

Involvement of Haptens in Allergic and Non-Allergic Hypersensitivity

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Abstract

The environment is used as a broad umbrella term for all sources of allergens which may cause allergic hypersensitive reaction of the immune system of humans. Westernization of life leads to increased average consumption of food additives (natural and xenobiotics) – starting from over 5kg (10lbs) annually with a strong tendency to intensify. Modern technologies of food production often employ substances improving quality, texture, colour, taste, acidity or alkalinity. Haptens present in food or drugs, penetrating organism via gastrointestinal tract, may be responsible for symptoms not only in the gastrointestinal tract itself, but also in peripheral organs. Mechanisms of hapten action within human body are different – they include reactions when the immune system is involved (allergic hypersensitivity) and not involved (food intolerance also known as non-allergic hypersensitivity). Food intolerance is a more frequent phenomenon diagnosed in 20-50% of cases; food allergy affecting 6-8% children and 1-2% adults. Our work elucidates mechanisms of hypersensitivity responses to haptens, and characterizes major haptens applied as food additives.

Keywords: allergic reactions, hypersensitivity reactions, health safety, food haptens, food allergens

Introduction

Predispositions for the Occurrence of Hypersensitivity Reactions

Today allergic reactions to environmental factors are more frequently recognized. Their number has tripled in the last forty years. Nowadays, the occurrence of IgE-dependent allergic diseases is estimated to affect 20% of the population [1]. This ratio increases if other pathological mechanisms

responsible for the occurrence of allergic disease symptoms (IgG-dependent, cellular) are included. Food antigens are particularly significant for triggering immunological hypersensitivity reactions. It is caused mainly by the fact that during life a person consumes about 10 tons of antigenically-varied food, which also contains significant amounts (from 10^2 to 10^{12}) of microorganisms per 1 cm³ of food. It also seems to be crucial that the estimated area of mucus in the gastrointestinal tract is approximately 300 square meters. Due to the vast contact surface with unfamiliar antigens, about 80% of daily antibody production takes place in the gastrointestinal tract (a few grams of immunoglobulins daily). Thus, the gastrointestinal tract is one of the largest body defence barriers.

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Food allergy (caused by food compounds) is an important, but still not well-understood problem, affecting 6-8% of children and 1-2% of adults [2]. The reasons why some individuals develop such a disorder are sought in genetic, anatomic-physiological, and environmental conditions. The progress observed in genomics has contributed to the discovery of chromosome-localized genes responsible for expression of receptors for IgE, synthesis of pro- and anti-inflammatory cytokines, synthesis of TCR receptors, adhesion molecule receptors, chemokines, and expression of MHC antigens [3-5]. The following chromosomal regions: 5q 31-33, 5q, 6p21.3, 11q13, 16p12 of chromosome 12 and 14, as well as 16p12 and 17q, were mainly found to be of particular importance. Among other things, the intensity of observed allergic reactions and the incidence of asthma are explained by a subtle genetic diversity observed among human races.

A substantial role in the incidence of allergic hyperactivity is played by environmental factors [6]. It is confirmed that the incidence and the level of symptom progression may be predisposed or modified without activation of immunological mechanisms, e.g. physical exertion or exposure to UV radiation. Moreover, immunity may be modified by increased exposure to various environmental antigens, e.g. via contact with bacteria, viruses, parasites, or microbiological cleanliness of the human environment [7-12].

The increased probability of incidence of hypersensitivity reaction may be a result of airway contacts, especially frequent and long-term, with toxic chemical compounds which may act as adjuvants, e.g. cigarette smoke chemicals and vehicle exhaust fumes (diesel asthma) [13]. A similar effect through frequent, direct contact, may be exerted by substances modifying physiological functions of skin and mucous membranes (ointments, cosmetics, antibiotics, disinfectants), as well as long-term treatment with some drug components [14]. Hypersensitivity reactions of the digestive tract may be demonstrated after consumptions of food products containing increased concentrations of natural compounds like histamine and tyramine, or dyes - for example: carmine (E120), indigo-carmine (E132), synthetic cochineal (E124) (also preserved with sulphites [15]).

A mode of contact is also of crucial significance, i.e. the size of a large single dose or repeated smaller doses of antigens, likewise chemical structure and penetration route and the ability to translocate and accumulate in specific tissues, organs and organisms.

According to the European Academy of Allergology and Clinical Immunology (EAACI), allergens are antigens triggering reactions of immunological hypersensitivity. Most frequently, these are proteins (lipo- or glycoproteins) of 3-160 kDa (predominantly, 20-40 kDa). The potential to cause allergy is also demonstrated by some haptens (i.e. chemical compounds of molecular weight lower than 1 kDa) most often by conjugation with organism proteins. These compounds include preservatives, dyes, and antibiotics present in food. Sometimes carbohydrates and their protein conjugates may become allergens. They are recognized by an immune system which leads to IgE production and development of type I allergy or specific TCR receptors and type IV allergy

according to Gell-Coombs classification of hypersensitivity [1]. Symptoms of type II and III hypersensitivity are less frequently triggered by consumption of food products.

The environment is used as a broad umbrella term for all sources of allergens which may cause hypersensitive reaction of the human immune system [16-18]. Due to their heterogenic structure, allergens are characterized by various biochemical, biological and physical properties. Some of them demonstrate enzymatic activity, which enables modification of mucous membranes and antigen penetration [19]. At the moment (May 2008), out of the total number of 2438 allergens described in Allergome database, 932 allergens were included and 788 identified and assigned to 139 protein families in the AllFam database [20, 21].

Considering a spectacular antigenic variety of food compounds, the above-mentioned interactions may affect a broad spectrum of antigen reactions. Thus, it is very important to evaluate health safety of food products, including the new generation and so-called functional food.

Physicochemical properties, nutritional and biological values, digestibility, and absorption are the main parameters to be analyzed. Antigenic and immunoreactive properties of food are rarely taken into account. This is particularly important in the case of food allergies when the immune response to food antigens is not typical (atopic).

Defence mechanisms of immune system are complex. Locally produced cells and mediators in gut-associated lymphatic tissue (GALT) may be transferred to peripheral organs via lymphatic and cardiovascular systems and, as a result, shape the final systemic immunity.

Until recently it was believed that haptens, due to their low molecular weight, have to be combined with peptides to gain antigenic properties. This belief was undermined by the discovery that there are haptens (e.g. mannitol) which are able to induce IgE-dependent reactions directly because of their similarity to protein-bound sugar (d-mannose) [22]. Haptens enter the body via mucous or serous membranes or skin; they sometimes penetrate to tissues during surgical procedures, leading to induction of inflammatory allergic reactions. Examples of haptens include metal ions or simple chemical compounds often found in drugs, preservatives, cosmetics, dyes or food ingredients. Some haptens may be released inside the body during digestion of complex chemical compounds (e.g. drugs) in the gastrointestinal system.

Modern technologies of food production often employ substances improving quality, texture, colour, taste, acidity or alkalinity. Westernization of life leads to increased average consumption of food additives (natural and xenobiotics) - starting from over 5kg (10lbs) annually with a strong tendency to intensify [23]. Haptens present in food or drugs, penetrating an organism via the gastrointestinal tract, may be responsible for symptoms not only in the gastrointestinal tract itself but also in peripheral organs, for instance leading to the urticaria, aggravated atopic eczema [24], haemolytic anaemia [25], and changes in central nervous system diagnosed as hyperactivity [26]. They may also provoke symptom manifestation during courses of autoaggression diseases, e.g. drug-induced allergic hepatitis [27] and lupus erythematosus [28].

Table 1. Preservatives used as food additives, which may trigger symptoms of non-allergic hypersensitivity. (Additive substances in food, 1999 [15]).

Preservatives which may trigger symptoms of non-allergic hypersensitivity	
E number	Preservatives
200	Sorbic acid
201	Sodium sorbate
202	Potassium sorbate
203	Calcium sorbate
210	Benzoic acid
211	Sodium benzoate
212	Potassium benzoate
213	Calcium benzoate
214-219	Esters and salts of p-hydroxybenzoic acid (PHB)
220	Sulphur dioxide, sulphite anhydride
221	Sodium sulphite
222	Sodium hydrogen sulphite
223	Sodium metabisulphite
226	Calcium sulphite
227	Calcium hydrogen sulphite
228	Potassium hydrogen sulphite

Data on 2D/3D structures, physicochemical properties, carrier proteins, and similarities among 7,500 haptens have been collected by computer databases. Super Hapten [29, 30] is one of the largest databases, and besides the above-mentioned data it also gathers about 10,000 hapten-related scientific publications.

Mechanisms of hapten action within the human body are different – they include reactions when the immune system is involved (allergic hypersensitivity) and not involved (food intolerance, also known as non-allergic hypersensitivity).

There is a clear lack of epidemiological data on hypersensitivity reactions caused by haptens. According to Johansson et al., haptens cause such reactions ‘rarely’ if compared to antigens [1].

Non-allergic food hypersensitivity (food intolerance) is a more frequent phenomenon, diagnosed in 20-50% of cases; however, the situation when both pathophysiological syndromes overlap leads to diagnostic problems.

Mechanisms of Allergic Hypersensitivity

There are four ways that haptens can gain their allergic properties:

a) by coupling to extracellular protein, e.g. in transcellular liquid. Metal ions bind to protein and change structural conformation of proteins, which leads to treating them as ‘non self’ by the immune system [31],

b) by coupling to intracellular proteins and Class I MHC presentation, involving the induction of cytotoxic cells [32],

c) by coupling outside the MHC groove and direct stimulation of CD4 TCR lymphocytes. This mode of T lymphocyte stimulation resembles stimulation by superantigens [33, 34]. Günther suggested the term superhaptens, especially for metal ions such as: Ni²⁺, Co²⁺, Cr³⁺, and Cu²⁺.

d) by direct coupling with cytoplasm of antigen-presenting cells (APC), if hapten has a lipid structure and can penetrate cytoplasmic membrane [29, 30].

Having gained their antigenic properties, haptens shift throughout the mucous membrane of the gastrointestinal tract and are internalized by APCs by endocytosis and pinocytosis. Most frequently, mature dendritic cells (presenting on their surfaces molecules CD80/86, receptors for chemokines, and increased density of Class I and II MHC antigens) and macrophages stimulated by IFN γ act as APCs. After internalization of allergen via pinocytosis, they release numerous cytokines such as TNF- α , IL-12, and IL-18. Moreover, the presence of IL-4 in transcellular spaces was observed in atopic patients contrary to healthy individuals. After enzymatic defragmentation and glycation allergens are presented to Th2 lymphocytes, which stimulate migration of eosinophils, granulocytes, and transformation of B lymphocytes into plasmatic cells producing antibodies (mainly IgE) via released cytokines: IL-4, 5, 6, 13, GM, and CSF. Other cell populations are also involved in the development of inflammatory allergic response. They include mastocytes, a small amount of basophils, monocytes and Treg lymphocytes, which are supposed to reduce and terminate the process. Currently, it is believed that a significant role in shaping the immune response of an organism to external antigen depends on the balance between Th1/Th2/Treg/Th17 lymphocyte subpopulations and lymphocyte-produced reaction mediators (IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IFN- γ (α/β), TGF- β). Such homeostasis, together with overlapping interactions of environmental micro-organisms and their metabolites, lies at the basis of studies on molecular mechanisms of allergy incidence [12]. Additionally, the increased level of pro-inflammatory cytokines (IL-17, TSLP and IL-6) in proportion to regulatory cytokines (IL-10 and TGF- β) may play an important role in diagnostics of inflammatory, allergic/autoimmune diseases and be a marker preceding disease signs in asymptomatic patients.

In 2008 McFadden et al. presented an interesting hapten-atopic hypothesis. According to this view, the increase in number of cases of atopic diseases observed during the last 40 years in Western countries is caused by consumption of processed products containing many artificially-created haptens. The authors believe that food, especially bread and milk, consumed in early stages of human life and containing numerous haptens, leads to reducing tolerance to allergens present in food. It happens because haptens activate different mechanisms than allergens. Destruction of natural barriers leads to inflammatory and allergy symptoms in organs, including skin, and to atopic dermatitis development [31].

Pathomechanisms of Non-Allergic Food Hypersensitivity

Chemical compounds characterized by small-sized molecules and present in food may have a negative influence on organisms without activation of immunity mechanisms.

According to Madsen, Denmark was the first country that in 1973 introduced a legal ban to food additives [32]. Since 1984 the ban has been extended on food dyes. Norway and Sweden followed Danish legislation with additional restrictions on food preservatives. However, a lack of consequence has been observed. The production of 'light' soft drinks and sweets (containing saccharine) increases systematically. Besides, the introduction of low-caloric semi-products (with fat partially replaced by water) and bread has seen the addition of emulsifiers, modified starch, carrageenans, glutamines, and antioxidants. As a result, consumption of food additives has increased [32]. They were tested on animal and human models.

Substances administered long-term to laboratory animals (as well as in high quantities) caused carcinogenic, or toxic- especially cardiotoxic changes, leading to behavioural disorders, induced diabetes and also element deficiencies. Significant differences between permissible doses and those causing acute or chronic toxicity in humans were observed. Therefore, food additives are safe for healthy individuals, but in patients suffering from allergy they may:

- act as adjuvants, non-specifically escalating the symptoms of atopic diseases: asthma and atopic dermatitis,
- change ratios of released prostaglandins and leukotrienes, like salicylates. Blocking of cyclooxygenase causes predominance of leukotrienes. Leukotrienes act chronically, constrictorally on bronchi and increase oedema of mucous membranes and mucus secretion. Asthma symptoms occur, although the reaction mechanism is exclusively biochemical.
- toxic cell destruction of mastocytes and basophils may lead to urticaria in children. A similar phenomenon was observed in prick-tests, leading to false positive results.
- demonstrate more advanced symptoms: blood pressure drop, tachycardia, skin hyperaemia, urticaria, rhinorrhoea. These symptoms, induced by haptens (mainly tartrazine), are caused by degranulation of a large number of mastocytes due to ionic disturbances (zinc ions are chelated by tartrazine),
- lead to hyperactivity and increased aggression. Such reactions in children were previously explained by swelling of the central nervous system (CNS) structures during the course of an allergy. It turned out that consumed aspartame, which doubles the concentration of phenylalanine in CNS, may be a 'non-immunological' reason. The increase in tyrosine concentration followed by a decrease in the serotonin precursor tryptophan was also reported. Low levels of serotonin in CNS of children were found to be responsible for hyperactivity and increased aggression.
- migraine attacks observed after consumption of chocolate, cocoa and sweets are also linked to direct influence

Table 2. Colouring materials used in food production, which may trigger symptoms of non-allergic hypersensitivity (pseudoallergy).

	E number	Colouring materials	Colours
Kind of dyes*	102	Tartrazine	lemon yellow
	104	Quinoline yellow	yellow
	110	Orange yellow S	orange yellow
	120	Carminic acid (carmin dye)	red
	122	Azorubine (azo dye)	red
	123	Amaranth (azo dye)	red
	124	Cochineal red A (azo dye)	red
	127	Erythrosine BS (azo dye)	red
	151	Brilliant black BN	black
	160 (b)	Bixin	orange
	161(g)	Canthaxanthin	orange yellow
	131	Patent blue	blue**

*Additive substances in food, 1999 [15]

**banned in Australia, USA and Norway [39]

of food compounds on mastocytes. It is explained by the low level of maturity of enzymatic systems in children, reduced ability of binding compounds to plasma proteins, and the ability of low-molecule compounds to penetrate a blood-brain barrier.

The Incidence of Selected Additives in Food

It is estimated that a person consumes about 12-60 different food additives during a single meal [23], thus a potential health threat for consumers suffering from non-allergic hypersensitivity is high.

Food additives used in food production are expected to make the product more attractive and prolong its shelf-life. Some of them, e.g. benzoates, disulphides, sulphates IV, aspartame, dyes, and sodium glutamate consumed regularly may cumulate in an organism and trigger symptoms of intolerance.

Benzoates are used as food preservatives and act by inhibiting enzymatic pathways of micro-organisms and their spores. Their excessive consumption may lead to allergy in asthmatics and allergy sufferers, and in people sensitive to aspirin may cause gastrointestinal disorders.

Disulphides, applied mainly as food and drink preservatives, are found in numerous products: bread additives, tea, sweets, seafood, jams, jellies, dried fruit and vegetables, fruit juices, vegetable preserves, potato powder, frozen potatoes, and soup concentrates.

Sulphates IV added to stabilize pharmacologically active substances may also be a direct reason for non-allergic hypersensitivity. It often happens that in such cases side-effects are falsely linked to the medicine and not to the additive itself.

Sulphur dioxide and sulphites at concentrations of more than 10 mg/kg or 10 mg/litre expressed as SO₂ should be disclosed on product labels, as they may be hazardous to consumer health [33].

The low-calorie sweetener aspartame has replaced sugar in many foods and drinks. Its compounds (methanol and genetically-engineered enzyme) and formaldehyde (a degradation product) cause a variety of symptoms. Previous studies suggest that it may cause vasomotor swelling of labia, eyelid, foot and hand swelling; however, their incidence caused by aspartame consumption is very rare and needs more detailed research.

Many natural and artificial colourings (especially the natural ones because of the character of the antigene), used as colour improvers in food products can be associated with adverse reaction, including allergenic hypersensitivity (Table 2) [15, 38, 39].

Dyes are widely applied in food production. Since 1996 the European Union has banned (with a few exceptions): erythrosine E127 (since 1992 it may be used only in fruit preserves containing black cherries, max. 150-200 mg/kg), canthaxanthine E161 (allowed only in Strassburg sausages, max. 15 mg/kg), tartrazine E102 (not allowed to be added to cakes, sweets, ice cream, hard cheeses in Poland).

Since 1976 amaranth dye (E123) has been banned in the USA, whereas in Poland it may be added to food, e.g. wines, spirits and caviar.

Monosodium glutamate (MSG) is a sodium salt of glutamic acid used as a flavour enhancer of characteristic broth-like umami (savoury) taste. The use of MSG is widespread in Asian countries, thus the symptoms of food intolerance triggered by it are also sometimes called 'Chinese Restaurant Syndrome'. However, scientific studies have not confirmed this link. The other ingredients of Asian cuisine, which are strong allergens, e.g. shrimps, nuts, peanuts or certain spices, are probably responsible for triggering allergic reactions (not additives) [34].

Numerous cases of hypersensitivity to food additives, e.g. disodium EDTA -(Ethylenedinitrilo) tetraacetic acid disodium salt, xanthan gum, and disulphides, are known; however, Bateman et al. (2004) comment critically on previous reports that suggest that artificial food additives such as colouring and benzoate preservatives are potentially linked to hyperactivity in children and that children with atopy may be at particular risk [35].

For educational and informational purposes Tables 1 and 2 present the most popular food additives that may trigger symptoms of non-allergic hypersensitivity (or rarely and in single cases – allergic hypersensitivity) [15, 39].

Aspects Linked to Health Safety of Consumers

Clinical consequences of food allergy are frequently serious, considering that a systemic anaphylactic reaction may pose a direct threat to life. Since avoiding allergens still seems to be the main therapeutic recommendation, people's education as well as support from food technologists and analysts to detect and identify potential allergens in food products are crucial.

To protect consumers from potential hypersensitivity, the European Union Commission Directive has announced that it will regulate the content of food labels [36]. Food labelling is mainly aimed at people suffering from food allergies, so label declaration must assure customers that a product is absolutely safe [37].

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