INTRODUCTION

Of the many cells that cause lower airway inflammation, perhaps none is more important than the mast cell. When activated, mast cell is a principal cell initiating asthma exacerbations ***(Metcalfe, 2000)***.

Mast cells are found in all airways and localize specifically to key tissue structures such as the sub mucosal glands and airway smooth muscle within asthmatic bronchi, irrespective of disease severity or phenotype. Here they are activated and interact exclusively with these structural cells via adhesive pathways and through the release of soluble mediators acting across the distance of only a few microns. The location of mast cells within the airway smooth muscle bundles seems particularly important for the development and propagation of asthma, perhaps occurring ***(Bradding, 2000)***.

Targeting this mast cell-airway smooth muscle interaction in asthma offers exciting prospects for the treatment of this common disease ***(Bradding et al., 2006)***.

Mast cells are present in most tissues characteristically surrounding blood vessels and nerves, and are especially prominent near the boundaries between the outside world and the internal milieu, such as the skin, mucosa of the lungs and digestive tract, as well as in the mouth, conjunctiva and nose ***(Prussin and Metcalfe, 2003)***.

The role of mast cells in asthma remains controversial, owing to limitations in the experimental models and a lack of access to human mast cells. Mast cells play a key role in the inflammatory process. When activated, a mast cell rapidly releases its characteristic *granules* and various hormonal mediators into the interstitium. Mast cells can be stimulated to totally or a partial degree of degranulate by direct injury (e.g. physical or chemical [such as opioids, alcohols, and certain antibiotics such as polymyxins]), cross-linking of Immunoglobulin E (IgE) receptors, or by activated complement proteins ***(Prussin and Metcalfe, 2003).***

AIM OF THE WORK

In the present study, we aim to describe the number, morphology, specific distribution and function of mast cells in an in-vivo model of induced asthma in guinea pigs, as compared to normal guinea pigs.

BIOLOGY OF MAST CELLS AND THEIR MEDIATORS

Introduction:

Over millions of years, the human immune system has evolved to become a highly complex, elegant, and efficient organ whose chief function is to protect the human host (*self*) from harmful offenders (*nonself*). *Antigens* are foreign (or self) molecules that will elicit an immune response. Immunologic responses to antigens in humans are coordinated by two immune systems: the ancient *innate* immune system, which humans inherited from invertebrates, and the recently evolved *adaptive* immune system, which is present in humans and vertebrates. The innate system is considered the first line of defense. Its effector components include mast cells, macrophages, dendritic cells, natural killer cells, granulocytes, antimicrobial peptides, complements, and cytokines ***(Kirshenbaum et al., 1999)***.

Mast cells are potent tissue-dwelling effector cells of hematopoietic origin. In addition to their role in allergy, they are implicated in innate immunity to bacterial and parasitic infections on the basis of animal studies. They are particularly abundant in a perivascular distribution in connective tissues and at mucosal surfaces ***(Daley et al., 2001)***.

Classic mast cell-mediated hypersensitivity (allergic) reactions are initiated by the binding of multivalent allergen to membrane-bound IgE that is coupled to the tetrameric high-affinity Fc receptor for IgE (FcεRI) on mast cells. IgE-dependent activation of mast cells results in their release of preformed inflammatory mediators that are stored in their secretory granules, including histamine, neutral proteases, preformed cytokines, and proteoglycans ***(Yeatman et al., 2000)***.

Additionally, mast cells activated via FcεRI secrete newly synthesized lipid mediators that are the products of endogenous arachidonic acid metabolism, such as prostaglandin (PG) D2, leukotriene (LT) B4, and LTC4, the parent molecule of the cysteinyl leukotrienes (cys-LTs). Finally, activated mast cells synthesize and secrete a host of proinflammatory cytokines. The net result of tissue mast cell activation thus includes the rapid development of plasma extravasation, tissue edema, bronchoconstriction, leukocyte recruitment, and persistent inflammation, with the clinically recognizable syndromes of anaphylaxis, urticaria, angioedema, and acute exacerbations of asthma ***(Gurish et al., 2006)***.

The importance of mast cells and their products in allergic diseases is supported by the fact that pharmacologic pretreatment of susceptible humans with cromolyn, an inhibitor of mast cell exocytosis, attenuates symptoms in response to allergen challenge ***(Vyas et al., 2006)***.

Histologic Identification of Mast Cells:

Mast cells were originally identified in 1878 by Paul Ehrlich in his doctoral thesis presented at Leipzig University, Germany. Ehrlich described them as granular cells in connective tissue that stained purple with aniline dyes, a property known as 'metachromasia' ***(Vyas et al., 2006)***. He noted that these cells had 'a tendency to collect around developing preformed structures in the connective tissue', such as blood vessels, nerves, secretory ducts, sites of inflammation, and neoplastic foci. Metachromatic staining of granules with toluidine blue remains a classic feature by which mast cells are identified and is a property that they share with basophils ***(Abonia et al., 2005a)***.

Morphology of Mast Cells:

Mature human mast cells range from 7 to 20μm in diameter and appear as round, spindle-shaped, or spiderlike cells in tissues with roundor oval nuclei. They have thin 1-2μm processes (microplicae) emanating from their plasma membranes. The most notable feature of mature tissue mast cells is their abundant cytoplasmic secretory granules that constitute about half of their volume. The metachromatic staining of mast cell granules reflect their content of sulfated proteoglycans that bind mediators such as histamine and proteases ***(Tsai and Orkin, 1997)***.

Reagents that detect mast cell-specific proteases, such as chloroacetate esterase activity (an indicator of chymotryptic proteases), or immunoreactivity for their trypsin-like proteases (tryptases) also provide reliable markers of mature mast cells. Mast cells in different tissue locations vary in their granule content ***(Masuda et al., 2001)***.

Inhuman tissues, mast cell granules may contain either tryptase alone (designated *MCT*) or a combination of tryptase, mast cell-specific chymase, carboxypeptidase A, and cathepsin G (designated *MCTC*). Each of these granule types predominates in the mast cells of particular locations. MCT are the primary type of mast cell in lung alveoli, the mucosa of the small intestine, and the mucosa in allergic eye disease. MCTC predominate in normal skin, blood vessels, the submucosa, and synovium. With mucosal inflammation, a selective increase in MCT occurs in the involved epithelial surface ***(Matsuzawa et al., 2003)***.

At the ultrastructural level, the secretory granules of human mast cells contain electron-dense material with crystalline features. With IgE-dependent stimulation, the crystalline structures become amorphous, and only amorphous material is discharged as the granules fuse with the plasma membrane. The crystalline granules contain one of three structural arrangements: scrolls; gratings (parallel electron-dense lines separated by lucent areas); and lattices (two sets of

parallel, electron-dense lines running in different directions) ***(Lora et al., 2003)***.

All three crystal patterns may be present in a single granule. The relative amounts of these granule structures differ among the mast cells at different tissue locations in humans and likely reflect corresponding differences in protease and proteoglycan content. The granules of mast cells in breast parenchyma, skin, axillary lymph nodes, and bowel submucosa contain relatively few scrolls ('scroll-poor' granules) but are rich in gratings and lattices, indicating the presence of chymase, whereas mast cells in the lung alveoli and in the bowel mucosa contain many scrolls ('scroll rich')but relatively few gratings and lattices ***(Weidner et al., 1991).***

These scroll-rich cells tend to lack chymase. Studies of human mast cells developing in the tissues of immuno-deficient mice that received infusions of umbilical cord bloodCD34+ progenitor cells indicate that protease content is determined by the specific tissue microenvironment rather than by the characteristics of the progenitor cell per se ***(Kambe et al., 2004)***. The distribution of MCT and MCTC in normal human tissue sections is summarized in Table (1).

Mast cell heterogeneity extends to their content of histamine and other biogenic amines, capacity for arachidonic acid metabolism, and functional responses to immunologic and pharmacologic stimuli. These features are summarized in Table (1).

Table (1): Mast cell heterogeneity.

Cell type	Human mast cells		Mouse mast cells	
	MC_T	MC_{TC}	MMCs	CTMCs
Biogenic amine	Histamine	Histamine	Histamine	Histamine, serotonin
Granule proteases	Tryptase	Tryptase, chymase, carboxypeptidase, cathepsin-G	Chymases, MMCP-1,2,4,9	Chymases, MMCP-3,4,5,8, tryptases MMCP-6,7
Proteoglycans	Heparin, chondroitin sulfate	Heparin, chondroitin sulfate	Chondroitin sulfate	Heparin, chondroitin sulfate
Granule ultrastructure	Scroll-rich	Gratings/lattices, 'scroll-poor'		
Tissue distribution	Mucosal surfaces, alveoli, bronchi allergic conjunctiva	Skin, submucosa, normal conjunctiva, synovium heart vascular wall	Mucosal surfaces	Connective tissues, peritoneal cavity
T-cell dependency	Yes	No	Yes	No
Staining	Metachromatic	Metachromatic	Formalin-sensitive, safranin-negative	Formalin-tolerant, safranin-positive
Arachidonic acid metabolism	PGD_2, LTC_4	PGD_2 (skin MCs)		
Responses to stimuli	Antigen, anti-IgE, calcium ionophore	Antigen, anti-IgE, calcium ionophore, compound 40/80 basic polypeptides, morphine sulfate fMLP peptides substance P VIP, somatostatin C5a		
Pharmacologic inhibition	Release inhibited by chromones	Chromones not effective		

MMCP, mouse mast cell protease; MC, mast cell; PGD_2, prostaglandin D_2, LTC_4, leukotriene C_4, VIP, vasoactive intestinal peptide.

Mast Cell Growth and Development:

Origin:

The origin of mast cells remained obscure for many years, it is now accepted that these cells originate from CD34/ c-Kit/CD13 pluripotent hematopoietic cells in the bone marrow ***(Toru et al., 1998)***. Mast cells are released into the circulation

in the immature state and undergo terminal maturation/differentiation after migration into tissues, where they ultimately reside. In early studies, mouse models were used to investigate the origin and development of mast cells:

(a) C57BL/6-bgj/bgj, beige Chediak-Higashi syndrome mice. In this model, the giant mast cell granules were used a morphological marker for donor-derived cells ***(Pardanani et al., 2006)***. Here, cells from the bone marrow, peripheral blood, liver, thymus, or lymph nodes of beige mice were transplanted into irradiated, congenic C57BL/6 mice, or alternatively, the beige mouse was parabiosed with a congenic mouse, (b) dominant white spotting (*W*)- or Steel (*Sl*)-locus mutant mice (vide infra) that are profoundly deficient in mast cells. Kitamura initially demonstrated that the mast cell content in skin and other organs in the adult *W/W v* mouse could be restored to normal levels after transplantation of bone marrow cells from congenic mice ***(Kitamura et al., 1978)***.

The relationship between human mast cells and cells belonging to other leukocyte lineages remains unclear. Although mast cells share several features with basophils, namely presence of cytoplasmic basophilic granules, expression of high-affinity IgE receptors (FcεRI), and release of histamine upon stimulation, the two cell types are considered to be distinct ***(Kitamura et al., 2006)***.

Unlike mast cells, basophils circulate in the blood as mature cells and are thought to be incapable of proliferation; that is, basophils undergo apoptosis after their recruitment and activation in the tissues ***(Tokarski et al., 2006)***. Studies of developmental pathways in murine hematopoiesis have not been conclusive as to how mast cell-committed progenitors are generated; that is, whether there is a shared bipotent progenitor for mast cell and basophilic lineages or whether mast cells are derived directly from multipotent progenitor cells ***(Arinobu et al., 2005)***.

Homing of Mast Cells and Their Progenitors:

The mechanisms involved in the recruitment and distribution of the circulating pool of committed mast cell progenitors, and the movement of mast cells in tissues, have only been recently explored. In mice, the small intestine contains an especially rich supply of mast cell progenitors, which constitutively traffic to this organ, likely ensuring the capacity for a rapid expansion of the mast cell population in the intestinal epithelium during the effector response to helminth infection ***(Morii et al., 2004)***.

Mast cell progenitor trafficking to the intestine depends on the expression of the α chemokine receptor CXCR2 (CD182) 16 and the β7 integrin subunit ***(Gurish et al., 2001)***. The α4β7 integrin (LPAM-1) interacts with vascular cell adhesion molecule 1 (VCAM-1, CD106) and mucosal addressin

cell adhesion molecule 1 (MAdCAM-1) for tissue-specific homing. Additionally, blockade of either α4 (CD49d) or β7 integrins inhibits the increase in mucosal mast cells in the intestinal mucosa of rats infected with *NippoStrongylus brasiliensis* ***(Issekutz et al., 2001).***

Although normal mouse lung tissue contains few mast cell progenitors (and few mast cells), these cells are recruited to the lung in substantial numbers in response to allergen-induced pulmonary inflammation. This inflammatory recruitment pathway requires α4β7 and α4β1 (VLA-4, CD49d/CD29) integrins as counter-ligands for inducible VCAM-1, whereas MAdCAM-1 is not expressed in the lung ***(Abonia et al., 2006)***. Several additional homing receptors have been reported on mast cells and/or their progenitors in humans and mice ***(Juremalm et al., 2005)***.

Mast cells of both humans and mice express several integrins, many of which are counter-ligands for extracellular matrix proteins such as fibronectin (α5β1), lamin in (α3β1 and α6β1), and vitronectin (αVβ3) ***(Columbo et al., 1995)***. Additional integrins such as αLβ2 (also known as lymphocyte function antigen (LFA)-1 or CD11a/CD18) and αEβ7 may be induced by inflammatory cytokines for interaction with their ligands on epithelial cells (intercellular adhesion molecule (ICAM)-1 (CD54) and E-cadherin, respectively) ***(Toru et al., 1997)***.

It seems likely that the process of mast cell homing and localization is regulated in a coordinated fashion in vivo, with each set of determinants acting in a tissue-specific and context-specific fashion.

Activating and Inhibitory Receptors of Mast Cells:

FcεRI:

When occupied by monomeric allergen-specific IgE and cross-linked by a multivalent allergen, FcεRI transduces signals that result in immediate granule fusion and exocytosis, arachidonic acid metabolism, and induction of cytokine and chemokine gene transcription. IgE-mediated activation of mast cells occurs exclusively via FcεRI and is thought to account for the clinical manifestations of rhinitis, conjunctivitis, urticaria, angioedema, and bronchoconstriction that immediately follow allergen challenge in susceptible hosts ***(Kepley et al., 2004)***.

It is not clear what physiologic role requires such high expression of FcεRI on mast cells. Mice genetically deficient in FcεRI are developmentally and immunologically normal and normally eliminate *Schistosomamansoni* from the intestine after experimental infection, although they do develop increased hepatic granulomas and hepatic fibrosis ***(Jankovic et al., 1997)***. Deficiencies in mast cells or IgE impair the ability of mice to eliminate *Haemaphysalis longicornis* ticks ***(Matsuda et al., 1990)***

suggesting a role for IgE-mediated hypersensitivity in resistance against this parasite.

Regulation of Mast Cell FcεRI Expression and Function:

FcεRI expression on the surface of mast cells and basophils is regulated by the level of circulating IgE, due to stabilization of receptors at the cell membrane ***(MacGlashan et al., 1997)***.Thus, decreased IgE concentrations in vivo lead to decreased levels of membrane expression of FcεRI. The administration of a humanized monoclonal anti-human IgE antibody to atopic individuals decreases the levels of FcεRI on the surfaces of their circulating basophils, skin mast cells ***(Beck et al., 2004)***, and lung mast cells ***(Djukanovic et al., 2004)***.

The deficiency of mast cell surface FcεRI in an IgE-deficient mouse strain was corrected by injecting the mice with exogenous IgE, and the incubation of in vitrobone marrow-derived mast cells (BMMCs) with IgE enhanced not only their FcεRI expression but also signal transduction and mediator release in response to FcεRI cross-linking ***(Yamuguchi et al., 1997)***.

Locally derived cytokines may also regulate FcεRI expression and signaling. Human mast cells derived in vitro from cord blood mononuclear cells cultured in the presence of stem cell factor (SCF) plus IL-6 respond to the addition of exogenous IL-4 with dose-and time-dependent enhancement of their surface FcεRI expression ***(Toru et al., 1996)***.

Similarly, human mast cells purified from dispersed intestinal tissue respond to recombinant IL-4 ex vivo with upregulated FcεRI expression and priming for IgE dependent activation ***(Bischoff et al., 1999)***. Unlike the effect of IgE, IL-4 causes an increase of mRNA encoding FcεRIα. Given that they act by separate mechanisms, it is not surprising that IL-4 and IgE are synergistic for FcεRI expression and FcεRI-mediated mast cell activation in vitro ***(Yamaguchi et al., 1999)***.

Fcγ Receptors:

While IgE-dependent mast cell activation and anaphylaxis require the intact FcεRI ***(Dombrowic et al., 1993)***, anaphylaxis can also occur in rodents through IgG-dependent activation of the low-affinity receptor for IgG, FcγRIII (CD16). This receptor shares β and γ subunits with the FcεRI but has not been identified on human mast cells. When ligated in vitro by antibody, FcγRIII induces mouse mast cells to degranulate and release LTC4, similar to their responses via FcεRI, and mast cell activation by FcγRIII in vivo can mediate anaphylaxis in mice ***(Miyajima et al., 1997)***.

Human mast cells exposed ex vivo to interferon-gamma (IFN-γ) inducibly express high-affinity FcγRI receptors (CD64) ***(Okayama et al., 2000)***, and FcγRIIa receptors (CD32) have been detected on skin-derived human mast cells ***(Zhao et al., 2006)***. In these studies, cross-linkage of either receptor resulted in generation of similar mediator profiles as did cross-linkage of FcεRI.