CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

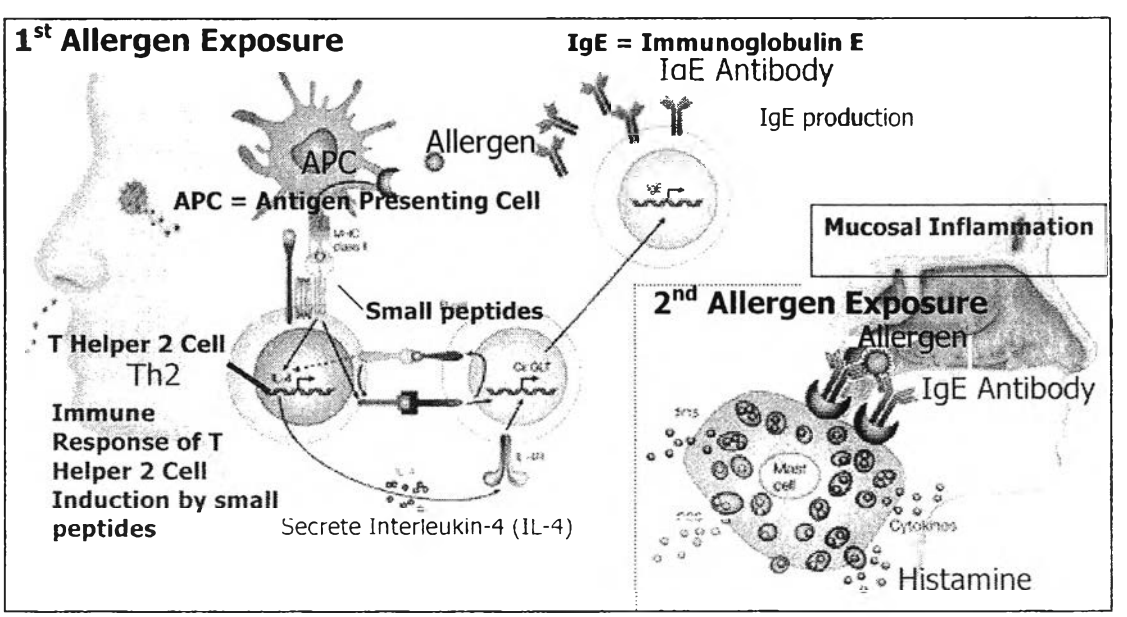
2.1 Allergy

An allergen is any protein substance which is able to induce immune response causing an allergic reaction in a genetically susceptible individual e.g. pollens, dust mites, cat dander, etc. The allergic symptoms are skin rash, urticaria, eye itching, sneezing, rhinorrhea, nasal swelling, and wheezing, etc.

When the patient exposes to the allergen, antigen presenting cell (APC) will process the allergen into small peptide and will present it on the surface of APC inducing T cell to be activated and to secrete some mediators i.e. interleukin-4 (IL-4), etc. The IL-4 activates B cell to produce immunoglobulin E antibody (IgE) which binds to IgE receptor on surface of mast cell and basophil. The re-exposure to the allergen causes the binding of allergen to IgE on mast cell. This causes the mast cell activation and degranulation, releasing several mediators, especially histamine which is an important mediator involving in the allergic reaction (Nisonoff, 1982).

Scheme 2.1

(Geha *et al.*, 2003 and the AstraZeneca group of companies)



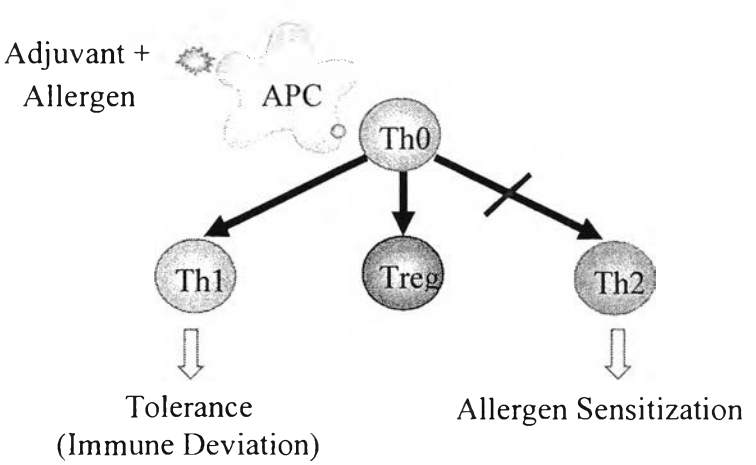
2.2 Allergen Immunotherapy

Allergen immunotherapy is a series of gradually increase culprit allergen administrating to the patients for a few years to induce the hyposensitisation state. For conventional method of allergen immunotherapy, an extracted allergen is administrated to the patient to induce the suppression of T helper 2 cell (Th2). The Th2 cell functions on producing IgE which is the cause of allergy. However, there will be some risks of severe allergic reaction during the immunotherapy course (Stites *et al.*, 1982 and Suri, 2006).

2.3 Allergen Delivery System

Allergen delivery system is a system of allergen and adjuvant that enhances the immune response. It may skew the Th2 immune response which is the cause of allergy to T helper 1 cell (Th1) and may induce the T regulatory cell (Treg) which functions on controlling balance between Th1 and Th2, thus the patient will be in a tolerance state (Gold *et al.*, 1970, Moingeon *et al.*, 2006 and Akdis, 2006) (Scheme 2.1).

Scheme 2.2



2.3.1 Adjuvant

Some allergens vigorously stimulate the primary antibody production when given by a single intravenous or intradermal injection, however some other weak allergens will ineffectively induce the response when injected alone. Whenever they are administered together with one of a number of non-specific stimulating agents or adjuvants, they strongly stimulate the antibody production (Gold *et al.*, 1970). Adjuvant has been discovered since 1930, however, to date, the acknowledge adjuvant for the usage in humans is alum (Bayler, 2002). Some recent studies revealed aluminium adjuvants have been associated with severe local reactions such as erythema (Jefferson *et al.*, 2004), subcutaneous nodules (Hindén, 2005, Vogelbruch *et al.*, 2000) and contact hypersensitivity (Bayler, 2002). Besides alum, many materials e.g. alginate, amino acid, and chitosan etc. have been reported (Scholl *et al.*, 2005) (Scheme 2.2).

2.3.1.1 Alginate Conjugate

In a hyposensitization study of allergic patients, the efficacy of grass pollen conjugated to sodium alginate was evaluated (Ortolani *et al.*, 1994). Nearly all treated patients benefited from the treatment regarding to the symptom/medication score, especially running nose and sneezing. As a drawback, 6 of 18 actively treated patients showed side effects like local swelling at injection site, 5 had rhinitis and 1 urticaria.

Alginate-coupled allergen extract was also used for injection of patients allergic to house dust mites (Corrado *et al.*, 1989). In this case, the development of asthmatic symptoms in rhinitis patients could be prevented. (?)

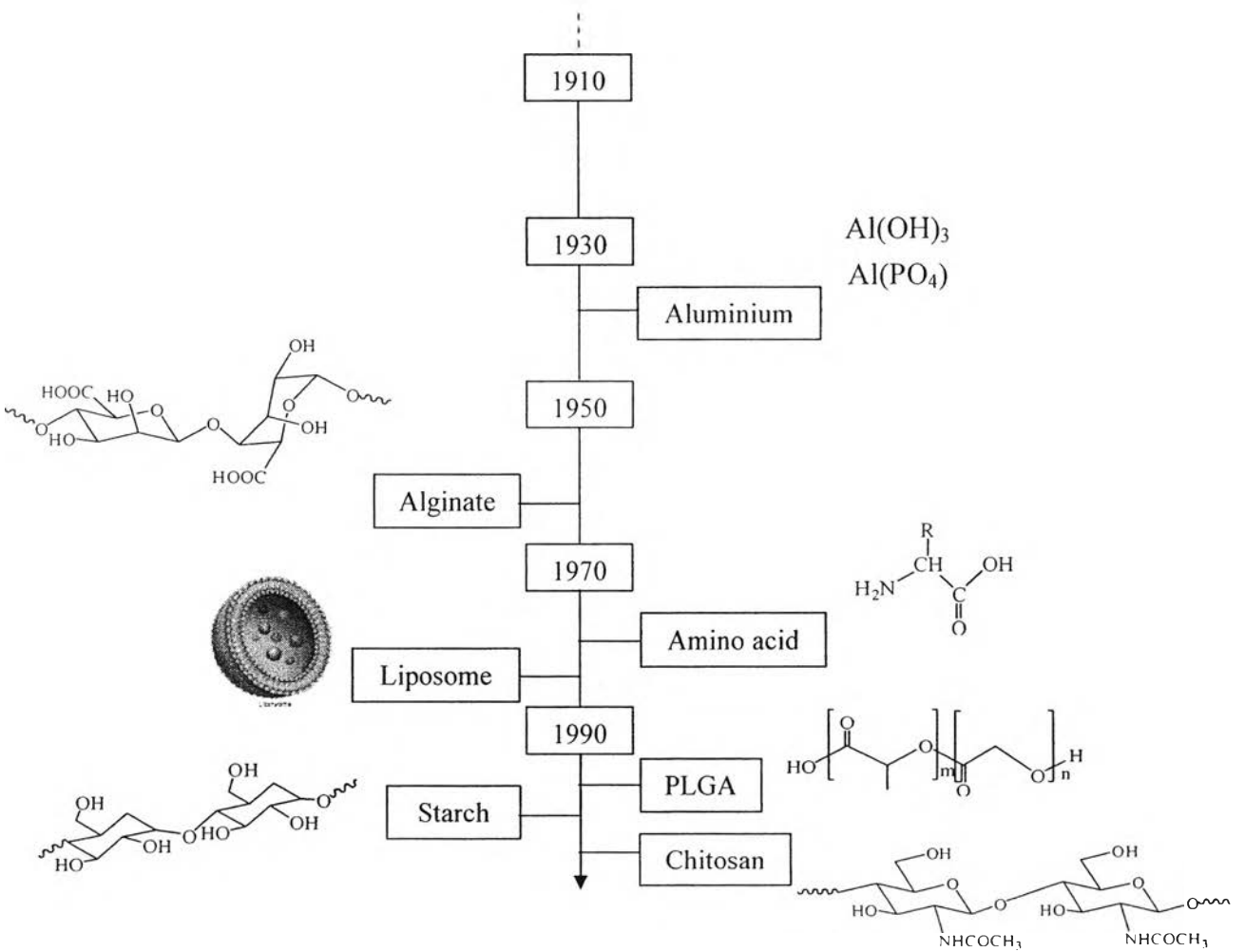
2.3.1.2 Amino Acid

Amino acid-based particles have been examined for their effectiveness in allergen immunotherapy (Butterfield *et al.*, 1981). Polymer of A:D-glutamic acid:D-lysine was linked to short ragweed extract and used for parenteral immunotherapy of ragweed-allergic patients. Both skin test sensitivity and symptom scores remarkably decreased. However, besides these positive effects, IgE increased and adverse reactions were observed, for example, swelling at the injection site or urticaria.

2.3.1.3 Poly-(D,L-lactic-co-glycolic) Acid Particles

Recently, Poly-(D,L-lactic-co-glycolic) acid (PLGA) particles were used for allergen delivery (Batanero *et al.*, 2002). Olive (Olea europaea) pollen-containing PLGA microparticles elicit a specific Th1-type immune response (Batanero *et al.*, 2003). PLGA protected the entrapped allergen from destruction during the gastrointestinal transit (Roth-Walter *et al.*, 2004). Its targetting M-cells in oral applications are capable of counter-balancing an allergic response.

Scheme 2.3 (Scholl *et al.*, 2005).

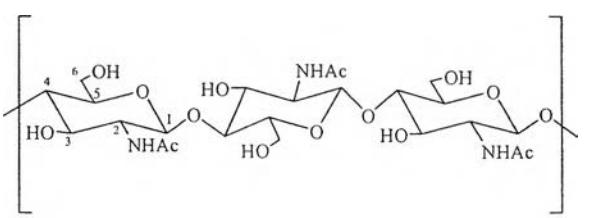


2.3.2 Chitosan: A Potential Material for Various Applications

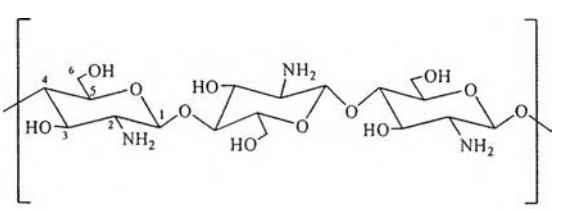
Chitin is a linear polysaccharide consisting of the random copolymer of distributed ß-(1-4)-D-glucosamine (deacetylation unit) and N-acetyl-D-glucosamine

(acetylation unit) found in the exo-skeleton of shellfish such as shrimp or crabs (Scheme 2.3). Chitosan is an N-deacetylated derivative of chitin. Chitosan is recommended for a functional material used in biosystem due to its specific properties on biocompatibility, biodegradability, and non-toxicity (Richardson *et al.*, 1999, Rao *et al.*, 1997, Yamamoto *et a*l., 1997 and Tomihata *et al.*, 1997). Up to now, chitosan has been proposed for various applications such as waste water treatment (Gidas *et al.*, 1999 and No *et al.*, 2000), wound dressing gel (Dutkiewicz, 2002), chitosan diet pills (Ni Mhurchu, *et al.*, 2005), post harvest agent (Prévost *et al.*, 2006) and plant growth promoter (Nge *et al.*, 2006). The development of chitosan has brought it to the commercialized products, for example textiles (Bofine Textile®), health-care and sanitary products (FibreNet®), food additives (Chitosan Kimchi Seogyun®), waste water flocculants (Gel-Floc®), fertilizers (Sugar Coat®), and cosmetics (Bio-Fiber soap®), etc.

Scheme 2.4



Chitin



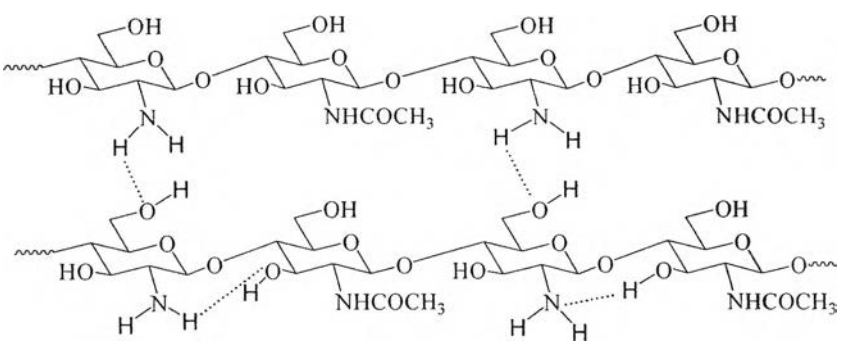
Chitosan

2.3.3 Chitosan Based Delivery System

Considering chitosan for biomedical field, various forms of chitosan and its unique potential applications (Zarzycki *et al.*, 2003), such as superabsorbent gel for wound healing (Dutkiewicz, 2002), nanosphere for drug delivery (Yamamoto,

et al., 2005), nanofiber for tissue engineering (liu *et al.*, 2005), have been proposed. It is important to note that one of the main problems is that chitosan is hardly dissolved in most organic solvents and water except the acids due to its inter and intramolecular hydrogen bonding network (Scheme 2.4). In the recent years, our group has succeeded in modifying chitosan chain with the hydrophilic and hydrophilic groups to control the solubility in organic solvents (Yoksan *et al.*, 2003). We also clarified the water soluble complex of chitosan and hydroxybenzyl triazole which offers the simple conjugation with carboxylic acid groups in water (Fungkangwanwong *et al.*, 2006).

Scheme 2.5



2.3.4 Chitosan-Allergen Delivery System

On the viewpoint of allergen delivery systems, recently, chitosan has been clarified for its function as adjuvant to deviate allergy-associated Th2 immune responses towards the T regulatory cell (Treg) and T helper 1 cell (Th1) (Seferian *et al.*, 2001 and Shibata *et al.*, 2001) and to transport through the tight junctions of mucosal epithelium. As a result, it allows the enhanced paracellular transport of co-administered drug across the cell epithelium (Scholl *et al.*, 2005, Schipper *et al.*, 1997 and 1999).

Sefarian *et al.* (2000) described the immune stimulating activity of an emulsion containing chitosan (ECC) and a zinc-chitosan particle (ZCP). The ECC was successful prepared by mixing 2% chitosan in 2% acetic acid solution with phosphate buffer saline containing antigen followed by neutralizing with NaOH.

The ZCP were prepared in similar way as ECC but adding 10% zinc acetate solution and was found to facilitate immunization with recombinant proteins containing a histidine tag. The ability of both ZCP and ECC elicits antibody responses more than 100 times of those elicited by recombinant protein without adjuvant.

The administration route (e.g. oral, nasal, subcutaneous, intramuscular or intravenous) also needs to be considered since allergen is a protein which is easily degraded in acid in stomach or the enzymatic environment and has poor permeability to cross the intestinal tract. Chitosan-DNA nanoparticles obtained from the acetic acid solution were reported for its possibility for oral gene vaccination. These nanoparticles can modify the immune system in mice even delivered in oral (Roy *et al.*, 1999 and Chew *et al.*, 2003).

Mucosal delivery is an attractive route of administration for inducing a protective immune response because many pathogens invade the body through mucosal surfaces. Chitosan could be a mucosa adjuvant of Helicobacter pylori vaccine (H. pylori vaccine). H. pylori vaccine with chitosan as adjuvant was reported for its anti-H. pylori infection, effective induction of immune response, the inhibition of Th2 induced by H. pylori infection and recovery the Th1/Th2 imbalance (Xie *et al.*, 2005). Nanoparticle chitosans and chitosan-coated emulsions were also reported that they can retain allergen on particle until uptaking into mucosal membrane and enhance immune response after administrating intranasal (Nagamoto *et al.*, 2004). In addition, chitosan microspheres (CMs) were developed to induce mucosal immunity in nasal cavity for the purpose of preventing atrophic rhinitis. The CMs show the high loading efficiency of Bordetella bronchiseptica dermonecrotoxin (BBD) (65-75%) to effectively increase the immune response (Kang *et al.*, 2006).

2.3.5 Cholic Acid and Deoxycholic Acid

Cholic acid and deoxycholic acid are in the family of steroid compounds. Naturally, cholic acid is a bile acid produced by the liver from cholesterol. Several cholic acid derivatives such as taurolithocholic acid, lithocholic acid 3-sulfate, taurolithocholic acid 3-sulfate, and glycolithocholic acid 3-sulfate were shown to inhibit selectively the replication of human immunodeficiency virus type 1 (HIV-1) in vitro (Baba *et al.*, 1990). Deoxycholic acid (DCA), which is a

secondary bile acid, is formed by bacterial action from the salt of cholic acid. DCA's main function plays an important role on an immunomodulator, which controls the activation of our unspecific immune system (Vlcek, R). It is reported that DCA is a major active constituent of Niuhuang (herb extract of schisandra chinensis) which is used to heal inflammations and immune deficiency (Chen *et al.*, 2002).

Both cholic acid and deoxycholic acid were used to modify the molecules to enhance hydrophobicity for use in delivery system (Nichifor *et al.*, 1999 and Kuen, *et al.*, 1998). Cholic acid was used to hydrophobically modify dextran to form nanoparticle aggregation with inner hydrophobic environment which is suitable for hydrophobic drug delivery system (Yuan *et al.*, 2006). Glycol chitosan bearing cholic acid was reported as a potential peptide drugs carrier, capable of forming nano-sized self aggregates and releasing peptide drugs in a sustained manner (Park *et al.*, 2004). Deoxycholic acid modified chitosan was also prepared for the use as a delivery carrier for DNA (Lee *et al.*, 1998).

2.4 Scope of the Work

As chitosan was reported for the potential adjuvant in developing chitosan-allergen delivery system, it is one of the practical approaches to develop chitosan incorporated with allergen and to investigate the optimal allergen incorporation conditions.

The present project aims to develop chitosan as an adjuvant with a specific interaction with allergen. Here, chitosan needs the modification to satisfy the conditions of (i) an effective interaction with the allergen, (ii) water solubility which is the preferable condition in the treatment, and (iii) the biocompatibility. In order to achieve the chitosan derivatives with the good performances mentioned above, the molecular design is shown in Scheme 2.5 which can be explained as follows. First, the strong inter- and intramolecular hydrogen bonds will be partly reduced via the substitution of the bulky groups to result in the water solubility. The bulky group used is cholic acid which is a bio-molecule and expected for the biocompatibility. Subsequently, the hydrophilicity of chitosan is also enhanced by introducing the polyethylene glycol chains. It is also one of our expectations that the good balancing

of hydrophilicity and hydrophobicity initiated from the modification at C-6 and C-2 will be the key factor to provide good interaction with allergen as well as the water solubility.

Scheme 2.6