V. DISCUSSION

The present study has shown that BMCs are mast cells, identified by the morphology, and mast cell specific staining characteristics. These cells are relatively large, round cells that contain numerous seed like granules, which tightly packed in the cell, often obscure the nucleus. They were of large size (10-25 μm in diameter), varied in shape and were easily identified by their granular appearances and their staining characteristics. To differentiate mast cell and distinguish mucosal (MMC) and connective tissue mast cells (CTMC), Toluidine blue and Alcian blue/Safranin histochemical staining were employed. Methods used for the identification of mast cells are based on their metachromatic properties (Ehrlich, 1877). Attribution of varying amounts of sulphated proteoglycans results to differential staining and reflect distinct types of mast cells (Seldin et al., 1985). The cells that stained metachromatically deep purple blue with Toluidine blue or stained red with Safranin, are CTMC containing significant amounts of heparin, a highly sulphated proteoglycans that is responsible for the metachromatic dye-binding (Kruger, 1974; Enerback et al., 1986) and for the red color of cells stained by Safranin (Dimitriadou et al., 1990). Toluidine blue- and Alcian blue-positive cells are represent MMC (Lambracht-Hall et al., 1990). Alcian blue stains low sulphated substances blue and it is known that the MMC preferentially synthesis chondroitin sulphate di-B/E proteoglycans but not heparin proteoglycans (Razin et al., 1982; Seldin et al., 1985; Stevens et al., 1986).

Basophilic metachromatic cells (BMCs) are the cells that stain basically with basic aniline dyes and metachromatically with Toluidine blue (Zeiger and Heller, 1993). It seems to be that BMCs are mast cells and/or basophils. Infact, basophils and mast cells have similarities and are believed to be important in allergy, but they also have different characteristics. They are come from different progenitor cells, proliferation and differentiation in different environments, and also localized in the mature stage at different tissues. Mast cells are of hematopoietic origin and originated from the bone marrow, can only mature within tissue and are particularly numerous in

the proximity to the external environment, i.e. the skin, gastrointestinal tract and respiratory tract. Whereas the proliferation and differentiation of basophils were found in blood circulation. Mast cells show unique ultrastructure appearances including cytoplasmic lipid droplets and a granular substructure composed of multilamellar arrays and scrolls, serving to distinguish human mast cells from blood basophils (Enerback et al., 1986). These evidences were indicated that mast cells are not tissue basophils.

The results showed the cells stain positive purple-blue color and have numerous tightly granules with Wright-Giemsa, and stain positive deep purple blue and red- or blue- color with Toluidine blue and Alcian blue/Safranin, respectively. These cells have similar morphologically characteristics with these dyes, suggesting that they are mast cells. Mast cells have granules that contain histamine and heparin, the potent mediator of allergic reactions. The presence of these sulphated glycosaminoglycan means that granules stain metachromatically with basic dyes (Toluidine blue and Alcian blue/Safranin). Some of granular contents, tryptase and chymase are found in mast cells. Tryptase, a neutral protease selectively concentrated in the secretory granules of human mast cells (but not basophils), is released by mast cells together with histamine and serves as a marker of mast cell activation (Schwartz et al., 1987). Tryptase is the proteoglycan, which imparts the metachromatic staining characteristic of mast cells when exposed to certain basic dyes. Chymase, a serine proteinase that found in high abundance within the secretory granules of mast cells and can used to differentiate mast cell type (CTMC) by staining with Alcian blue/Safranin. Mast cells that contain both tryptase and chymase usually found at submucosa/connective tissue layer that are rarely found at mucosa layer in the normal conditions.

In this study, there was no difference in the number of mast cells in any dye stainings. With Wright-Giemsa stain, the total number of mast cells per 50 oil immersion fields gradually increased in mild, moderate, and severe AR, respectively, and also found similar results in both Toluidine blue and Alcian blue/Safranin stains.

Mast cells are normally distributed throughout connective and mucosal tissue, but in several pathological conditions accumulation of mast cells occur. This accumulation is probable due to directed migration of mast cells and they are subjects for migration at least two different occasions: 1) when they are recruited as progenitor

cells from the blood into the tissue; 2) when they are recruited to sites of inflammation as mature mast cells.

The study of tissue mast cells in mucosal biopsies found the recruitment of mast cells (but not basophils) into the epithelium (Enerback et al., 1986) that similarly found in this study, suggesting that the increasing of these mast cells (especially CTMC from connective tissue into the mucosa) will be the part of human allergic mucosal response. The mechanisms of these mast cell accumulations are not well understood. Due to the rapid kinetics for the accumulation of mast cells it is most likely that redistribution of neighboring mast cells by directed migration, or other possible, mechanisms of proliferation, prolonged survival, and differentiation of recruited progenitors from the peripheral blood. The recruitment of cells depends on adhesion and chemoattractants. Possibly, the presence of these CTMC in nasal mucosal epithelium of AR might be response to chemoattractants from lymphocytes or degranulated mast cells. However, the number of known mast cell chemoattractants has recently increased considerably. Examples of human mast cell chemoattractants are SCF, TGF-β, PAF, C3a and C5a, CCL5/RANTES, CCL11/eotaxin, CXCL12/SDF-1α, CXCL8/IL-8, and CXCL1/fractalkine (Olsson, 2000).

The mast cells in nasal scraping of AR showed various in morphology, which were classified into three categories; normal, slightly to moderately, and extensively degranulation (Supajatura et al., 2002). Highly degranulated mast cells especially extensively degree was markedly observed in severe AR. Furthermore, the degree of degranulation was parallel to the degree of clinical symptoms, suggesting that degranulation of mast cells was responsible for AR reactions.

Mast cells presented in this study were collected from nasal mucosal layer since the presence of epithelium cells were found in all specimens, and should not be technical errors of CTMC collection because of there was absence of red blood cells (normally found in submucosal/connective tissue layer) in specimens or at the sites of collection. The morphology of CTMC found both in intact and degranulated cells, and also correlated with the severity of AR. This indicated that they had functions and involved in the part of allergic response. The pressure during specimen collection was not the stimulated factor for mast cell degranulation.

Increasing of CTMC which have more potent mediators than MMC and after response to stimuli, accelerate the elimination of the causative of tissue injury or, paradoxically, may lead to chronic inflammatory response. Usefulness of this finding 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.

Since it has been reported that leukocyte recruitment after infection is crucial for host protection from diseases. In this study, nasal cytology in acute AR inflammation was investigated. The examination of mast cell and leukocyte recruitment into the nasal mucosa were performed and determined whether the recruitment associated with the clinical symptoms in AR allergic inflammation.

Mast cells and polymorphonuclear cells were found in specimens of AR but not found in nonallergic healthy subjects. Significantly increasing of mast cells was presented in AR groups. The presence of nasal eosinophils could be observed in nasal lavage fluid within one hour after allergen challenge procedure prior to a dramatic increase 7 to 11 hours, thereafter (Bascom et al., 1985; Bascom et al., 1988). This late increase of eosinophils is associated with an increase in the number of neutrophils, basophils, and mononuclear cells. In addition, the number of mast cells in the nasal mucosa also increases after allergen exposure (Pipkorn et al., 1988; Kleinjan et al., 2000). As found in this study, the number of mast cells increased in nasal scraping specimens of AR and also correlated with the severity of nasal symptoms, markedly presented in severe AR group, whereas mild AR and moderate AR groups were found relative higher number of eosinophils and neutrophils. Eosinophils are critical cells, usually found in allergic imflammation site that similarly found in this study which in severity dependent pattern. Since the release of mast cell mediators characterizes the early allergic nasal response, eosinophils with accompanying basophils play an important role in the late phase nasal response; the increased mast cells in nasal mucosa should be effect to eosinophils and polymorphonuclear recruitment. The presence of eosinophils and polymorphonuclear cells was absent in nonallergic group. The previous reports found the number of nasal eosinophils were higher in the allergic

group than in the control group (Igarashi et al., 1995). On the other hand, the presence of nasal polymorphonuclear cells of any degree, commonly found in healthy subjects (Cohen et al., 1985; Jirapongsananuruk and Vichyanond, 1998). The presence of these cells in nasal specimens of those studies, however, was found to be evidence substantiating the diagnosis of nasal infections, particularly sinusitis. Thus, nonallergic group in this study were appropriate representative because of none of any inflammatory cells was observed.

Although several studies have previously shown some factors that modify the pathogenesis of AR (Sorri et al., 1980; Peterson and Saxon, 1996), the association between bacterial colonization and AR has not been well documented (Till et al., 1997; Shinogi et al., 1998). For other atopic disorders, including bronchial asthma and atopic dermatitis, there have been many reports described the association of bacteria (Falanga et al., 1986). In this study, nasal scraping specimens were cultured and identified for predominant bacteria, the predominant representative bacteria in AR and nonallergic groups were compared. The dominant bacterial flora in specimens of patients with AR in comparison with nonallergic healthy subjects was different. Coagulase-negative *Staphylococci* and *Corynebacterium* species were the most frequently found in both groups, similar to the reports from several studies (Winther et al., 1984; Savolalinen et al., 1986; Jousimies-Somer et al., 1989; Rasmussen et al., 2000). Focusing on the existence of a higher carriage rate of *S. aureus* (44.44%) in patients compare with nonallergic subjects (3.85%). The colonization of *S. aureus* in the nasal cavity varies according to the population studied. Previous studies have reported that the mean carriage rate in the general population is 37.2 %, with a range of 19.0% to 55.1% (Kluytmans et al., 1997). There are many factors that may influence the rate of *S. aureus* nasal carriage. The adherence of the bacteria is mediated by receptors on the cell wall for fibronectin and laminin present on the mucosal epithelial cells (Bibel et al., 1983; Lopes et al., 1985). The allergic nasal mucosa may provide better nutrition and less host defense. The increased *S. aureus* carriage rate in AR may be partly due to increased hand-to-nose contact caused by blowing, picking, or rubbing. However, the reason for the higher carriage rates in patients with AR is unclear in detail.

The number of *S. aureus* (CFU/ml) was significantly higher in AR group compared with nonallergic group ($p<0.01$). This observation suggests that the nasal carriage of *S. aureus* in AR may have an influence on the pathogenesis of AR or may give rise to infectious rhinitis symptoms. However, there was no significant increasing in the number of *S. aureus* according to the severity of AR groups. It might be due to the elimination of nasal secretion before scraping specimen collection, or some of these patients might previously be taken antibiotics before enrollment.

Recently report published that staphylococcal superantigens activated T cells and produced Th 2 type cytokines in peripheral bloods of patients with AR (Shiomori et al., 2000). However, that report showed no significant difference between the nasal symptom scores of the nasal carriage of *S. aureus*-producing superantigenic exotoxins group and the nasal carriage rate of the group with *S. aureus* not producing superantigenic exotoxins. It was suggested that production of superantigens from *S. aureus* colonizing in the nasal cavity does not influence the symptoms or otherwise that *S. aureus* in the nasal cavity may not produce superantigenic toxins in situ. Other staphylococcal products may also be involved in AR.

Since the report demonstrated that peptidoglycan (PGN) from *S. aureus* can activate mast cells degranulation and lead to cytokine production of skin mast cells (Supajatura et al., 2002), PGN should be involved in the nasal mast cell induction. The role of PGN-induced nasal response should be further investigated. In addition, other gram-positive bacterial cell walls, lipoteichoic acid (LTA) enhances allergen-specific IgE production in mice that might explain the role of *S. aureus* in AD patients (Matsui and Nishikawa, 2003). This finding might be one of the reasons that explain the role of nasal carriage with *S. aureus* in AR patients. The clarification of the mechanisms of mast cell activation by gram-positive bacterial cell walls may elucidate the important role of nasal colonization of bacteria to pathogenesis of AR. If colonization with *S. aureus* contributes to the inflammation in patients with AR, one would expect a therapeutic effect from an antimicrobial treatment. Taken antibiotics together with other allergic treatments might result in a sustained eradication of *S. aureus* and consequently improve the nasal condition in patients with AR.

House dust mite allergens are important in the pathogenesis of allergic asthma, atopic dermatitis and should be involved in allergic rhinitis. Group 1 allergens of the mites *Dermatophagoides pteronyssinys* (Der p 1) and *Dermatophagoides farinae* (Der f 1) are the most significant allergens; 80% to 95% of patients allergic to dust mites have an elevated IgE response to them (Platts-Mills et al., 1992). In Thailand, the distribution of Der p and Der f are very common (Malainual et al., 1995). The study of allergen sensitization among Thai asthmatic patients by skin prick test (SPT) were revealed the incidence of positive SPT to *Dermatophagoides* is the most common allergens sensitized by both pediatric and adult asthmatics (Daengsuwan et al., 2003). Some of these patients were sensitized to storage mites, *Blomia tropicalis* (Blo t). It was interesting to know the involvement of these mites in the pathogenesis of AR. If these mites, Der p, Der f, and Blo t are sensitized mites in patients with AR, especially via type I hypersensitivity, it should be examined specific - IgE in sera of patients.

In order to evaluate the specific-IgE against different mite species, the recombinant Der p 1, r Der f 1, and r Blo t 1 which are the major allergen, were used in this study. Using the indirect ELISA, specific-IgE antibodies were evaluated from AR and nonallergic healthy subject sera. The patients were performed skin tests with dust mite extracts, and interpretation by allergist before enrollment. The mean plus threefold standard deviation of the optical density (OD) reading from nonallergic subjects was set as the cut-off value for positive IgE reactivity. The results showed the AR positive for IgE antibodies to r Der p1 (25/55, 45.45%) and r Der f 1 (13/55. 23.64%) but not to r Blo t 1. All patients' sera that were positive IgE antibody to r Der f 1 also positive to r Der p 1. Although some of patient's sera were not reacted with any mite allergens, there were probably reacted with other allergens.

The value of OD varied by many factors such as concentration and quality of conjugate and substrate being used. Therefore, the OD of a sample assay in laboratory is not the same when it is assayed in another laboratory. Similarly, antibody expresses in only OD values could not be compared among laboratories. Quantitation of the antibody was performed by constructing a standard curve. In this study, it was done by coating the mouse anti-human IgE on a microtiter plate and reacting to standard amounts of human IgE follows by mouse anti-human IgE biotin conjugate, avidin-HRP conjugate and substrate. Each day of assay, a standard curve

of IgE concentrations was performed along with the test system. The antibody concentration of test sera was estimated from the standard curve obtained.

In this study, the OD or concentration above the cut-off point of mean + 3SD of allergic group was considered as positive antibody to each mite. Some of nonallergic sera were positive with r Der p 1 and/or r Der f 1 allergen; it is possible that these cases with low positive OD might previously be sensitized with Der p and/or Der f but no clinical symptoms.

The concentration of antibody against Der p1 and Der f 1 was significantly higher in AR than in nonallergic group. Interestingly, the mean estimated IgE concentration against r Der p1 was higher than that r Der f 1. It is possible that these patients were more sensitized with Der p than with Der f or Der p 1 is an immunogen stronger than Der f 1. This study detected low level concentration of the specific-IgE against r Blo t 1 in a few sera of AR patients, but it was not significantly difference between AR and nonallergic group. Blo t 1 might be not good allergen resulted to low level detection of the specific-IgE, or these patients might be sensitized with another Blo t allergen groups. The distribution of mite allergen in patient's environment may describe and confirm these data. Since the incidence of Blo t has not been reported in Thailand, these data might be provided an update epidemiologic contribution of Blo t.

In order to correlate IgE levels with the patient severity, the specific-IgE concentration against different mite species was plotted with severity scores. The result showed low correlation between specific IgE antibody to Der p 1, Der f 1, and Blo t 1 with the severity scores. However, the specific IgE antibody to Der p1 and Der f 1 was detected from most of the severe AR group. In patients with AD, serum IgE concentration correlated to severity and cutaneous lesion of disease (Johnson et al., 1974; Wuthrich B., 1978; Laske and Niggemann, 2004). Increasing the number of patient sera and enrollment of AR from various geographic areas might be help to clarify the correlation of specific IgE concentration and severity of patients with AR.