara h 2 and raised a monospecific antiserum against this epitope to obtain a positive control for future epitope resolved diagnostics. First, a MAP of epitope 3, having a molecular mass of 7770 Da, was synthesized, purified, and its structure confirmed by liquid chromatography-mass spectrometry (electrospray ionization) (LC-MS(ESI)), matrix assisted laser desorption/ionization-time of flight (MALDI-TOF), and Edman sequencing. The MAP was then used to raise high titer antibodies in rabbits using the adjuvant Titermax™ and to characterize the specificity of IgE from allergenic patients sensitized to Ara h 2. The antiserum exclusively detects Ara h 2 in crude peanut extract with a titer of 10^7 by Western blot and reacts specifically with epitope 3 shown by epitope mapping for a library of solid-phase-bound synthetic 15-mer peptides covering the entire sequence of Ara h 2. Such IgE-reactive epitopes are of high analytical relevance as they could constitute the basis for epitope-specific detection systems for use in quality control in the food industry or for forensic purposes in cases of fatal reactions to otherwise undetected peanut proteins.

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Effect of roasting history and buffer composition on peanut protein extraction efficiency

Peanut is a major allergenic food. Undeclared peanut (allergens) from mis-formulation or contamination during food processing pose a potential risk for sensitized individuals and must be avoided. Reliable detection and quantification methods for food allergens are necessary in order to ensure compliance with food labelling and to improve consumer protection. The extraction of proteins from allergenic foods and complex food products is an important step in any allergen detection method. In this study, the protein extraction efficiency of various buffers prepared in-house and some extraction buffers included in some commercial allergen enzyme-linked immunosorbent assay (ELISA) test kits for peanut determination in food products were tested. In addition, the effect of roasting history on the extractability of peanut protein was investigated by the biuret and the bicinchoninic acid (BCA) assays. Elevated roasting temperatures in food processing were found to have a major impact on protein extraction efficiency by reducing protein yields of oil and dry roasted peanuts by 50 – 75% and 75 – 80%, respectively, compared with the raw material. Extraction buffers operating in the higher pH range (pH 8 – 11) showed best yields.

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Systematic survey on the prevalence of genes coding for staphylococcal enterotoxins SElM, SElO, and SElN

Staphylococcus aureus remains a leading cause of food-poisoning with substantial impact on public health. Using a multiplex polymerase chain reaction-DNA enzyme immunoassay (PCR-DEIA), we studied the presence of genes encoding staphylococcal enterotoxin-like (SEl) superantigens *sem*, *sen*, and *seo*, associated with the enterotoxin gene cluster (*egc*), in 429 clinical *Staphylococcus aureus* isolates. 294 (68.5%) isolates tested positive for at least one of the three SEl genes. In contrast to the fixed gene combination *seg*/*sei* also located on *egc*, a substantial number of isolates ($n = 108$) were found to bear only one or two of the genes encoding SElM, SElN, and SElO. Regarding the origin of the *S. aureus* isolates, a significant difference ($P = 0.022$) was found for the possession of *seo* (61.2% of blood isolates *versus* 42.9% of nasal strains). Also *sem* (not significantly) was found more common in blood isolates (52.1% *versus* 40.5%). The survey of the newly described SEl genes *sem-seo* supports the concept that most clinical *S. aureus* isolates harbor subsets of pyrogenic toxin superantigens. The potential contribution of *seo* and *sem* to the pathogenic potential of *S. aureus* has to be further evaluated.

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Expression profiles of effector proteins SopB, SopD1, SopE1, and AvrA differ with systemic, enteric, and epidemic strains of *Salmonella enterica*

The presence and expression of *sopB*, *sopD1*, *sopE1*, and *avrA* genes encoding virulence associated effector proteins were studied comparatively in 405 *Salmonella enterica* strains. They belong to different serovars and clonal types (genotypes, phage types) and originated from different clinical (systemic infection, focal enteritis, enterocolitis) and epidemic sources (epidemics, sporadic cases). The *sopB* and *sopD1* determinants were commonly prevalent, but *sopE1* and *avrA* genes only in 55% and 80%, respectively. A correlation of this pattern of absence and presence of the respective genes to the epidemic and clinical origin could not be detected. In contrast, the expression of the respective genes appeared differently: SopB and SopE1 proteins are well produced, but SopD1 and AvrA proteins only rarely under the applied standard culture conditions. However, using a range of different environmental signals (temperature, pH, cations, *etc.*) some of the *S. enterica* nonproducer strains (*e.g.*, *S.* Agona, *S.* Bovismorbificans, *S.* Virchow, *etc.*) begin to produce AvrA and SopD1. They turned now into an expression profile which was found typically for the epidemic strains of *S.* Typhimurium and *S.* Enteritidis. Also *S. enterica* strains from systemic infections could be characterized by their strong SopB and SopE1 expression while SopD1 and AvrA proteins were missing. Although it is premature to outline generally a correlation of these expression profiles and the clinical and epidemiological potency of Salmonellae, the reported results allow a first understanding how a fine tuning of their virulence will take place.

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Molecular characteristics of *Escherichia coli* serogroup O78 strains isolated from diarrheal cases in bovines urge further investigations on their zoonotic potential

We investigated the virulence properties and clonal relationship of 21 *Escherichia coli* strains of serogroup O78 isolated from diarrhoeic cattle and calves. Isolates were screened for 18 genes representing virulence features of different *Escherichia coli* pathotypes. None of the strains harboured enterotoxin-genes *estIa/Ib*, *eltIa*/*Ib,* or Shiga toxin (*stx*) genes, genes involved in adhesion (*eae*, *f5*, *f41*) hemolysin gene *hlyA* or invasion gene *ipaC*. With a high prevalence we detected enterotoxin *astA* (61.9%), genes involved in iron acquisition, like *fyuA*, *irp* (each 57.1%) and *iucD* (81.0%), and the operon sequence of Colicin V plasmids (38.1%). Some strains possessed toxin genes *cdt-IIIB* and *cnf1/2* (both 14.3%), the invasion gene *tia* (23.8%), and the serine protease encoding gene *espP* (23.8%). Moreover, we could show that *E. coli* O78 strains under investigation were able to adhere to and invade MDBK-cells with varying efficiencies. The results indicate that the closely related O78 strains, constituting two major PFGE-clusters, harbor various virulence features for bovine intestinal disease but cannot be grouped into one of the common *E. coli* intestinal pathogenic or other pathotypes according to their virulence gene pattern. Nevertheless, the ability to adhere, invade or harbor toxin genes lets us suggest that O78 strains isolated from diarrheal cases in bovines urges further investigations on the zoonotic potential of these strains.

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A rapid method for the discrimination of genes encoding classical Shiga toxin (Stx) 1 and its variants, Stx1c and Stx1d, in *Escherichia coli*

Subtyping of Shiga toxin (Stx)-encoding genes by conventional polymerase chain reaction (PCR) is time-consuming. We developed a single step real-time fluorescence PCR with melting curve analysis to distinguish rapidly stx_1 from its variants, stx_{1c} and stx_{1d}. Melting temperatures (T_m) of 206 Stx-producing *Escherichia coli* (STEC) identified to harbor stx_1 or stx_{1c} were analyzed using a specific hybridization probe over the variable region. 170 of 171 stx_1-harboring STEC displayed T_m of 69°C to 70°C, whereas 34 of 35 strains containing stx_{1c} had T_m of 65°C – 66°C. This constant and reproducible difference of 4°C demonstrated that melting curve analysis is a reliable technique to differentiate stx_1 from stx_{1c}. Two isolates displayed atypical T_m. Sequence analysis showed that one of them was 100% identical to stx_{1d} within a 511 bp DNA stretch. Our data demonstrate that real-time PCR is a rapid and reliable tool to differentiate stx_1 from stx_{1c} and stx_{1d} and to detect new stx_1 variants. Because stx_1-harboring STEC cause diarrhoea and hemolytic-uremic syndrome, whereas those containing stx_{1c} are often shed asymptomatically, a rapid differentiation between stx_1 and its variants using the procedure developed here has both clinical implications and a direct significance for the risk assessment analysis of STEC isolated from foods.

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Occurrence of antibiotic-resistant enterobacteria in agricultural foodstuffs

Antibiotic-resistant bacteria or their corresponding resistance determinants are known to spread from animals to humans *via* the food chain. We screened 20 vegetable foods for antibiotic-resistant coliform bacteria and enterococci. Isolates were directly selected on antibiotic-containing selective agar (color detection). Thirteen "common vegetables" (tomato, mushrooms, salad) possessed $10^4 – 10^7$ cfu/g vegetable of coliform bacteria including only few antibiotic-resistant variants ($0 – 10^5$ cfu/g). All seven sprout samples showed a some orders of magnitude higher contamination with coliform bacteria ($10^7 – 10^9$ cfu/g) including a remarkable amount of resistant isolates (up to 10^7 cfu/g). Multiple resistances (up to 9) in single isolates were more common in sprout isolates. Resistant bacteria did not originate from sprout seeds. The most common genera among 92 isolates were: 25 *Enterobacter* spp. (19 *E. cloacae*), 22 *Citrobacter* spp. (8 *C. freundii*), and 21 *Klebsiella* spp. (9 *K. pneumoniae*). Most common resistance phenotypes were: tetracycline (43%), streptomycin (37%), kanamycin (26%), chloramphenicol (29%), co-trimoxazol (9%), and gentamicin (4%). The four gentamicin-resistant isolates were investigated in molecular details. Only three (chloramphenicol) resistant, typical plant-associated enterococci were isolated from overnight enrichment cultures. In conclusion, a contribution of sprouts contaminated with multiresistant, Gram-negative enterobacteria to a common gene pool among human commensal and pathogenic bacteria cannot be excluded.

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Molecular monitoring of environmental conditions influencing the induction of ochratoxin A biosynthesis genes in *Penicillium nordicum*

A real-time polymerase chain reaction (PCR) system specific for the ochratoxin A polyketide synthase gene (*otapks*PN) of *Penicillium nordicum* has been used to analyze environmental conditions, influencing the induction of that key gene of the ochratoxin A biosynthetic pathway. Generally, the induction of that gene coincides very well with the biosynthesis of ochratoxin A, demonstrating that its induction can be used as a molecular signal to monitor ochratoxin A production. It could be shown, that the expression of the *otapks*PN gene is greatly dependent on the media used. In YES medium expression is highest, followed by minimal medium which support ochratoxin A production and minimal medium which suppresses ochratoxin A production. The amount of ochratoxin A produced shows the same tendency. The amount produced is highest on YES medium and decreases successively to the two minimal media. The system was also used to determine the influence of environmental parameters like temperature, pH and NaCl concentration on the expression of the *otapks*PN gene and on ochratoxin A production in parallel. It could be shown that under acidic conditions, below pH 5.0, the expression of the *otapks*PN gene as well as the ochratoxin A concentration were reduced. In case of salt concentration again both measures coincide, having both highest values at increasing NaCl concentrations. In case of the temperature, however, expression of the *otapks*PN gene was uncoupled to ochratoxin A production. The expression was high at all temperatures tested, however, clear differences in the biosynthesis of ochratoxin A by *P. nordicum* could be observed at the different temperatures, showing highest production at 25°C. The importance of these data are discussed with reference to the natural habitat of *P. nordicum.*

