I. INTRODUCTION

1. Principles and rationales

Allergic Rhinitis (AR) is one of allergic diseases defined as disorder of the nasal mucosa that react to foreign substances mediated through immunoglobulin (IgE) - antibodies. It is common throughout the world with a high burden of morbidity and cost. The high prevalence of AR and its effect on quality of life have led to its being classified as a major chronic respiratory disease (Strachan et al., 1997; Bousquet et al., 2001). It is reported to affect 10% to 40% of the global population and it prevalence is increasing both in children and adult (Spector et al., 1997; Lundback, 1998; Quraishi et al., 2004). In Thais, the prevalence of AR has been increased from 20% to 40% (Vichayanond et al., 1998), and found 30% of Chiang Mai province population (Pothirat, 2002). Allergic rhinitis can significantly reduce quality of life, impairing sleep and adversely affecting leisure, social life, school performance and work productivity (Simons, 1996; Blance et al., 2001; Tripathi and Petterson, 2001). The predominant symptoms of AR are sneezing, itching, nasal congestion, runny nose/sniffing, and postnasal drip/snorting. Some patients with AR are symptomatic only during the pollen season, which lead to seasonal symptoms while many others are allergic to multiple allergens including indoor allergens such as animal dander, cockroaches, and fungi, appear throughout the year, which lead to perennial symptoms.

The pathophysiology mechanism of AR is characterized as type I hypersensitivity. Immunoglobulin E (IgE) is the proximate cause of AR. Circulating IgE antibodies bind to the high affinity IgE receptor on mast cells. Mast cells are known to play a central role in the immediated phase reaction of allergic diseases. IgE antibodies, bound to the mast cell receptors crosslinked by allergen, initiate the secretion of inflammatory mediators including histamine, leukotrienes, and cytokines. These mediators can induce both acute and chronic changes that result in symptoms of allergy. Many therapies are approved for the treatment of allergic rhinitis including

allergen avoidance, pharmacotherapy and immunotherapy. Allergen avoidance is the mainstay of therapy for many patients but is not always practical. For those patients who have not responded to appropriate medications, allergen specific immunotherapy may also be effective. Effective therapy for AR requires understanding the pathophysiology of the disease, as well as the role of various inflammatory mechanisms.

Evaluation and diagnosis of AR is usually performed by assessment of history, physical examination and diagnostic tests. One of effective methods for the evaluation of AR is nasal cytology. It has been utilized as a diagnostic tool for differentiation of rhinitis. Nasal cytological studies of AR patients showed the large cells that contain numerous granules in the cytoplasm, stain basically with basic aniline dyes and metachromatically with Alcian blue and Toluidine blue dyes that called Basophilic Metachromatic Cell (BMC) (Hastie et al., 1979; Okuda et al., 1985; Enerback et al., 1989; Zeiger and Heller, 1993; Otsuka et al., 1995).

The first report in Thai patients found that the number of BMC in nasal scraping of AR patients was significant more than nonallergic rhinitis patients and normal people (Jareoncharsri et al., 1995). This finding suggested that the number of BMCs in nasal mucosa could be considered as another tool to diagnose AR. In children, nasal cytology revealed that the scores for nasal eosinophils and basophilic metachromatic cells between the AR and the control groups were distinctive differences and were correlated significantly with sign scores (Jirapongsananuruk and Vichyanond, 1998). These evidences showed BMC predominantly found in the nasal mucosa of AR patients and correlated with the nasal symptoms, and suggested that this cell might be an important inflammatory cell in the pathogenesis of AR. So far, which kind of BMC is not yet exactly identified. In this study, identification whether BMC is mast cell will be performed. Since mast cells predominate in submucosa and mucosa layer those called connective tissue mast cell (CTMC) and mucosal mast cell (MMC), respectively. These mast cells show functional difference according to chemical containing in granules. CTMC contains more harmful mediators than MMC. If BMC is mast cell, what is the type of mast cell involve at the sites of AR inflammation. Moreover, over production of IgE and allergen triggers cause mast cell degranulation. The released mediators cause a variety of allergic symptoms. How

individual AR shows different in severity is not well understood. The degree of mast cell degranulation may involve in the clinical symptoms. The purposes of this study also to investigate the type of mast cell contribution at the site of reaction and the degree of mast cell degranulation correlated with the severity of AR. Nasal scraping specimens of AR were stained with Wright-Giemsa stain for nasal cytology and differential cell count. Mast cell identification and classification were examined by Toluidine blue and Alcian blue/Safranin staining.

Genetic predisposing clearly plays a critical role in the pathogenesis of AR. However, the role of the environment is also important since allergens, which are ubiquitous in virtually all environments, initiate and trigger immune response. Atopic dermatitis (AD), one of atopic diseases has pathophysiology similarity with other atopic diseases. The atopic eczematous skin of AD found more than 90 % of *Staphylococcus aureus*, the best known gram positive bacteria, colonizes at skin lesions and the number of colonized bacteria and an increased number of activated mast cells are well correlated with the severity of the eczematous lesions (Mihm et al., 1976; Damsgaard et al., 1997). Recent study showed that peptidoglycan (PGN) from *S. aureus* directly stimulates mast cells to produce cytokines and results in mast cell degranulation (Supajatura et al., 2002). These released mediators and proinflammatory cytokines triggered skin symptoms and inflammation, leading to infiltration of inflammatory cells especially neutrophils and eosinophils. Since bacteria play a role in the pathogenesis of AD, it is important to clarify whether the type and number of colonized bacteria in nasal cavity of AR and nonallergic healthy subjects are different, and correlate to severity.

Exposure to house dust mite is an established risk factor for exacerbation of allergic asthma. It has been demonstrated that high level of exposure to house dust mite is associated with more severe asthma (Zock et al., 1994; Custovic et al., 1996). The majority of patients with asthma present with symptoms of AR. In epidemiological studies, AR was found to occur in up to 80% of patients with asthma (Leynaert et al., 2000). Allergic rhinitis may be a predisposing factor of allergic asthma. Thus, it is possible that house dust mite may be an important factor that triggers AR symptoms.

It has been demonstrated that a major allergen in house dust is related to the presence of mites of the genus *Dermatophagoides*. Worldwide and in Thailand, the most prevalent mites found in homes are *D. pteronyssinus* and *D. farinae* (Malainual et al., 1995; Arlian and Platts-Mills, 2001). *Blomia tropicalis* may also be prevalent in some temperate geographic areas especially in Singapore and in Malaysia (Chew et al., 1999; Mariana et al., 2000) but it has not been reported in Thailand. House dust mites, usually found in mattresses and pillows, sensitize and induce allergic symptoms, its may also be allergen in AR as possible as in asthma. In this study, specific IgE anti-house dust mite allergens, Der p 1, Der f 1, and Blo t 1, in sera of AR patients was examined by using indirect ELISA method. The data obtained will be analyzed to prove whether house dust mites are sensitized allergens and to determine the correlation between the amount IgE and the severity of symptoms. Information of mites induced- IgE production in this study will provide an epidemiologic contribution of mites in Thailand.

2. Objectives

The main objectives of this study are:-

1. To identify whether Basophilic Metachromatic Cell (BMC) is mast cell, investigate the type of mast cell contribution and the degree of mast cell degranulation.
2. To determine type and number of colonized bacteria in nasal cavity of AR and nonallergic healthy subjects, and correlate types and numbers of these bacteria with the severity of AR.
3. To examine specific IgE anti-house dust mite allergens in sera of AR patients, and correlated it with the severity of AR.

3. Education/Application advantages

1. No published study has been reported to identify which kind of BMC. This study identified whether BMC is mast cell by using specific mast cell staining,

Toluidine blue and Alcian blue/Safranin dyes. BMCs were positive stained in deep purple blue color and orange-red color with Toluidine blue and Alcian blue/Safranin respectively, suggesting that they are mast cells. BMC in nasal cytological study should be reported as mast cell. It is a novel finding that CTMC were found in nasal scraping specimens. It may migrate from connective tissue submucosa to the mucosa layer, and the degree of degranulation was parallel to the severity, suggesting that it responsible for AR reaction. The increasing of CTMC in mucosa will elucidate the complexity of allergic reaction and may offer new insight into the treatment of AR. Manipulating mast cell migration, inhibition of CTMC recruitment to mucosa, may be an important strategy for controlling the outcome of allergic and inflammatory response, in addition to well-known treatment with antihistamine and mast cell stabilizer.

2. Finding of the type and number of bacteria in nasal cavity of AR patients different from those of nonallergic healthy subjects demonstrated that some of bacteria, especially *Staphylococcus* spp., might be involved in the pathogenesis of AR. This evidence could be the supporting data for antibiotic manipulation together with other treatment, as use as in AD treatment.

3. Examination of specific IgE anti-house dust mite allergen, Der p and Der f, was clarified to demonstrate involvement of house dust mites in the allergen-sensitized AR symptoms. Specific IgE anti-Blo t 1 antibody was not detected. This data represent an etiology of AR and an epidemiology of mites in Thailand.