First East Asian Symposium on Asthma

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First East Asian Symposium on Asthma

Advances in the Pathogenesis and Management of Asthma

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M.L.C. Centre
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Introduction

The occasion of the First East Asian Symposium on Asthma provided an excellent opportunity for scientists and specialists in respiratory disease to meet and discuss the pathogenesis and treatment of asthma. Delegates came from all over the world – from Canada, the United Kingdom, Switzerland, Australia, Hong Kong, Singapore, Malaysia, Indonesia, Taiwan and the People's Republic of China. The Symposium was held in three centres – Singapore, Taipei and Hong Kong – and provided an unparalleled opportunity for the interchange of ideas.

Asthma is a disease in which the approach to therapy is changing. Many clinicians now feel that it is not appropriate simply to treat acute attacks of asthma, but rather to try to prevent their occurrence. Patients should not be allowed to wheeze their way from one acute attack of bronchospasm to the next, but rather should receive the appropriate amount of therapy not only to prevent the onset of acute attacks but also to allow them to take part in all the normal activities of life. The successful prophylactic treatment of this disease should mean that the troublesome symptoms of night-time cough and waking, and wheezing induced by changes in temperature, contact with atmospheric irritants and on exercise, should not occur. This approach necessitates the administration of regular therapy with drugs such as ketotifen, sodium cromoglycate and inhaled corticosteroids, supplemented by bronchodilator drugs, such as the β-adrenoceptor agonists or theophyllines. Much was heard in this Symposium about ketotifen, its pharmacology and the results of many controlled and uncontrolled studies throughout the world which should enable clinicians to assess its rightful place in the management of asthma.

Investigations into the pathogenesis of asthma have been dominated in the last decade by studies on mast cells and their mediators and on the underlying abnormality present in all asthmatics, namely increased responsiveness of their airways to non-specific stimuli – bronchial hyper-reactivity. New developments are occurring in both of these fields. First, the recognition that there is a heterogeneity amongst mast cells and that these cells present in different sites within the same organ may behave differently, both in response to stimuli and to therapeutic agents. Although it seems likely that the mast cell remains pivotal in the development of allergic asthma, more attention is being focussed on the role of other inflammatory cells attracted to the site of the allergic reaction by chemotactic factors.

The role of the neutrophil, eosinophil, macrophage and even lympho-

cyte in the development of inflammation in the airways and subsequent increased responsiveness of the airways is being intensively researched. These inflammatory cells may be the main source of potent mediators, such as platelet-activating factor and the leucotrienes, which may be central to the pathogenesis of bronchial hyper-reactivity. Although it is clear that there is an interaction between the allergic response and the development of increased responsiveness of the airways, bronchial hyper-reactivity may be present in individuals who are not experiencing allergic reactions or indeed symptoms. Several epidemiological studies have shown that substantially increased airway responsiveness can occur in up to 15-20% of the population. The mode of inheritance of this feature and its relationship to the development of the clinical symptoms of asthma remain to be determined. Nevertheless, it remains possible that extrinsic allergic asthma will occur in those individuals who are both atopic and who have bronchial hyper-reactivity. Exposure of these individuals to potent allergens in the environment leads to sensitization and subsequent allergic reactions, thus increasing the underlying level of bronchial hyper-reactivity with the development of persisting symptoms of asthma. The next few years promise to be even more exciting than the last with regard to our understanding of this common disease.

There were several areas where opinions from the East and the West differed. In the West, we consider that asthma is a disease with a very high prevalence in the community. Few would consider that it affected less than 5% of the population, whilst more recent studies suggest that the prevalence in the first and second decade of life may be as high as 12%. Although asthma is a disease of major importance in Southeast Asia, we heard time and time again of the much lower prevalence rates of the disease. For example, in studies from the People's Republic of China and Hong Kong, the prevalence appears to be only 0.5-1%. Whether this is a true difference in prevalence or the result of differences in the criteria accepted for diagnosis, remains to be elucidated. Nevertheless, my own feeling is that, even allowing for differences in diagnostic criteria, there is a real difference at the present time between the frequency of this disease in different countries around the world.

Differences also appeared in the attitude of participants towards assessment of drug therapy and in the most suitable method for administration of therapeutic agents. The importance or otherwise of controlled double-blind clinical trials was a source of lively debate. Participants from the English-speaking world were obsessed by the need for double-blind randomized studies, whereas participants from the East were often more concerned to know whether the patient himself or herself actually benefitted from administration of the drug. Whilst

delegates from the English-speaking world considered that the only proper way to take a drug for the respiratory system was by inhalation, this was certainly not the view of the delegates from many other parts of the world who, I suspect rightly, considered that it was much easier to take a tablet than inhale an aerosol. As always, the truth probably lies in a compromise and the great benefit of the First East Asian Symposium on Asthma was that it allowed an active interchange of ideas between participants from all over the world. I am sure a great deal was learned by all those attending. There was no shortage of questions for discussion and all of us look forward to the opportunity to continue our discussions.

Robert Davies
Chairman
First East Asian Symposium on Asthma

Mast cells and mediators in allergic respiratory disease

R.J. DAVIES

Academic Unit of Respiratory Medicine, St. Bartholomew's Hospital, West Smithfield, London, UK

INTRODUCTION

In 1873, the English physician Charles Harrison Blackley showed that provocation with grass pollen allergens could reproduce the symptoms of rhinitis and asthma. Whilst it is well known that an immediate attack of asthma can result from the inhalation of common allergens, such as those from the house dust mite, it has become apparent that such immediate asthmatic, and probably nasal, responses are frequently followed by the development of more severe and prolonged episodes of airway narrowing. This secondary response is referred to as late reaction.

It is even more extraordinary to discover that the inhalation of an allergen for just a short period of time can lead to quite dramatic and important changes in airway function. Figure 1 shows the development

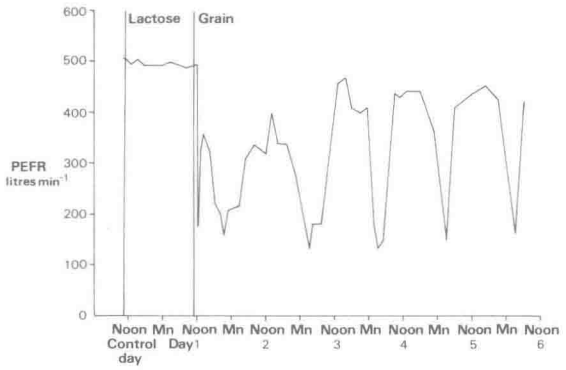

Fig. 1. The development of immediate, late and recurrent asthma in a farmer, following a 30-minute exposure to grain infested with *Glyciphagus domesticus.* Mn = midnight, Lactose = control bronchial provocation, PEFR = peak expiratory flow rate

of a late asthmatic response following the inhalation of allergens from *Glyciphagus domesticus,* and the subsequent recurrent asthma, lasting many days.[1] This phenomenon is probably linked to the development of bronchial hyper-reactivity which has been shown to occur following late reactions.

MAST CELLS AND MEDIATORS

Many years of study of mast cell-mediated allergic responses have resulted in the development of an attractive hypothesis to explain these phenomena which, in fact, occur in man. Much of the original work on mast cells. Such techniques as have been used to isolate and concentrate the rat, from which it is possible to obtain large quantities of purified mast cells – for example, from the peritoneal cavity. The situation regarding man is, of course, much more complex. It is not possible to obtain pure populations, even of circulating basophils, let alone tissue mast cells. Such techniques as have been used to isolate and concentrate mast cells from the human lung involve physical and chemical processes which may themselves alter the activity of the cells.

Any hypothesis regarding the role of mast cells and associated mediators must be interpreted in this light. The possible involvement of granule-associated and membrane-derived mediators in the development of early- and late-phase asthmatic reactions and subsequent sustained bronchial hyper-reactivity is illustrated in Figure 2, as is the important role of inflammatory cells.

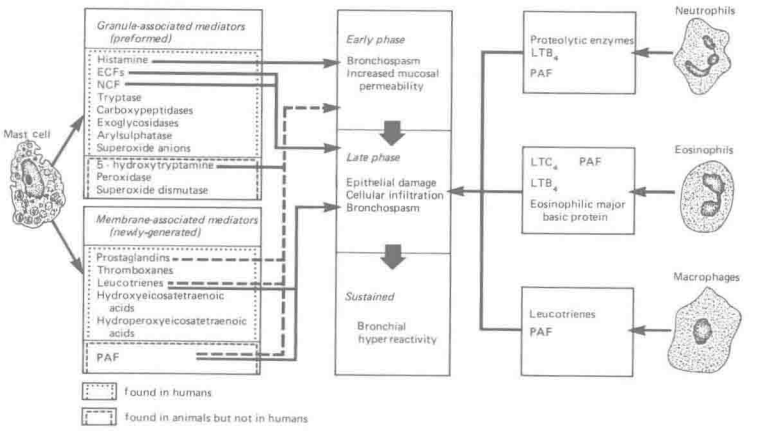

Fig. 2. Mediator release and the inflammatory response following activation of the mast cell.

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At the present time, the suggestion that white blood cells, in particular neutrophils, eosinophils and macrophages, are involved in late-phase allergic reactions and subsequent inflammation, comes largely from studies of skin-window techniques. These studies indicate that the neutrophil cell is attracted to the site of the allergic response at about two hours, followed by eosinophils and subsequently macrophages some 8-24 hours later. It is now clear that these inflammatory cells can release both chemo-attractant and inflammatory factors that may cause not only increased inflammation, but also its continuation and even progression.

As might be expected, there are no human studies on the histological changes occurring in the bronchial wall during allergen-induced immediate and late asthma. Nevertheless, examination of sputum during prolonged asthmatic attacks indicates the involvement of eosinophils in the reaction since these are present in large quantities, as are bronchial epithelial cells.

It is currently considered that release of major basic protein from the eosinophils may have an important role in producing the inflammation that leads to denudation of the epithelium. Histological studies of lung tissue from patients dying from severe asthma indicate the extensive

TABLE 1

Evidence for mediators and inflammatory cells in human asthma

Mediators		Early	Late	Recurrent/progressive
Histamine	Plasma	↑ ?	↑ ??	Unknown
	Urine	↑	→	Unknown
	Antihistamines	+	(–)	(–)
NCA		↑	↑	(↑)
Leucotrienes		↑ (Nasal secretions)	Unknown	Unknown
	FPL-55712	–	+ (Sheep studies)	Unknown
Cells				
Mast cells		+	?	?
Neutrophils		–	+	+
Eosinophils		–	+	+
Macrophages		–	+	+
Lymphocytes		–	–	+

↑ = increase; + = effective or present; – = ineffective or absent

nature of the inflammatory response. Samples contained eosinophils, neutrophils and macrophages, as well as large numbers of lymphocytes. The latter cells possess a whole array of inflammatory and chemotactic factors which may, additionally, contribute to the progression of the disease.

Rigourous proof that any of the mediators outlined in Figure 2 are involved in the development of asthma in man requires three criteria – the demonstration of release under appropriate circumstances, the induction of an expected effect and pharmacological antagonism of such effects by agents known to suppress clinical and experimental asthma. It remains regrettably true that these stringent but necessary criteria have not yet been met for any of the compounds that have emerged as possible mediators. The results of the in vivo studies, to date, on the main mediators thought to be of importance in the development of asthma in man are outlined in Table 1.

HISTAMINE

Many investigators have attempted to measure histamine release into the circulation following allergen challenge, but technical problems have made this approach difficult. Histamine has a short half-life in the circulation, rendering sampling problematic. In addition, with the recent advent of a highly specific and sensitive radio-enzymatic assay for histamine, it has become clear that early reports of elevation of circulating histamine in experimentally-induced asthma probably overestimated the levels, due to: (1) lack of specificity in the assays used; and (2) failure to take into account the contribution of whole blood histamine by circulating basophils.

Even with new assays, careful sample handling to avoid basophil-derived histamine is very important, particularly because basophils of asthmatics may release histamine more readily than those of normal subjects. It has been shown, for example, that the simple procedure of separating plasma from red blood cells by centrifuging gives rise to increasing quantities of histamine in the supernatant, depending on the number of basophil cells present.[2] This emphasizes the great care with which samples must be handled and the need to reject at least 1 ml of plasma above the separated cells.

The evidence that plasma histamine is elevated during immediate asthmatic reactions is not conclusive. Nevertheless, some studies have shown that there may be a slight increase in the level of metabolites of histamine found in urine following immediate reaction. There is no such evidence concerning late-phase reactions. Antihistamines, both

inhalant and intravenous, have been shown to inhibit the immediate-phase reaction induced by allergen. This lends weight to the concept that histamine may, indeed, be involved in the development of asthma.[3]

LEUCOTRIENES

A great deal of attention has recently been given to the leucotrienes as potent mediators of asthma,[4] and the release of leucotrienes from allergen-challenged human lung in vitro has lately been reconfirmed. The sulphidopeptide compounds (leucotrienes C4, D4 and E4) have been shown to induce bronchial smooth muscle constriction in vivo in animals and in man, and to cause airflow obstruction when administered intravenously and by inhalation. Indeed, the in vitro potency of inhaled leucotrienes C4 and D4 in causing contraction of bronchial tissue is approximately 3,500-fold greater than that of histamine.

In addition to bronchial smooth muscle contraction, the sulphidopeptide leucotrienes also stimulate mucus production from human airways in vitro and increase airway microvascular permeability. Both of these features are consistent with a role in asthmatic reactions. Leucotriene B4, produced by hydrolase conversion of leucotriene A4, is a potent chemo-attractant for both eosinophils and neutrophils, which, as outlined above and in Figure 2, may play an important role in the development of airway inflammation and hyper-reactivity.

The demonstration of the release of leucotrienes in vivo has proved difficult because of the instability of biological samples. Such release has been claimed for sulphidopeptide leucotrienes in greyhounds and small elevations of leucotrienes B4, C4 and D4 have been noted following bronchial allergen challenges in man. These results have still to be widely confirmed.

Leucotrienes have been shown to be present not only in sputum of patients with asthma, but also in sputum of patients with chronic bronchitis and cystic fibrosis. Increased quantities of leucotrienes have been demonstrated in nasal secretions following allergen challenge. The cellular source of these leucotrienes, however, remains undetermined. The only in vivo clinical trial of a leucotriene antagonist, FPL55712, showed that it had little activity in the small number of patients studied. The situation in animal experiments is different and a recent study with sheep has shown that the inhalation of this compound can block the development of the late asthmatic response following the inhalation of *Ascaris suum*.[5] These results suggest that the late-phase reaction may be partially due to the effects of leucotrienes. However, these com-

pounds may be generated from other inflammatory sources rather than mast cells present in the airways.

PLATELET-ACTIVATING FACTOR

Since the discovery of platelet-activating factor (PAF) as a lipid-like molecule released from IgE-sensitized rabbit basophils, this compound has emerged as one of the most potentially important of the putative mediators of asthma. In addition to potent haemodynamic and vascular activities, it has a constellation of properties that are entirely appropriate to a role as a pharmacological mediator for asthma. Furthermore, its efficacy has been demonstrated in both animal and human models.

NEUTROPHIL CHEMOTACTIC ACTIVITY

The presence of a serum factor in asthamatic patients capable of attracting neutrophils was first described in 1977. Neutrophil chemotactic activity (NCA) was found to increase during asthma induced by exercise and allergen but not by hyperventilation. It was also shown to be elevated in cold, solar and heat urticaria and even in milk-induced wheeze. The fact that NCA could also be generated by challenge of chopped human and guinea-pig lung indicated that this mediator might be released from lung mast cells.

Initial studies suggested that NCA had a high molecular weight of at least 600,000 and an iso-electric point of 6-6.5. Its exact nature remains to be determined but our recent studies have shown that it has both chemotactic and chemokinetic activity.

The fact that NCA may be released from cells additional to mast cells has been shown in our studies of levels of this mediator in serum of patients admitted to hospital with asthma, pneumonia and chronic bronchitis. Levels of NCA in these three groups were equivalent and significantly higher that in serum from patients admitted to hospital for various surgical operations or from non-hospitalized healthy volunteers.

The separation of blood from patients with atopic asthma into its cellular components indicated that NCA could be released from those fractions containing lymphocytes on stimulation with house dust mite allergen or goat antihuman IgE, or even Conconavalin A. Purification to achieve a 90-100% pure lymphocyte fraction, with monocytes as the only contaminating cells and subsequent stimulation with agonists, indicated that the lymphocyte was a source of NCA. Separation of lymphocytes into T and B cells showed that NCA originated from the

T lymphocyte. Incubation of lymphocytes with salbutamol at concentrations around that achieved during treatment of patients with asthma showed that NCA release could be inhibited.

Further studies comparing NCA released from human lymphocytes with that released during allergen-induced reactions in man showed that the materials were identical in terms of molecular weight upon separation on Superose 6B columns. NCA consisted of at least four separate proteins, the largest with a molecular weight of approximately one million daltons and the others having molecular weights of 600,000, 400,000 and less than 60,000 daltons, respectively.[6]

The conclusion from our studies is that NCA can be released from human T lymphocytes upon stimulation with allergen, anti-IgE and Conconavalin A. Furthermore, this release can be inhibited by stimulation of the β 2 adrenoceptors known to be present on the lymphocyte cell surface. Whether NCA is a true lymphokine, or whether it is associated with the cell surface in the way that some acute-phase proteins are attached, has yet to be determined. Release of NCA from lymphocytes during the chronic inflammatory process occurring in asthma could be one of the factors that might maintain the inflammatory response.

The difficulties associated with the demonstration of release of putative mast cell mediators during allergic reactions in man led to the more direct study of mast cell morphology. The human nose is a readily accessible part of the human respiratory tract that is covered by a mucous membrane. There have been few studies of mast cells in the epithelial surfaces in man. The majority of such work, to date, has been performed on the human gastro-intestinal epithelium, particularly that of the small intestine. Studies of tissues obtained from this organ suggest that human mast cells found in the epithelium and lamina propria above the muscularis mucosa may be of a different type to those found below the muscularis mucosa.[7] These two populations of mast cells have been labelled mucosal and connective tissue mast cells, respectively. The nasal mucous membrane consists only of the epithelium and the lamina propria; there is no sub-mucosal region below an equivalent of the muscularis mucosa.

Extensive studies in animals have indicated that the two populations of mast cells differ in origin and behaviour. The mucosal type cell is probably derived from persisting cells (of T lymphocyte origin), as a result of stimulation by lymphocyte growth factors, Interleukin 3, or persisting cell stimulating factor (PSF).[8]

A major difference in the behaviour of these two types of cell is that the granules of mucosal, but not connective, mast cells are 'sensitive' to aldehydes and cannot be studied in tissues processed in routine fixa-

tives, such as formol saline. Mast cells present in the human nasal mucosa have been studied, biopsying the mucous membrane 1 cm distal to the anterior end of the inferior turbinate under local anaesthetic with 10% Xylocaine. Nasal tissues obtained in this way from 10 subjects were divided equally and half fixed in formol saline, the other half in Carnoy's solution (a mixture of absolute alcohol, chloroform and glacial acetic acid in proportion 6:3:1). Sections were cut and stained with both alcian blue and safranine and also with the chloro-acetate esterase technique. The number of mast cells identified per 10 random high-power fields in tissues fixed in formol saline was only a quarter of those identified in tissues fixed in Carnoy's. Further, 90% of the remaining mast cells in tissues fixed in formol saline showed evidence of degranulation compared to only 20% of those in tissues fixed in Carnoy's.

These results would suggest that the majority of mast cells in the human nasal mucous membrane are of the mucosal type. The granules in these cells are considered to have proteoglycans that are poorly sulphated and thought to consist of chrondroitin sulphate, whereas the classical connective tissue type mast cell contains proteoglycans that are more sulphated and largely consist of heparin.

These proteoglycans stain differently in the alcian blue/safranine technique. Granules containing chrondroitin sulphate stain blue, whereas heparin-containing mast cell granules stain reddish-brown. Examination of the nasal mucous membrane indicates that all the identifiable cells stain blue, consistent with the presence within the granules of chrondroitin sulphate.

Additional studies on the type of mast cell present in the nasal mucous membrane can be performed by examining the effect of incubation with amine-releasing agents, such as 48/80, since the granules of the mucosal type mast cell are relatively resistent to the activity of this compound. The importance of these findings is that different types of mast cell may behave differently to therapeutic agents.

Initial studies on the protective role of such compounds as sodium cromoglycate were performed using the rat passive cutaneous anaphylaxis (PCA), a phenomenon involving degranulation of mast cells of the connective tissue type. Not only is it reasonable to suggest that there may be wide differences between species in the response of mast cells to drugs, but also between the types of mast cell involved in the reaction. On this basis, the efficacy of so-called 'anti-allergic drugs' may well require re-examination.

The human nose presents an ideal site for direct study of the behaviour of mucosal type mast cells in response to provocation by specific and non-specific factors and for the study of the possible protec-

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