

Novel Formulations for Oral Allergen Vaccination

Natalija Polovic and Tanja C. Velickovic*

Faculty of Chemistry, Department of Biochemistry, University of Belgrade, Belgrade, Serbia

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Abstract: Allergen specific immunotherapy, comprised of subcutaneous injections of increasing doses of allergen extracts, has been shown to be the only treatment able to influence the natural progression of allergic disease.

Different forms of local immunotherapies, involving oral, sublingual and nasal routes of allergen administration, have also been considered in clinical practice. The inability of the protein to survive gastrointestinal physiological barriers is a generally encountered problem in oral administration of protein drugs.

In order to overcome the problems of low allergen bioavailability and absorptivity, during oral immunotherapy, several stabilization strategies have been outlined in the recent years. This review focuses on interventions including: hexose monosaccharide, ethyl alcohol and water vehicles, oxygen-containing metal salt based preparations, particles with enteric coating, and poly (lactic-co-glycolic) acid microspheres.

Regarding the enormous potential of oral responsiveness and/or oral tolerance, research that focuses on new and improved carriers or vehicles for safe allergen oral delivery has great potential in treating allergic diseases. This article also reviews some of the recent patents related to the field.

Keywords: Oral immunotherapy, oral allergen delivery, allergy.

ALLERGY AND ALLERGEN SPECIFIC IMMUNOTHERAPY

Allergy is a common health problem whose worldwide prevalence is rising. Nowadays, about 25% of the population in industrialized countries suffers from some kind of allergic symptoms e.g. rhinitis, conjunctivitis, asthma [1]. The pathoetiology of allergy involves several immunopathological mechanisms. Hypersensitivity type I is essentially based on increased production of IgE antibodies against unharmed foreign substances, known as allergens, after a first exposure to the allergen source (sensitization process) [2]. Allergens are molecules which predominantly have protein or glycoprotein structure, with molecular masses ranging from 10 to 100 kDa [1, 3]. Allergens are present in naturally occurring grass, tree or weed pollens, animal fur or dander, food, moulds, dust mites or insect venoms. IgE is an immunoglobulin with a life time of a few hours in circulation, but by binding to the high affinity receptor (FcεRI) on the surface of effector's cells: blood basophiles or tissue mast cells; its life time is prolonged. The later contact of the allergen with IgE bound to mast cells or basophiles triggers the release of histamine and other mediators, causing an inflammatory reaction and leading to the symptoms of allergy [4]. T cells involved in maintenance of allergic reactions belong to the Th2 subset and produce Th2 specific cytokines, like IL5, involved in IgE class switching and recruitment and activation of eosinophiles. Infiltration of eosinophiles into lung tissue is the essential feature of asthma [5].

Classical clinical treatments of allergy are based on the usage of anti-histaminics and corticosteroids. Although pharmacotherapy is very efficient in relieving symptoms of allergic inflammation in patients, it does not treat the pathogenesis of allergy. So far, the only causative treatment of allergic diseases able to influence the natural progression of allergy, has been allergen-specific immunotherapy. The treatment was introduced almost 100 years ago by Noon, who was the first to try desensitizing an allergic patient by repeated subcutaneous injections of the causative allergen (grass pollen extract).

The immunological mechanisms of a successful allergen vaccination are still not entirely resolved, but they are believed to involve modulation of antigen-presenting cells, B cell and T cell responses [6]. The proposed mechanisms place T-cells as preferential targets of a successful allergen vaccination, regardless whether the underlying immunological mechanism is induction of a state of anergy and/or deletion of allergen-specific T cells or immune deviation (a shift from predominantly Th2-like to a more Th1-like functional phenotype) [7, 8]. Lately, T regulatory cells have also attracted attention as possible target cells for a successful immunotherapy [9]. Another mechanism by which immunotherapy has been suggested to act is via antigen-specific blocking IgG antibodies. Production of blocking antibodies, especially the IgG4 subclass, leads to inhibition of allergen-induced release of inflammatory mediators from mast cells and basophiles, inhibition of IgE facilitated allergen presentation to T cells, blocking late-phase response, and also further boosts of IgE production [10, 11].

Conventional immunotherapy is the treatment of allergic patients with increasing doses of allergens or allergenic extracts administered by a series of subcutaneous injections. Despite the clinical success of conventional immunotherapy,

*Address correspondence to author at the Department of Biochemistry, Faculty of Chemistry, Studentski trg 16, 11 000 Belgrade, Serbia;
Tel: +381113336661; Fax: +381112184330;
E-mail: tcirkov@chem.bg.ac.yu

there is a great demand for safer approaches, because of the severity of immediate and late-phase side effects [12].

In order to comply with this demand, several new methods have been suggested. Nowadays, different strategies exist in designing novel allergen formulations for specific immunotherapy. These include chemically modified forms of allergens [13-15], genetically modified allergens (hypoallergens) [16, 17], allergen-derived peptides [18], hybrid allergens [19] and allergens bearing immunostimulatory molecules, or adjuvants [20], which foster Th 1 immune responses. Many of them have been proven as safe and reliable in clinical trials [21-24].

Because of the pivotal role of IgE in sensitization, manifestation and maintenance of allergic diseases, a new therapeutic strategy has recently been launched. A monoclonal antibody to IgE (Omalizumab) inhibits IgE binding to Fcε receptors on mast cells, thus preventing degranulation of effector cells and the release of mediators into surrounding tissue [25]. So far it has been proven safe and reliable in treating allergic symptoms. More long-term studies are needed to prove the benefits of using this particular, rather expensive, therapy over traditional injection specific immunotherapy.

Additionally, non-injectional forms of immunotherapy (oral, sublingual, nasal immunotherapy) [26-29] have been introduced into clinical practice and have been shown to be effective, with an improved safety profile in comparison to subcutaneous administration and with their efficiency rising with increasing allergen dosages.

THE IMMUNE SYSTEM OF THE GUT AND ORAL TOLERANCE

The gastrointestinal mucosal immune system has a demanding task in distinguishing between desired and unharmed dietary antigens and intestinal bacterial populations and, on the other hand, possible pathogenic molecules and organisms. The specific environment of the intestinal lumen leads to the development of a powerful tolerogenic mechanism known as oral tolerance [30].

In the intestine, there are several pathways of antigen entry into the organism and their presentation to the mucosal immune system Fig. (1).

A very important portal for antigens present in the gut lumen are the organized lymphoid tissue structures known as Peyer's patches (PPs), which are distributed in subepithelia along the intestinal wall, below specialized epithelial cells known as M cells (microfold cells). M cells are specialized in uptaking of particulate antigens. Antigens entering through the M-cell dependent mechanism are eventually delivered to underlying APSs (DCs or macrophages) which migrate into the PPs, priming immune response. It is thought that Peyer's patches are involved in immune reactions and not in the development of tolerance (ablation of Peyer's patches does not affect tolerance and the studies show that soluble antigens not presented by PPs lead to systemic tolerance, whereas particulate antigens generally prime immune responses) [31-33].

Another important portal for antigen entry and presentation is via subepithelial dendritic cells (DCs). It has

recently been shown that intestinal dendritic cells are able to open the tight junctions between adjacent epithelial cells and to capture bacteria from gut lumen directly across the mucosal epithelium [34]. In respect of invasive or noninvasive pathogens, DCs are able to induce tolerance or reaction [35, 36].

Antigens can be uptaken and presented to the immune system by intestinal epithelial cells which might act as non-professional antigen-presenting cells (APCs) and modulate local immune response through the activation of intraepithelial CD8⁺ T cells with regulatory functions [37, 38].

Antigens passing through intestinal epithelial cells might also escape phagocytosis by APCs and enter into capillaries that culminate in the portal vein [39].

ORAL ADMINISTRATION OF PROTEIN DRUGS

The inability of the protein to survive gastrointestinal physiological barriers is a generally encountered problem in oral administration of protein drugs and leads to low bioavailability of administered protein drugs [40]. Acidic pH in the stomach and the presence of great amounts of proteases in the gastric compartment, intestinal lumen, and the brush border of the enterocytes, influence dominant loss of protein structural integrity necessary for triggering the mucosal immune system. Besides enzymatic barriers, there are significant physical barriers to peptide drug absorption in the intestinal wall. The membranes of adjacent epithelial cells form tight junctions, and this together with the presence of a glycocalyx, the negatively charged mucus layer on the luminal surface of enterocytes, provides the obstacle for protein absorption in the intestine [41-43].

Despite all of the above mentioned obstacles to peptide and protein absorption, there is both clinical and experimental evidence that large molecules, to some extent, may penetrate the intestinal mucosa [44, 45].

The way to enhance protein survival in gastrointestinal conditions is to try to formulate transporting model systems which should protect proteins from the acidic and proteolytic environment of gastrointestinal tract. Also, it would be desirable to create therapeutic protein transporting systems with characteristics such as charge or hydrophobicity, which might adhere to and exploit the intestinal wall features and result in increased systemic uptake of therapeutic proteins [42, 43].

ORAL IMMUNOTHERAPY OF ALLERGIC DISEASES

Oral delivery, the popular and convenient method for drug delivery, is the alternate route of administration. However, orally administered protein drugs are easily degraded in the gastrointestinal tract by the same enzymes that degrade dietary proteins. One of the characteristics of the small intestine is also its high proteinase content, and poor absorption of therapeutic proteins.

Although the most convenient administration method, oral immunotherapy (OIT) of allergic diseases showed very limited efficacy, probably due to significant loss of therapeutic allergens by protease attack in the gastrointestinal tract and/or limited allergen absorption in intestinal

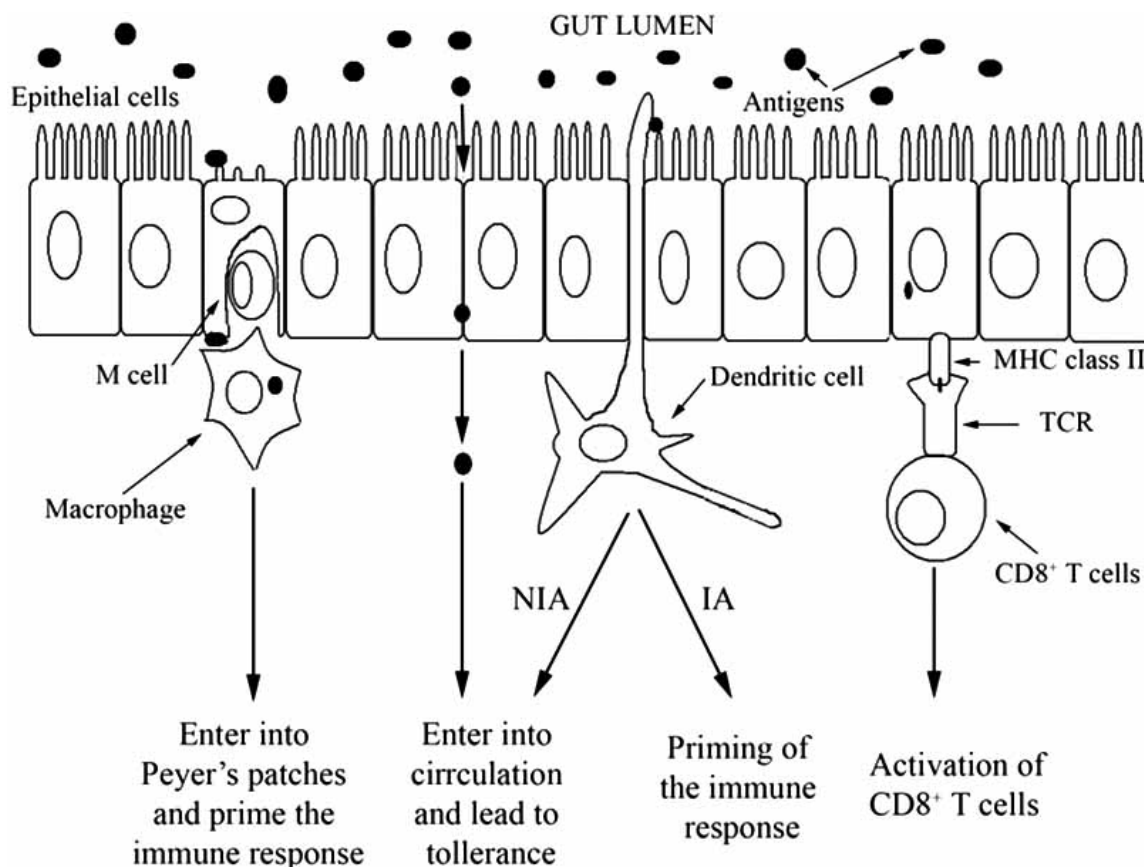


Fig. (1). Possible pathways for entry of antigens present in the gut lumen into the organism and the subsequent immunological consequences. NIA - non-invasive antigens (e.g. gut bacterial flora), IA - invasive antigens (e.g. flagellated pathogens).

compartment. Nine oral immunotherapy trials with grass, birch and ragweed pollen, or dust mite and cat allergenic extracts, were conducted in the last two decades of the twentieth century, but just three of them had shown considerable clinical improvement of the classical symptoms of rhinitis and asthma [46-48]. On the other hand, allergen doses necessary to provoke beneficial outcomes were hundreds times higher than those used in subcutaneous immunotherapy, leading to low cost-effectiveness of OIT. For these reasons, its clinical use was abandoned [49, 50].

In order to overcome the problems in low allergen bio-availability and absorptivity during OIT, several stabilization strategies have been outlined in the recent years. This review focuses on: hexose monosaccharide, ethyl alcohol and water vehicles, oxygen-containing metal salt based preparations, particles with enteric coating, and poly (lactic-co-glycolic) acid microspheres.

HEXOSE MONOSACCHARIDE, ETHYL ALCOHOL AND WATER VEHICLES

The intervention published in 1994, in the European Patent Database, describes a method of preparing therapeutic allergen doses in a liquid vehicle comprising of a defined amount of selected hexose monosaccharide and ethyl alcohol [51].

Most commonly, protection of an orally administered drug from premature destruction has been achieved by enteric coating of a tablet or capsule. Under these conditions,

particles react as solids in the stomach and intestine. Since the active component of an enteric coated dosage form will not be released until the dosage form passes into the intestinal tract, gastric emptying time becomes an important factor. Tablets or capsules may be retained in the stomach for a few minutes or as long as twelve hours. Liquids appear to empty continuously at a more predictable, possibly exponential, rate.

The author claimed that the liquid vehicle described in the invention appears to protect allergen extract during rapid passage through the stomach and could be expected to provide uniform and predictable delivery of allergens to the small intestine.

Hexoses found in nature are aldohexoses (monosaccharides of six carbons, having an aldehyde function) and ketohexoses (monosaccharides of six carbons, having a keto function). The aldohexose sugars are of interest in the described invention.

Therefore, up to now, there are no clinical or animal studies that can support these findings and the usage of described liquid vehicles in allergy treatment.

METAL SALTS BASED PREPARATIONS CONTAINING OXYGEN

Aluminum salts, especially aluminum hydroxide (alum), have a long history in usage as adjuvants in vaccination of human subjects. However, it is shown that alum enhances

Th2 specific immune response [52], as well as the formation of granulomas at the injection sites [53] making alum less suitable for injective allergy treatments.

Still, despite these negative effects of alum usage in conventional allergen immunotherapy, there is a potential in using aluminum salts with adsorbed allergens in oral form of immunotherapy. The authors of the patent WO0045847A1 [54] claimed that adsorption of proteins on oxygen containing metal salts can be modulated with pH changes during the adsorption process or by changes in the working concentration of the metal salt, as well as in the concentration of biologically active molecules, e.g. allergens.

The term "biologically active substance" refers to any substance that has a biological effect in itself or any substance that interacts with or influences other substances, reactions in the body and organs resulting in a biological effect. A wide range of these biologically active substances may be applied in the invention: antigens, allergens, allergoids, peptides, haptens, as well as their analogues or derivatives.

A common feature of delivery systems is that they conserve, retain, deliver, and may enhance the effect of the biologically interactive substance. It is believed that the active substance is adsorbed or coupled to the oxygen-containing metal salt, and this adsorption contributes to the efficacy of the mucosal delivery system. Several factors are of importance for the process of adsorption to the oxygen-containing metal salt. These include pH, the length of time the adsorption reaction is carried out for, mixing conditions, concentration of the active substance, buffer, storage and excipients.

Only metal salts with extremely low water solubility can be used as carriers in mucosal delivery systems. Furthermore, oxygen-containing metal salts can exhibit a depot effect and provide gradual release of allergens from the formulation. Also, of crucial importance is that formulations based on oxygen containing metal salts possess certain entrapment and buffering properties, thus protecting biological substance from the microenvironmental changes, especially preventing acid and proteolytic degradations of entrapped proteins, thereby allowing them to be delivered to the mucosal membrane. A further feature of the oxygen containing metal salts of the mucosal delivery systems can be their capability to adhere to the mucosal membrane, prolonging the time allowed for the interaction of protein therapeutics with the target mucosal tissue, leading to increased transport of proteins via the mucosal membranes and resulting in a cellular and/or humoral immune response.

Regardless to proposing a great number of different salts to be used as potential mucosal delivery systems, the authors used alum adsorbed allergens of *Phleum pratense* in an animal study of oral immunization of mice. The same doses of allergen extract of *Phleum pratense*, with or without alum, were used for the oral immunization of groups consisting of 8 mice. The positive control consisted of intraperitoneally (i.p.) immunized mice. The obtained results suggest that the usage of alum causes a lower oral immune response, yet still comparable with the response in the i.p. immunization protocol.

PARTICLES WITH ENTERIC COATING

There have been many attempts to overcome acid and proteolytic destruction of allergens in the stomach by enteric coating of allergens, a procedure of encapsulation of allergens in a coating which dissolves at basic pH, thus enabling coated particles to pass the stomach and release encapsulated allergens in the intestine. Enteric coating consists of adsorbing allergens from an aqueous solution onto nonpareils - small round particles of pharmaceutically inert materials. The nonpareils formed from sugar/starch core were used in OIT of ragweed pollen allergy [55-57]. Those nonpareils were coated with ragweed allergens by spraying and subsequently enterocoated with polymethacrylic acid. Microencapsulated ragweed allergens' particles were resistant to acid at pH 1.2 for at least 6 hours and dissociated rapidly at a pH greater than 6.0, releasing the proteins in their native form [55]. Although, clinical studies have shown that enterocoated ragweed extract has some beneficial effect on symptom scores and raises blocking IgG levels while suppressing seasonal IgE levels, the effective doses of allergens were higher compared to injectionous immunotherapies.

A method for making an enterocoated allergen preparation, further stabilized by addition of gastrointestinal proteolytic enzymes' inhibitors as ovalbumin (OVA) or soy proteins, was described in 1997 [58]. Generally, the transport enteric coated allergen system is made as described above, with the exception of using an enteric coating preparation based on hydroxyl-propyl-methyl-cellulose-acetate-succinate and with addition of 10 fold the amount of OVA (known to have anti-trypsin activity) in comparison to the allergens content.

The obtained transport particles were used in the conducted *in vitro* gastrointestinal release study. The amount of released ragweed proteins was estimated by inhibitory ELISA. It was shown that none of the ragweed proteins can be detected within 2 hrs of incubation in gastric fluid, but the release of the complete amount of ragweed proteins was finished in half an hour of incubation in intestinal fluid *in vitro*. Also, immunogenicity of encapsulated OVA and ragweed was tested in a mice study. It was shown that coencapsulation of ragweed proteins and OVA leads to a significantly higher immune response than encapsulation of ragweed without OVA.

POLY (LACTIC-CO-GLYCOLIC) ACID MICROSPHERES

The poly (lactic-co-glycolic) acid (PLGA) polymers are shown to be completely biocompatible and biodegradable and thereby suitable for oral delivery of antigens. PLGA particles loaded with antigen are proven to have a depot effect and can release entrapped antigen in a manner which mimics repeated immunizations [59, 60].

In recent years, a few studies on using PLGA particles in oral vaccine development have been published [59-64].

The study of Pecquet *et al.* showed that orally administered milk allergen β -lactoglobulin (BLG) loaded PLGA microspheres are potent tolerogens, inducing specific systemic and mucosal oral tolerance in BALB/c mice.

Tolerogenic response was more pronounced with the usage of BLG loaded PLGA particles than with soluble BLG, probably because of both the protection of protein from degradation in GIT and the depot effect [62].

Also, upgrading of PLGA microspheres with molecules (as nontoxic *Aleuria aurantia* lectin) that specifically target intestinal M cells has recently been reported [64, 65].

PLGA microspheres containing major birch allergen Bet v 1, with covalently linked *Aleuria aurantia* lectin, were potent in provoking IgG immune response specific for Bet v 1 after a two-time oral immunization of a group of 5 mice, while soluble Bet v 1 did not provoke any significant response. Furthermore, ELISA determination of allergen, after two hours of treatment with simulated gastric fluid, showed that the microencapsulated allergenic protein is more efficiently protected from gastric degradation than the non-encapsulated allergen.

It was also shown that PLGA allergen containing microspheres, with covalently linked *Aleuria aurantia* lectin, adhere to the intestinal epithelial origin cell line Caco-2 both in qualitative and quantitative terms, leading to the prolongation of time exposure in the intestinal compartment and specific adsorption of allergens.

Furthermore, oral treatment of birch pollen allergic BALB/c mice with the obtained microspheres results in significantly higher levels of birch pollen-specific IgG2a, compared with the control groups of mice, while with no alteration in the IgG1, IgE, and IgA levels. Allergic mice treated with the microspheres also start to produce significant levels of interferon γ [64].

All these findings lead to the conclusion that PLGA allergen containing microspheres, with covalently linked *Aleuria aurantia* lectin, provide an immune shift toward Th1 specific response in an ongoing Th2 response, and thus can be used in oral treatments of allergic diseases.

CURRENT & FUTURE DEVELOPMENTS

Regarding the enormous potential of oral responsiveness and/or oral tolerance, research focusing on new and improved carriers or vehicles for safe allergen oral delivery has great potential and is a promising new approach to treating allergic diseases. The field of preparing and optimizing orally stable allergen formulations is still open.

It could be helpful to optimize existing pharmaceuticals delivery systems for the delivery of allergens. One of the examples can be the usage of chitosan as a DNA oral carrier [66]. It was shown that chitosan is mucoadhesive and can be transported across the mucosa through M cells or can transfect epithelial cells [67, 68]. Because of all these properties, the chitosan particles loaded with peanut or house dust mite allergens' DNA, were successfully used for oral vaccinations in mice models of allergy [69, 70]. Chitosan particles could be promising agents in the future development of oral allergy treatments, especially for transporting acidic proteins, as chitosan is known to be a positively charged carrier.

On the other hand, oral vaccines based on genetically modified organisms (for example, expression of allergenic

T-cell epitope peptides of major Japanese cedar pollen allergens in rice) showed considerable efficacy in oral immunotherapy for the treatment of pollinosis in the mouse allergy model [71].

Improved and efficient formulations, preferably with controlled release features of targeted allergen, are still lacking in the field of oral delivery. A suitable, improved carrier could maximize the great potential of oral tolerance and wide-spread use of oral delivery in treating allergies.

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CONFLICT OF INTEREST

The authors claim no conflict of interest.

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