CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Allergy

An allergy, in another word allergic disease, occurs when immune system was stimulated by an allergen. Dust-mite, cockroach, animal dander, and pollen are common allergens and capable to stimulate a type-I hypersensitivity reaction in atopic individuals. The allergic symptoms are itchy, cough, running nose, nasal congestion, wheezing, vomiting, diarrhea, and skin rash etc. according to the part of the body contacted by the allergen (Suri, 2006). The reactions and symptoms can be explained by molecular-molecular interactions between antigen and its corresponding immunoglobulin E (IgE antibody) (Hoffmann *et al.*, 2009). After the first exposure to the allergen, antigen presenting cell (APC) will process allergen and present it on surface of APC inducing T helper 2 cell (Th2) to be activated and to secrete some mediators i.e. interleukin-4 (IL-4) etc. The IL-4 will induce B cell to produce IgE on plasma cell, then the IgE will bind to IgE receptor on surface of mast cell (in tissue) and basophile (in circulation). If function of T helper 2 cell (Th2) is equal with T helper 1 cell (Th1), allergy and symptoms are not occurring. But when the function of Th2 is dominant, the second allergen exposure will bind with IgE on mast cell. The mast cell activation and degranulation will occur, chemicals will be released, particularly histamine resulting in allergic symptoms (Scheme 2.1). At this time, there are many treatments that have been developed for allergic diseases such as using anti-inflammatory drug, allergen immunotherapy, and allergen delivery system.

2.2 Treatments

2.2.1 Medication

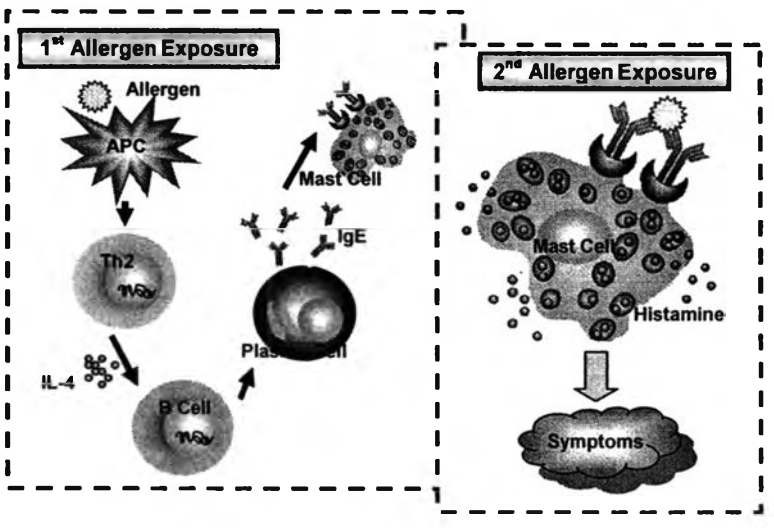
Medication is an act of treating with medicines that prevent or alleviate the symptoms of disease. Medicines, such as antihistamines, corticosteroids, and beta-2 agonists (Rolland *et al.*, 2009) are taken for allergic diseases. This

treatment is effective at controlling symptom. However, it is not preventing the onset of allergies. Moreover, there are some side effects of medicine administration in prolonged use.

2.2.2 Allergen Immunotherapy

Allergen immunotherapy is gradually tolerated to sensitize allergen by a series of increasing administration of allergen (known as vaccine), resulting in reduction of allergic sensitization (Huggins and Looney, 2004). For this treatment, an extracted allergen is administrated to the patient to induce the suppression of Th2 and reduce the production of IgE which is an important culprit of allergy. However, there are some risks of severe allergic reaction from live-attenuated vaccine (extracted allergen which has a possibility for living again) during the immunotherapy. Moreover, this treatment gives the low ability of stimulating immune responses from subunit vaccine.

Scheme 2.1



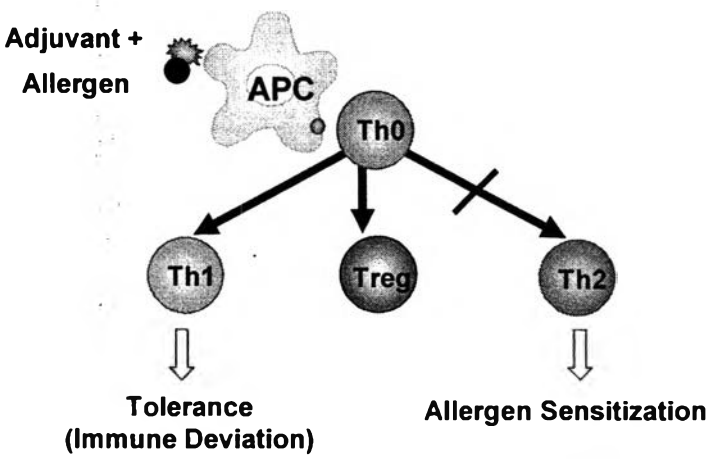
(Geha *et al.*, 2003)

2.2.3 Allergen Delivery System

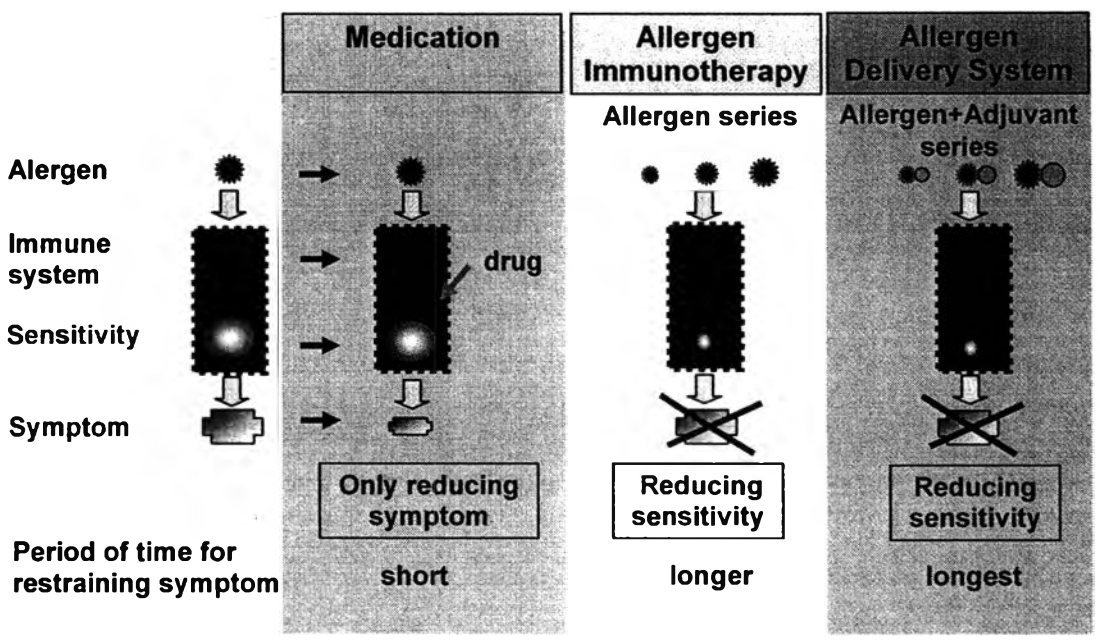
Allergen delivery system is a system of allergen administration which uses adjuvant to enhance immune response. This treatment can control immune

activation with an appropriate level. In general, T helper 2 (Th2) immune response is decreased of dominance. While, function of T helper 1 cell (Th1) increases and induces the T regulatory cell (Treg) which controls balance of Th1 and Th2 (Scheme 2.2) (Mallapragada and Narasimhan, 2008).

Scheme 2.2



Scheme 2.3

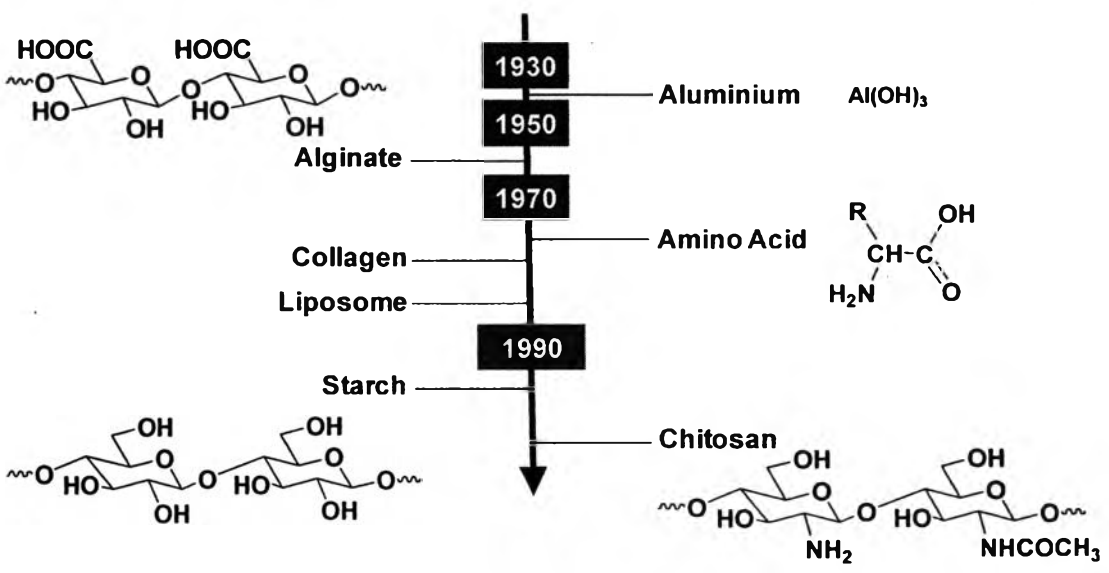


Scheme 2.3 shows a summary of three kinds of treatments which can function in different ways for restraining symptom. Allergen delivery system is a combination of a series of adjuvant and allergen for the treatment which the immune sensitivity in the cell is not significant and at the same time it needs the long period of restraining symptom until the immune system is able to develop the immune response by itself.

2.3 Adjuvant

Adjuvant is a substance which increases allergen activity in stimulating immune response. Moreover, it can decrease allergen concentration and times of administration due to being longer period of time in body. Adjuvant has been discovered for over 80 years (Scheme 2.4). The first adjuvant developed by Freund is alum (Aluminum salt) which is well known as Freund's adjuvant and use in various vaccine such as influenza vaccine (Sutane, 2009). The others such as alginate, liposome, gelatin, amino acid, starch, and chitosan have been reported to use as adjuvants (Scholl *et al.*, 2005) for the past three decades.

Scheme 2.4



(Scholl *et al.*, 2005)

2.3.1 Alginate Conjugates

Alginate is derived from brown seaweed. Alginate-coupled allergen extract was injected to house dust mites' allergic patients. The specific IgE levels decreased significantly, whereas other immunoglobulins levels simultaneously increased (Corrado *et al.*, 1989).

In a multicenter hyposensitization study of allergic patients, the effectiveness of grass pollen extract conjugated with sodium alginate was evaluated. Almost all patients benefited from the treatment, especially the reduction of running nose and sneezing. However, there are some side effects, like local swelling at the injection site, rhinitis, and urticaria (Ortolani *et al.*, 1994).

2.3.2 Collagen

Collagen is a polymer which composes of three polypeptide chains in triple helix form. It has already been used in several medical applications such as treatment and prevention of bone and soft tissue infections, wound healing, as well as ophthalmic and periodontal treatment (Ruszczak and Friess, 2003). Although the collagen is biocompatible, the optimization for sustained delivery is difficult due to the release is hardly to control. Sano *et al.* (2003) developed a novel system by creating mini pellets as carriers for release various types of proteins. Although mini pellets have low antigenicity, they elicited antibody responses similar or superior to conventional subcutaneous vaccination with alum.

2.3.3 Amino Acid

Amino acid-based particles have been examined for their effectiveness in allergen immunotherapy. Polymers of A: D-glutamic acid: D-lysine were linked to short ragweed extract and used for parenteral immunotherapy of ragweed-allergic patients. Skin test sensitivity and symptom scores decreased in the treated groups. However, IgE was not decreased. Adverse reactions also were observed, for example, swelling at the injection site or urticaria (Butterfield *et al.*, 1981).

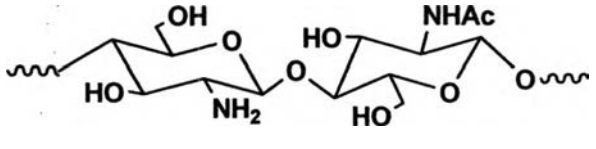
2.3.4 Liposome

Liposome is non-toxic, biodegradable and bio-compatible and forms as phospholipid vesicles (Kersten *et al.*, 1995). Mite allergens incorporated into liposome were tested in clinical studies (Basomba *et al.*, 2002). Asthmatic patients treated subcutaneous for a year had fewer bronchial, nasal and ocular symptoms as well as reduced drug consumption. There are further studies try to clarify the long-term effects of liposome immunization. It has been observed that non-IgE mediated psudoallergy against liposomal formulations can be induced (Szebeni, 2001).

2.4 Chitosan

Chitosan is a linear copolymer of D-GlcN and D-GlcNAc units (Scheme 2.5) (Prashanth and Tharanathan, 2006) and has D-GlcN level higher than D-GlcNAc which can be reported in percent of deacetylation. Specific properties of chitosan for biosystem are biocompatibility, biodegradability, and non-toxicity. The potential application of chitosan is multidimensional, such as in food and nutrition, biotechnology, material science, drugs and pharmaceuticals, agriculture and environmental protection, and in gene therapy (Prashanth and Tharanathan, 2006).

Scheme 2.5



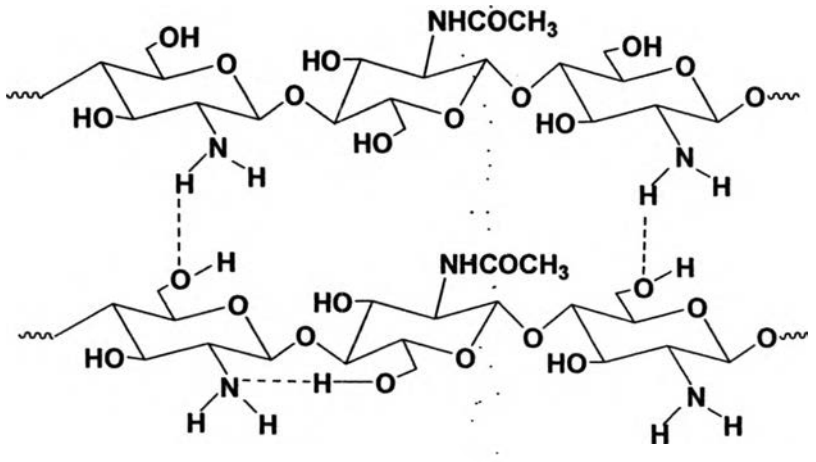
2.4.1 Chitosan for Water-based System

In general, chitosan is hardly dissolved in most organic solvents and water except acid due to its inter and intramolecular hydrogen bonding network (Scheme 2.6). While human body is water-based, study of chitosan which can be dissolved in water is needed. In recent year, Yoksan *et al.* (2003) succeeded in modifying chitosan chain with the hydrophilic and hydrophobic groups to control the solubility in organic solvent. Moreover, Fangkangwanwong *et al.* (2006) clarified the

water soluble complex of chitosan with hydroxybenzotriazole. This system offers a simple conjugating reaction of carboxylic acid groups to water soluble chitosan.

For water-based drug delivery, Park *et al.* (2006) reported that glycol chitosan beared cholic acid has capability to form nano-sized via self-aggregate system. Furthermore, deoxycholic acid modified chitosan was prepared for the use as a DNA delivery carrier (Lee *et al.*, 1998; Kim *et al.*, 2001).

Scheme 2.6



2.4.2 Chitosan-allergen Delivery System

Recently, chitosan has been clarified for using as adjuvant. Its function is deviate allergy-associated Th2 immune response to the Treg and Th1 which can decrease allergen sensitization. Sefarian *et al.* (2001) described ability of two adjuvant formulations containing chitosan to stimulate antibody response. One is the formulation in an emulsion reaction between chitosan and antigen in phosphate buffer saline by using ECC as coupling agent. Another is the formulation in a zinc-chitosan particle (ZCP) prepared by similar method as ECC but adding 10% zinc acetate solution. Both were found to facilitate immunization with recombinant proteins containing a histidine tag. The ability of both ECC and ZCP could elicit antibody responses more than 100 times of those elicited by recombinant protein without adjuvant.

Kumar *et al.* (2003) reported an approach to cure ovalbumin (OVA) sensitized mice. Animals were intranasal treated with chitosan nanoparticles

containing plasmid DNA coding with IFN-γ (which is cytokine for cell communication) and resulted in reducing of airway hyper-responsiveness and lung eosinophilia.

Chitosan could be a mucosal adjuvant of Helicobacter pylori vaccine (H. pylori vaccine). H. pylori vaccine with chitosan as adjuvant was reported for its anti-H. pylori infection. The inhibition of Th2 induced by H. pylori infection and recovery the Th1/Th2 imbalance (Xie *et al.*, 2005). Alpar *et al.* (2005) prepared chitosan microsphere contained BSA by spray drying and showed an increase in immune response as compared to that of the pure BSA. In addition, chitosan nanoparticle and chitosan-coated emulsions were also reported that they can retain allergen on particle until up taking into mucosal membrane and enhance immune response after administrating intranasal (Nagamoto *et al.*, 2004).

2.5 Amino acid

Amino acids, the monomeric units of proteins and bio-origin materials, are chiral molecules with a relative low molecular weight and various properties due to their various side chains (acidic, basic, hydrophobic, etc.). Yi *et al.* (2009) reported improving polymer supported for immobilization of lipase by amino acid modified chitosan. The result showed activity of the immobilized lipase on amino acid modified chitosan was high comparing with unmodified chitosan. Moreover, hydrophobic alkyl side chain of each amino acid could improve the activity of the immobilized lipase. In addition to enhancement of immobilized lipase, amino acid conjugated chitosan could increase the removal of heavy metals (Ishii *et al.*, 1994).

Chen *et al.* (2009) showed that pH-responsive polymer, poly(L-lysine *iso*-pthalamide), synthesized by grafting L-valine, L-leucine, and L-phenylalanine increased membrane disruptive activity. They also investigated an *in-vitro* cytotoxicity of grafted polymers by using a propidium iodide fluorescence assay and confocal microscopy. The results showed that the grafted polymers, especially grafted with L-phenylalanine, could induce a significant release of endocytosed materials into the cytoplasm of HeLa cells which was a feature critical for drug deliver application.

Yoksan and Akashi (2009) accomplished grafting of L-phenylalanine onto low molecular weight chitosan, an alternative promising carrier for negatively charged active molecules, by using carbodiimide as a coupling agent. This material could form complex with DNA. The release of DNA from the complex was very fast in high pH media and relatively slow or more sustainable in neutral and low pH ones.

2.6 Nano-structured Chitosan

Nanoparticles are generally defined as engineered structures with at least one dimension less than 100 nm. In recent years, an increase number of products composed of these nanostructures are available in the market such as cosmetic, food packaging, and pharmacy related products (Kroll *et al.*, 2009). The advantages of being nanostructure are, for example, high ability of absorption and release (Singh and Jr, 2008), effective drug transportation via nasal (Urrusuno *et al*, 1999). Gref *et al.* (1994) showed a preparation of PEG–PLGA nanoparticles to be smaller than 200 nm using a nanoprecipitation method. The nanoparticles were stable in aqueous solution and could be re-dispersed in water even after being lyophilized without cryoprotective agents. The PEG–PLGA nanoparticles not only reduced drug uptake by the liver, but also prolonged drug retention time in the blood. In the case of chitosan, nanoparticles could be made by various techniques such as emulsion technique, ionic gelation technique (Calvo *et al.*, 1997) in high molecular weight (MW) chitosan (CS) solution. For low molecular weight chitosan, Vila *et al.* (2004) investigated the potential utility of low MW CS in the form of nanoparticles as new long-term nasal vaccine delivery. Tetanus toxoid (TT), a model antigen, was entrapped within CS nanoparticles by an ionic cross-linking technique. TT-loaded nanoparticles elicited an increasing and long-lasting immune response as compared to the fluid vaccine. Confocal laser scanning microcopy (CLSM) images indicated that CS nanoparticles can cross the nasal epithelia.

2.7 Points of the Present Work

Chitosan was reported for effective adjuvant used in allergen delivery system. In addition, the development of nanoparticles was successful in our group in the past. It comes to our viewpoint to develop molecular design and synthesis to obtain nanoparticle chitosan with a target of allergen delivery system under the adjuvant property of chitosan.

The main idea is to functionalize chitosan with biomolecules to achieve biocompatible nanoparticles whereas the use of nanoparticulate morphologies is important in term of incorporating allergen. Herein, we focus on the conjugation of amino acid molecules and polyethylene glycol on low molecular weight chitosan via a simple conjugating reaction. Moreover, the preliminary dust mite allergen incorporation and the fundamental qualitative and quantitative analysis are investigated.