ASTHMA

Emerging Concepts in Pathophysiology and Clinical Management

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Medical Grand Rounds
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INTRODUCTION

There have been significant changes in our knowledge of the pathophysiology of asthma that have important implications regarding approaches to managing these patients. As a result, this review represents an update on important developments, particularly as they relate to advancement of our knowledge of pathophysiologic processes and clinical management.

In the past we have viewed asthma as being nearly completely caused by the release and direct action of mast cell mediators on the airway smooth muscle, subepithelial glands, epithelium and vasculature. This dated concept is presented in Figure 1. In this model, bronchospasm and increased mucus production in patients with asthma is viewed as an outcome of mast cell mediator release caused by IgE sensitivity to either environmental aeroallergens (in "extrinsic" or allergic asthma) or unknown internal allergens ("intrinsic asthma"). While this model is supported by a great deal of data that demonstrated both the release of these mediators by IgE crosslinking and the ability of mast cell mediators to cause appropriate responses in relevant end organs, its suitability has become diminished as our clinical knowledge of asthma and our ability to study it with new procedures have developed.

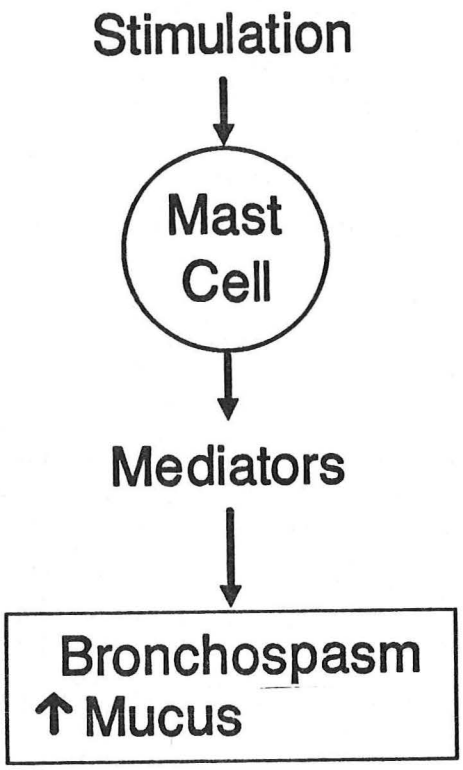


Figure 1

While the mast cell is still likely to have significant importance in the evolution of asthma, it is not solely responsible for the pathophysiological processes that occur. A more realistic view is provided by the summary provided in Figure 2. Inflammation occurs either as a result of mast cell mediators or other mechanisms and is likely essential in the evolution of chronic asthma. In this view, the mast cell is considered important in establishing and perpetuating an inflammatory response, but the contributions of other cells are equally important.

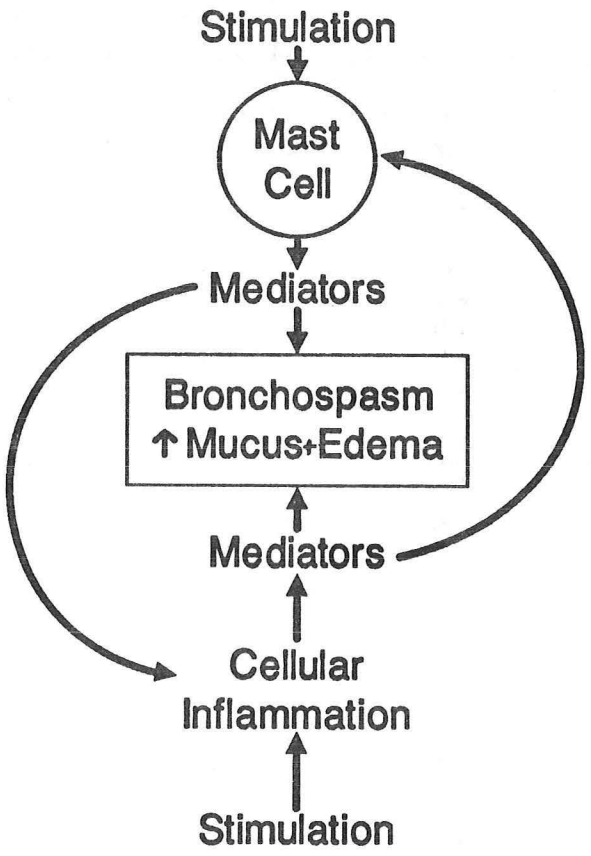


Figure 2

What follows is a series of brief reviews of a number of related but distinct topics that expand upon aspects of the concepts presented in Figure 2 or led to its development. None are dealt with in great detail since each could stand alone as a grand rounds topic. The reader is urged to use the references at the end of each section as a point of departure for more serious study. In the first two sections the concept of nonspecific bronchial hyperreactivity will be discussed as an important parameter of chronic asthma followed by its important association with late phase asthmatic responses. Recent studies documenting the existence of a brisk inflammatory reaction in patients with asthma are presented in the following section on bronchoalveolar lavage/antigen challenge. Because inflammation involves a variety of cells and seems to be

quite important in the evolution of late phase reactions and nonspecific brohchial hyperreactivity, the individual cells involved in these reactions will be discussed in in subsequent sections that describe evidence that supports their importance.

The therapy of asthma is discussed as it relates to changes that are suggested by this different view of asthma. Agents that are newly available or will be shortly are also discussed. For more detail on the basic therapeutic modalities, the reader is referred to Dr. Sullivan's Grand Rounds or any current Allergy or Pulmonary textbook. We are increasingly coming to understand that the inflammatory nature of asthma has important and optimistic therapeutic implications. The ability to antagonize previously unsuspected inflammatory mediators produced by other cells (such as neuropeptides and platelet activating factor) as well as addressing the process of inflammation itself through conventional steroid agents or less conventional agents such as methotrexate and gold therapy may provide us with the opportunity to more adequately treat patients with significant asthma. Because nonspecific bronchial hyperreactivity has a unimodal distribution with asthmatics representing simply those patients who have the greatest reactivity to nonspecific stimulatory agents, we may suspect that treating patients vigorously may be able to shift this reactivity into the normal range and in some situations provide clinical remissions by inductive and subsequent maintainence therapy.

It seems appropriate to feel that we are on the threshold of new and exciting approaches in both the understanding of the inflammatory mechanisms that lead to asthma as well as the therapy that may benefit a large number of patients.

BRONCHIAL HYPERREACTIVITY

Methods of Assessment

A variety of methods have been used to quantitate the bronchospasm that occurs as a result of exposure of the airways to either systemically or inhaled agents. Patients with allergic sensitivity (with IgE antibodies directed against a specific allergen) can be challenged by exposure to increasing concentrations of aerosolized allergen every 15 to 30 minutes until a fall in FEV_1 is noted. This procedure (antigen inhalation challenge) is useful for documenting that a given antigen is responsible for an individual's asthmatic complaints (particularly in occupational asthma). Alternatively, inhalation of putative mediators of bronchial asthma can help to determine their role in asthma and the effectiveness of pharmacologic agents designed to block their action.

Nonspecific bronchial hyperreactivity (NSBH) is a parameter of considerable interest both in the investigation of the mechanisms involved in allergic and nonallergic asthma as well occasional clinical situations where the diagnosis is in question. NSBH is defined as the propensity of an individual to develop airway narrowing (and obstruction to air flow) after exposure to a mediator that is able to provoke immunologically nonspecific bronchoconstriction. Over the years, two agents have been used most frequently to challenge patients during the assessment of NSBH - methacholine and histamine. Although there are relative advantages to the use of each agent, these issues are of less importance in the current discussion. Methacholine has recently been approved by the FDA for studies of NSBH in the evaluation of patients with suspected asthma. Histamine is not approved.

Although the method of evaluating NSBH varies, certain features are common to most laboratories. Bronchodilating agents are with held for 6-24 hours (depending on the agent) and challenge is limited to those patients whose asthma is fairly well controlled. After assessing baseline parameters of pulmonary function of interest (FEV_1, total airway resistence, specific conductance, or other parameters of interest to the investigator), patients inhale nebulized saline followed by a series of solutions of either methacholine or histamine of exponentially increasing concentration. Nebulized solutions are inhaled by tidal breating usually for several minutes followed by measurement of pulmonary function 30 seconds and several minutes after the inhalation of histamine or methacholine. If a suboptimal or no response takes place, the concentration is increased until the investigator achieves the degree of bronchospasm that is desired. Figure 3 illustrates studies of the bronchial reactivity of 4 patients (three asthmatics and one normal). In each situation, with the exception of the normal individual, there is a progressive reduction of FEV_1 with increasing dosages of methacholine. A specific parameter is calculated by extrapolation - in this example, the PC_{20} (provocative concentration that elicits a 20% reduction in FEV_1). The lower the PC_{20}, the greater the sensitivity of the patient. Using this technique groups of

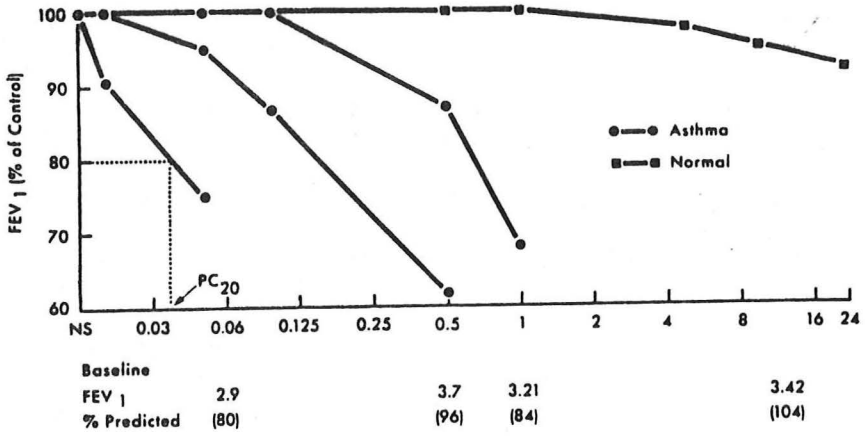


Figure 3

patients's may be compared with each other or with themselves to assess temporal changes in NSBH. As Fig. 4, illustrates there is very good reproducibility within a given patient on a day to day basis in the absence of clinical change, but quite significant differences exist between patients. As a result of patient to patient variability, most assessments of NSBH in patient groups are described using geometric means because of the exponential increase in concentration that is required.

NUMBERS OF CELLS IN BRONCHOALVEOLAR LAVAGE IN 10 NORMAL DOGS AND IN LAVAGE AFTER OZONE EXPOSURE IN 6 DOGS GIVEN NO HYDROXYUREA TREATMENT, AND IN 6 OTHER DOGS STUDIED DURING HYDROXYUREA TREATMENT, AND AGAIN 6 WK AFTER TREATMENT

Cell Type	Normal Values	No Treatment	Hydroxyurea Treatment	6 Wk After Treatment
Neutrophils	0.8 ± 0.2*	7.7 ± 2.1†	0.6 ± 0.2	13.4 ± 3.1
Epithelial Cells	0.3 ± 0.1	4.0 ± 0.8†	2.9 ± 1.3†	3.9 ± 1.0†
Macrophages	12.7 ± 1.9	10.9 ± 2.5	7.3 ± 1.3	6.7 ± 1.2
Lymphocytes	1.3 ± 0.3	0.9 ± 0.2	0.5 ± 0.1	0.7 ± 0.3
Eosinophils	1.3 ± 0.5	0.2 ± 0.2	0.3 ± 0.2	1.0 ± 0.6
Total Cells	16.4 ± 2.0	23.3 ± 1.9†	9.5 ± 3.1	25.9 ± 4.9†

* Units are numbers of cells × 10^4/ml of fluid recovered from lavage (mean ± SEM).
† Value significantly different from normal value. ($p < 0.008$).

Figure 4

NSBH has been used in a variety of clinical situations in the investigation of the pathophysiology of asthma over the last decade. First, it was initially shown that patients with asthma had markedly greater NSBH (lower PC_{20}'s) than did patients with allergic rhinitis or normal patients. Although the degree of difference between asthmatic patients and normal patients is great, patients with allergic rhinitis differ from asthmatics only by approximately

4-fold. This test is useful in the diagnosis of asthma in patients in whom bronchospasm could not be documented or in whom isolated nocturnal symptoms or cough exists. Of note is that NSBH in population studies has been shown to have a unimodal rather than a bimodal distribution. While patient with allergic asthma have much greater sensitivity than most normal individuals, there are asthmatics that fall within the normal range and nonasthmatic patients who fall within the asthmatic range. While this issue may seem trivial, it has important significance in that it suggests that perhaps patients with asthma may be susceptible to therapies which reduce their NSBH and return them to normal rather than simply placing them in the high end of a limited and abnormal distibution.

Animal Models Investigating the Mechanism(s) of NSBH

Because of its clinical relevance, NSBH has been examined in a number of animal models. Exposure of dogs to ozone results in a transient increase in airway resistence and a more prolonged increase in bronchial hyperreactivity. Of note in this model system is that bronchoalveolar lavage (BAL) fluid from animals exposed to ozone contains an increased number of neutrophils concurrent with the evolution of NSBH. Pathologic studies demonstrate the loss of airway epithelium - a finding similar to autopsy studies of patients with asthma with nonasthmatic causes of death. These observations suggest that either epithelial loss or neutrophil infiltration may be responsible for the acquisition of NSBH. Subsequent studies showed that depletion of circulating neutrophils by the use of hydroxyurea or nitrogen mustard abrogated not only the infiltration of neutrophils (Fig. 4) but also the evolution of NSBH. The observation that neutrophil depletion failed to alter epithelial cell loss during ozone exposure coupled with the finding that other pulmonary diseases demonstrate epithelial loss without a change in NSBH suggests that neutrophil infiltration is of greater importance than epithelial loss in the development of NSBH.

These obervations do not diminish the possibilitiy that epithelial cells are of importance, particularly in neutrophil infiltration. Although discussed in more detail in a subsequent section, it has been shown that epithelial cells subjected to noxious stimuli release arachidonic acid that is converted to LTB_4 - an extremely potent chemotactic factor for neutrophils.

Inhalation of a variety of chemical mediators felt to be important in contributing to bronchospasm have not been able to increase NSBH (cholinergic agents, histamine, prostaglandins, leukotrienes, neuropeptides and bradykinin). These data would suggest that a single mediator is not responsible for NSBH and that perhaps the presence of neutrophils is important for the evolution of this reaction. More recent observations demonstrate the possible importance of platelet activating factor (PAF or PAF-acether) in the evolution of NSBH (discussed in more detail in a subsequent section). Inhalation of PAF by baboons and other model systems (and more recently in humans) induces an increase in nonspecific bronchial hyperreactivity that may persist for three to 14 days. The

mechanism(s) underlying these findings are uncertain and likely to be complex.

Clinical Studies of NSBH

A number of clinical observations made in patients with asthma shed light on the mechanisms that might contribute to the development of NSBH as well as the importance of NSBH as a parameter of importance in patients with asthma.

The role of antigen exposure in patients with IgE sensitivity (and allergic asthma) is now well established. Cholinergic NSBH was greater in grass pollen sensitive patients during the grass pollen season and that this sensitivity was reversed after the end of grass pollen season (Figure 5). Platts-Mills demonstrated that in house

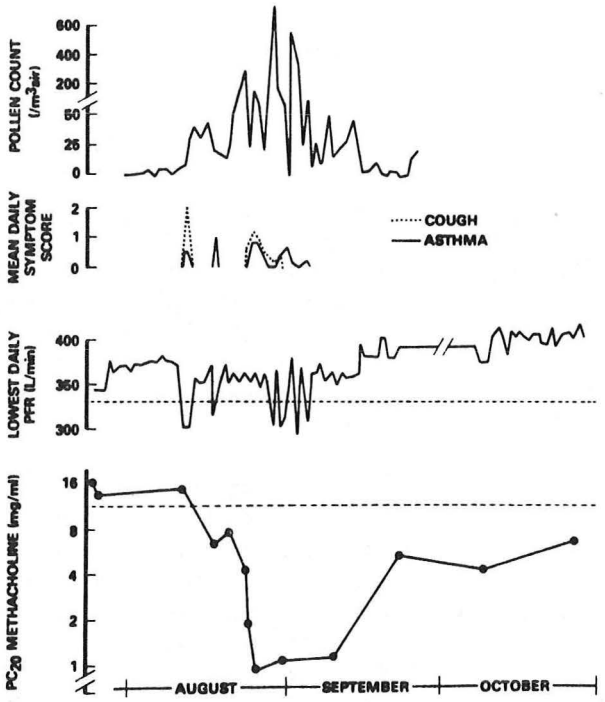
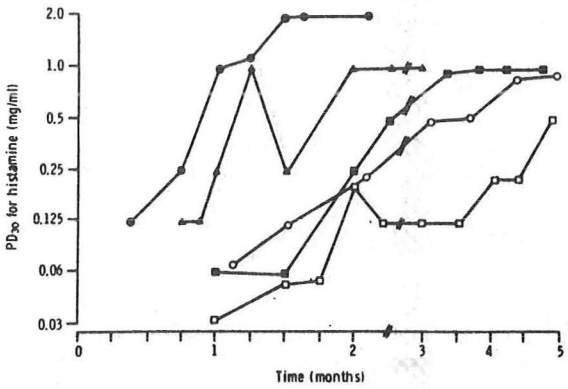


Figure 5

dust mite sensitive asthmatics, near complete removal of house dust antigen by living in a hospital room showed a marked decrease for the need for medications and an eight fold reduction in NSBH to histamine ($PD_{3\emptyset}$) over 6-12 weeks (Fig 6). In addition, Hargreaves demonstrated that men with normal bronchial reactivity who were exposured to toluene diisocyanate (TDI - an agent fairly frequently causes occupational asthma) developed a progressive increase in frequency of asthma and NSBH that abated after exposure was



Time course of changes in bronchial reactivity to histamine in five patients showing eight-fold or greater increase in PD_{30}.

Time is given in months after admission. Normal range for PD_{30} histamine in our laboratory is 2·0 to >8·0 mg/ml.

Figure 6

discontinued. In a subsequent study of grass pollen sensitive asthmatics out of season (in whom their NSBH was minimized), brochial inhalation challenge with grass pollen antigen resulted in an increase in NSBH but only if the patient demonstrated a late phase reaction (not if an isolated early reaction was observed). Pollen-associated seasonal increases in NSBH in patients with IgE sensitivity were shown to be reversed with treatment with systemic or topical glucocorticoids (agents that also ablate the late phase asthmatic response during antigen challenge). A variety of studies have been shown that NSBH may increase for as long as several months after a single inhalation challenge.

As illustrated in Figure 7, a study of normal college students demonstated the association of atopy (those individuals with + skin test reactions to regionally appropriate antigens) and NSBH. Of note however, is that while the frequency of asthma increased with greater NSBH a significant number of normal patients without asthmatic or rhinitic complaints had significantly abnormal bronchial reactivity. This suggests that the propensity to develop clinical allergy (atopy) and NSBH are not likely inherited as a single trait although variable penetrance could be argued. This hypothesis is additionally supported by studies of children in rural and urban New Guinea that suggest that NSBH may be genetically determined but is not expressed until it is activated by unspecified immunologic or nonspecific pulmonary insults.

NSBH and Late Phase Asthmatic Responses

The association of NSBH with late phase reactions (LPR) is of substantial interest and importance. Late phase reactions were initially examined in the skin, but have been demonstrated to occur

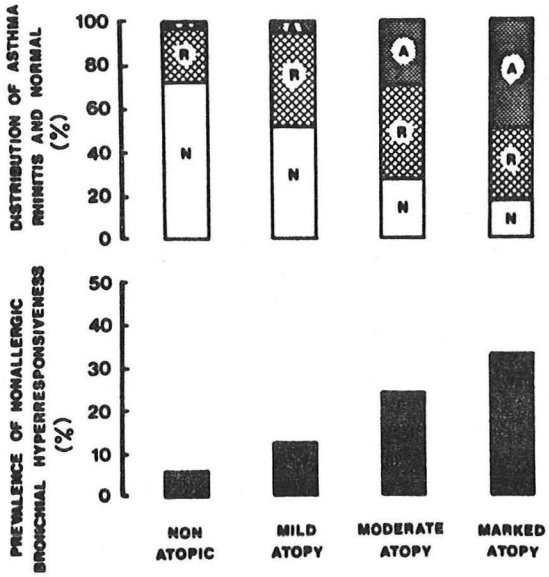


Figure 7

in the nose, lungs and skin after exposure to antigen in individuals with appropriate IgE sensitivity (discussed in more detail in a separate section). Neutrophil depletion in animal studies of antigen inhalation results in a parallel ablation of neutrophil infiltration, the late asthmatic repsonse and the increase in NSBH. Glucorticoid pretreatment of patients with late asthmatic repsonses after antigen challenge abolishes the late phase but not the early phase bronchospasm and prevents the development of NSBH. Cromolyn inhalation has been shown to ablate both the early and late phase asthmatic responses after antigen challenge, but agents that eliminate only the early phase have no effect on the evolution of NSBH. Additionally, it has been shown that treatment of patients with either intrinsic or extrinsic asthma with cromolyn sodium or inhaled or systemic steroids reduces NSBH in parallel with improvements in their status. This observation underscores the importance of NSBH as an indicator of clinical status and a parameter that may be very useful in investigational settings.

The previous discussion suggests mechamisms that may contribute to the development of NSBH in patients with **allergic asthma** but the mechanism(s) by which it evolves in "intrinsic" asthma is (are) less clear. Exposure of asthmatics to a number of materials or clinical situations causes increases in NSBH. These include viral infections, certain immunizations, chemical irritants, hypotonic mists and pollution. Most of these situations are transient and therefore the mechanism that perpetuates an increase of NSBH in nonallergic asthma is not known at present, but it seems reasonable to propose that individuals may have a genetic predisposition toward the development of NSBH and that frequent albeit transient exposure to the agents described above may exceed a "threshold" for the evolution of a positive feedback loop that results in sustained asthma (Figure 2).

Support for the possibility that these reactions can be triggered exogenously but become self sustaining comes from the observations in patients with Western Red Cedar occupational asthma. Removal of antigen exposure resulted in resolution of symptoms in only 40% of patients while the remaining 60% had symptoms consistent with "intrinsic asthma" despite the absence of asthma prior to occupational exposure. The patients who improved had marked reduction in NSBH while those that did not had no improvement in NSBH suggesting that once inflammation and increased NSBH are established it may become self propagating and more difficult to eradicate.

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ALLERGIC LATE PHASE REACTIONS IN THE LUNG

Early Observations

That asthmatic reactions take place over a variable time course after exposure to allergen has been known for some time. Reactions have been categorized as being either isolated early reactions (taking place from 10-30 minutes after exposure), isolated late reactions (taking place from 3-10 hours after exposure), or dual reactions (with both early and late reactions). Examples of an isolated early response and a dual response are shown in Figure 8.

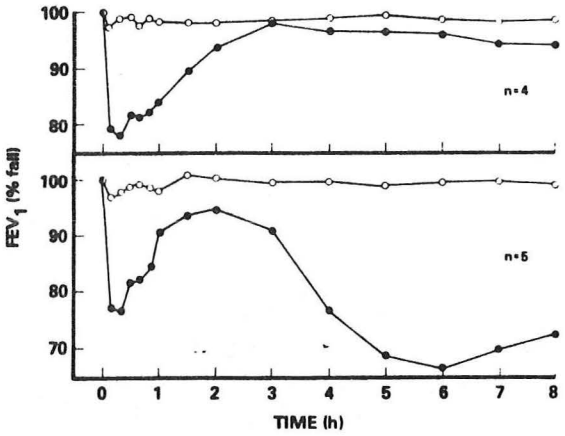


FIG. 3. Mean percent fall in FEV_1 after laboratory exposure to an aerosol of ragweed extract. In four subjects there was an early response, with little or no late response. In five others there was an early response followed by a late response, with a fall in FEV_1 of more than 15% between 3 and 8 hr.

Figure 8

Although the existence of late phase reactions to inhalation of relevant allergens has been observed clinically for years, the generality of this observation for antigen challenge in cutaneous and nasal models was not recognized until more recently. After cutaneous injection of small amounts of antigen in a patient with significant sensitivity (presence of antigen specific IgE), one usually observes the classic wheal and flare reaction which peaks 15 to 20 minutes after challenge and is gone within 30 to 40 minutes. This constitutes an isolated early reaction that represents the result of the release of vasoactive mediators from mast cells that cause increased vascular permeability and vasodilation of postcapillary venules. Late phase reaction can be reproducibly induced by the injection of greater quantities of antigen and occur three to eight hours after antigen challenge. The reaction is quite different from both clinical and pathological perspectives. The late phase lesion is erythemetous, warm, modestly pruritic, indurated and mildly

painful and biopsy demonstrates the presence of a vigorous neutrophil infiltrate. When these reactions had been seen by earlier investigators they had been attributed principally to Arthus reactions involving antigen IgG antibody complexes. The finding that IgE alone can passively sensitize recipients suggested that the immune complex hypothesis was not sufficient. The relationship of this cutaneous inflammatory reaction to the late asthmatic reaction in patients with allergic asthma was uncertain, but provoked interest in its investigation since it appeared that mast cell stimulation in the skin could not only produce immediate reactions, but could also result in the influx of cells that might secondarily release compounds that might be important as mediators of chronic asthma.

Subsequent studies in the nose demonstrated that challenge with appropriate antigens in sensitized individuals resulted the release of mediators known to cause physiologically relevant reactions in allergic rhinitis. Along the same lines, it was shown that the biphasic reactions occurred in approximately 50% of antigen challenges, but did not occur if patients were challenged with histamine alone. Because the lower airway was less accessible to the recovery of putative cells and mediators, data regarding the cellular nature and the mediators involved in late phase asthmatic reactions in humans lagged those of allergic rhinitis despite the clinical observations that had been well known for years. Studies described in a subsequent section related to bronchoalveolar lavage suggest that the kinetics of the late asthmatic response (LAR) is similar or follows rapidly the influx of neutrophils and eosinophils into the airway and surrounding structures suggesting an important contribution by these cells to the evolution of LAR.

Evolution of Perspectives on the Late Asthmatic Response

The initial view held by many investigators was that the biphasic response to antigen exposure in a subset of patients with allergic asthma occurred as a result of biphasic release of mediators from mast cells. They felt that an appropriate model of chronic asthma was that mast cells were somehow able to release mediators chronically as a result of antigen exposure even though the latter might be episodic. A more recent and divergent view is that the presence of neutrophils in the cutaneous lesions of the late response and the inability of antihistamines to block the late response suggested that mediators derived from other cell types might be of great importance in the pepetuation of asthma and that the mast cell might be most important in triggering exacerbations. It seems likely that the truth lies between these two positions - principally the mast cell (and to a lesser extent the macrophage) release mediators that cause the early response and initiate the late response and the perpetuation of the late response in clinical asthma is likely due to a bidirectional interplay of the mast cell with a variety of cells including neutrophils, eosinophils, platelets, macrophages and their products.

Clinical Relevance

Although the existence of late phase reactions occurring 3 to 10 hours after stimulation by appropriate antigens has been known for some time, the importance of these reactions was not emphasized until their presence was found to have predictive value in patients with asthma. Patients with antigen-specific IgE with or without asthma (during an asymptomatic period or who are well controlled) and who have significant late asthmatic responses to antigen challenge demonstrate a significant increase in nonspecific bronchial hyperreactivity (NSBH - see section devoted to this topic) after resolution of the late phase reaction which persists for 2 to 9 days. Patients with equivocal late phase reactions had less frequent evolution of increased NSBH. Conversely, patients who demonstrate isolated early reactions without any late phase reactions failed in nearly all circumstances to show increases in NSBH (Figure 9).

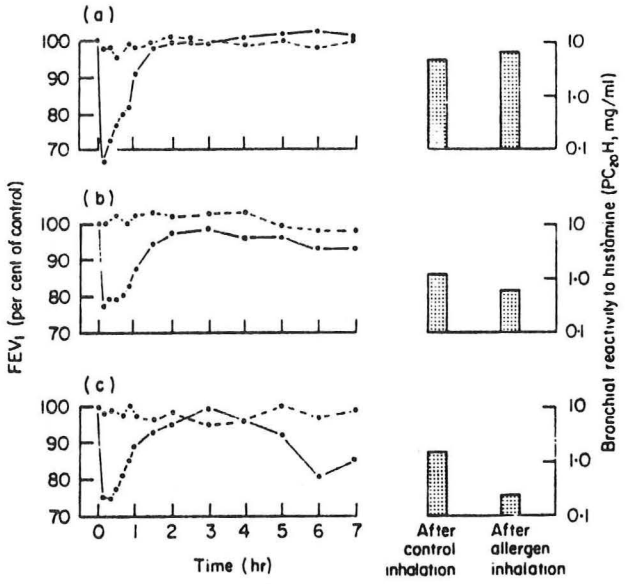


Figure 9

Additionally, the magnitude of the late phase reaction correlated with the reduction of PC_{20} for histamine suggesting that the presence of the late phase reaction was both qualitatively and quantitatively predictive of the increase in NSBH (Figures 10 & 11).

Following initial studies suggesting the importance of late phase asthmatic responses in the evolution of clinically meaningful asthma, a number of pharmacologic studies were performed in an effort to develop an appropriate model that might explain these findings. Unfortunately this approach not only failed to move our thinking forward, but the relatively simplistic assumption that the use of pharmacologic inhibitors would be able to dissect an extremely complicated pathologic process resulted in interpretations that hampered the development of thinking.

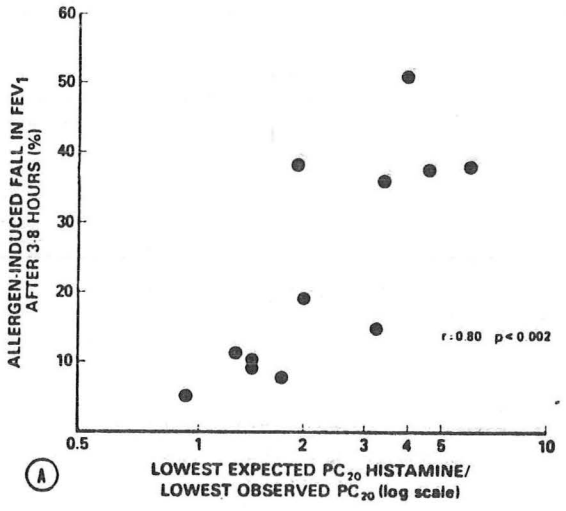


Figure 10

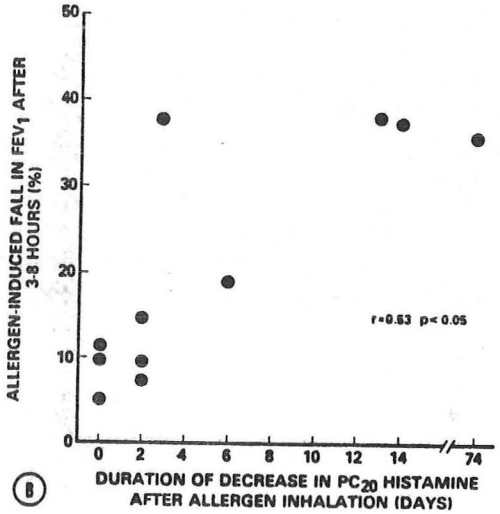
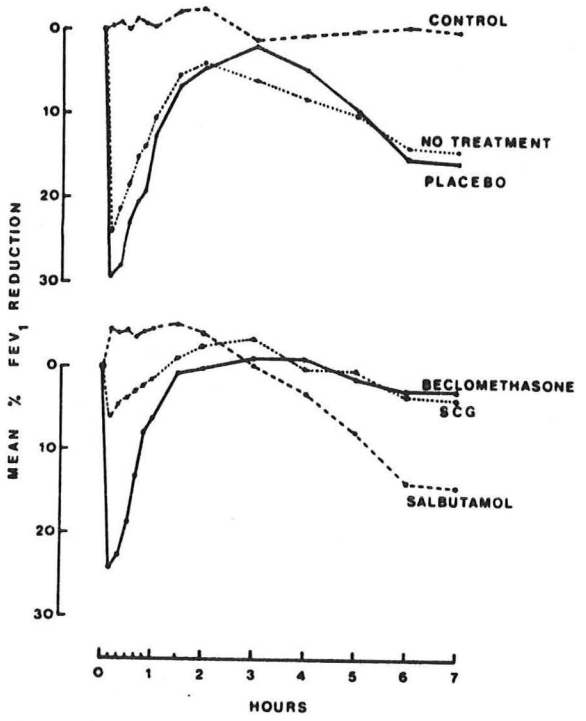
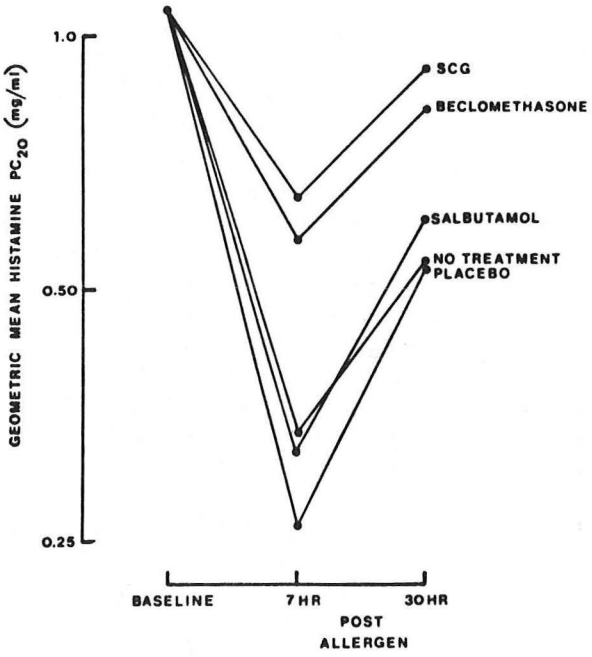


Figure 11

Pretreatment of patients with inhaled cromolyn sodium abolished both early and late phase reactions in patients with dual reactions to anigen inhallation challenge (Figure 12 - next page). Cromolyn was found to inhibit mast cell mediator release <u>in vitro</u> and was therefore felt to block the dual response solely by inhibiting the mast cell directly. Complicating these observations was the finding that pretreatment with beclomethasone for several days or more failed to inhibit the early asthmatic response to antigen challenge, but successfully inhibited the late reaction (Figure 12). Further, inhaled or systemic glucocorticoids were able to markedly reduce NSBH in patients with dual reactions challenged with antigen (Figure 13). Investigators were in a quandary as to how steroids were able to block late mediator release from the mast cell but not the early response but most simply proposed that the initial phase of mediator release from mast cells was less subject to inhibition by either topical or systemic steroids. Similarly complex is the interpretation of data illustrated in Figure 12 that show that the use of beta-agonists were successful in ablating the early asthmatic response, but had a minimal effect on the late asthmatic response and almost no effect on the development of increases in NSBH (Figure 13). Thus, the model of biphasic mast cell release in dual responses and of sole causation of physiologic events of asthma by direct effects of the mast cell became difficult to support.

More recent observations suggest that cromolyn sodium (and its experimental, but more effective replacement nedocromil) probably acts at a variety of levels on nearly all cells involved in the inflammatory response of asthma. Its mast cell effects are likely important, but the erroneous assumption that it was limited to the mast cell retarded the evolution of thinking related to the


CONTROL
NO TREATMENT
PLACEBO
BECLOMETHASONE
SCG
SALBUTAMOL
MEAN % FEV1 REDUCTION
0
10
20
30
0 1 2 3 4 5 6 7
HOURS


GEOMETRIC MEAN HISTAMINE PC20 (mg/ml)
1.0
0.50
0.25
SCG
BECLOMETHASONE
SALBUTAMOL
NO TREATMENT
PLACEBO
BASELINE
7HR
30HR
POST
ALLERGEN

mechanisms of the evolution of late phase asthmatic responses. The unifying concept of this presentation is that a multicellular response likely takes place that results in a plethora of mediators that all contribute to physiologically relevant increases in nonspecific bronchial hyperreactivity.

Several studies of immunotherapy also suggest the importance of late phase reactions in the evolution of clinically relevant allergy. First, studies performed by Dr. Lichtenstein's group demonstrate that in a patients with ragweed allergic rhinitis who were treated with immunotherapy had a marked decrease in the late cutaneous reaction to administration of antigen but a minimal change in the early reaction compared to untreated control patients. Additionally, in patients with allergic asthma and who had been treated with immunotherapy for one year, antigen challenge during a time when patients were not exposed to the relevant antigen failed to alter the early response, but caused a 67 $\pm$ 9% (mean $\pm$ SEM) reduction of the late asthmatic response as well as clinical improvement.

Recent Studies of the Basic Mechanism of Late Asthmatic Responses

The evolution of bronchoalveolar laveage (BAL) in patients with asthma, assays of relevant mediators, and the perspective that cellular inflammation may be important in the development of late phase reactions permitted a variety of interesting studies to shed light on the mechanisms of late asthmatic responses. In one study by Dr. Kay's group, individuals with early asthmatic responses or patients with dual responses were studied with respect to the release into the blood of several mediators found in mast cells. As shown in Figure 14 antigen challenge resulted in prompt increases in plasma histamine that occurred over a 10 to 20 minutes period and returned to baseline within 40 to 60 minutes. However, only patients with late asthmatic responses showed a secondary rise in plasma histamine 3-10 hours after antigen inhalation challenge. Because histamine is present in both basophils and mast cells and could be derived from either, this group sought to evaluate the presence of neutrophil chemotactic activity (NCA) that is released only by mast cells and is not present in circulating basophils. As shown in Figure 14, and similar to histamine, NCA increases in a biphasic manner only in patients with dual reactions and not in patients with isolated early asthmatic responses. This data strongly supports the release of mediators from pulmonary mast cells in a biphasic manner although does not provide any information regarding the mechanism of secondary mast cell mediator release. Subsequent studies by Dr. Wasserman's group that involved more rigorous purification of both eosinophil and neutrophil derived chemotactic activities showed that in patients with dual responses, there was release of both activities early and late, but in patients who had isolated late responses there was no early release of either eosinophil or neutrophil chemotactic activities but brisk late release (Figure 15). The mechanism of isolated late asthmatic responses involving only a late mast cell mediator release is not well understood, but may evolve as a result of the release of mediators fron alveolar macrophages (that possess low affinity IgE receptors) that cause an inflammatory response, a

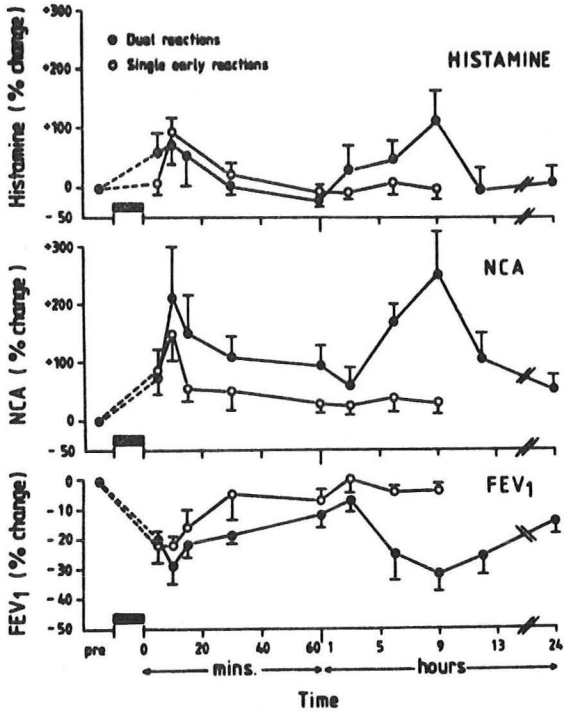


Figure 14

component of which includes the late release of mediators from the mast cell.

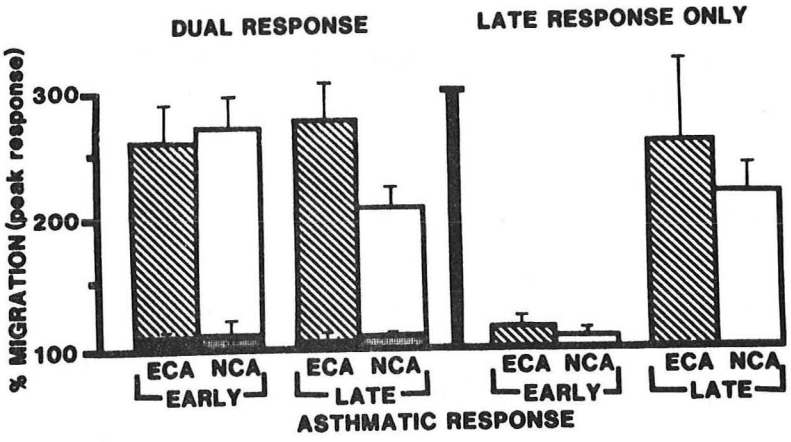


Figure 15

The mechanism of the late asthmatic response is under considerable investigation and is not well understood although some exciting new data have created viable hypotheses. Bronchial

inhalation challenge using a variety of mediators (including histamine, cholinergic compounds, kinins, neuropeptides and leukotrienes) in humans and laboratory animals fails to cause either dual responses or isolated late asthmatic responses. Further, the inability of these compounds to induce increases in NSBH suggests that either a combination of these compounds or that their presence in association with the presence of appropriate inflammatory cells is required for the expression of late phase responses in NSBH. These observations have been disappoining and point toward a more complex mechanism than had been suspected. Additionally these findings suggest that the development of antagonists to these mediators will not alone be sufficient to block the evolution of asthma.

More recent studies investigated the role of platelet activating factor have suggested that perhaps this compound may be very important for late asthmatic responses. In a number of animal model systems, inhalation of PAF causes an increase in NSBH and late bronchoconstriction. Although its name implies action on platelets, PAF has profound effects on a variety of other cells and seems to have a major role in an influx of the eosinophils and neutrophils into the lungs 2-12 hours after antigen challenge. Inhalation of PAF in humnans has been limited but reports demonstrate both late phase reactons and increased NSBH occur in nearly all of these patients (who did not have asthma and were not known to be atopic) which lasted from several days to several weeks despite resolution of initial bronchospasm in less than 12 hours. PAF antagonists and PAF are discussed in more detail in a subsequent section. PAF and antagonist compounds have received a great deal of attention by clinical investigators, basic scientists and pharmaceutical firms in the hope of disabling the evolution of NSBH and late phase reactions in all forms of chronic asthma.

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ANTIGEN CHALLENGE/BRONCHOALVEOLAR LAVAGE IN ASTHMA

Methodologic Approach

During the last 4 years investigators have obtained bronchoscopic lavages of inflammatory mediators and/or cells from the airways and alveoli of patients with asthma without adverse outcome. Studies have included challenge protocols using either inhaled antigen or bronchoscopic instillation of antigen and have been similarly safe and well tolerated. Table I lists the guidelines used by Dr. Hunninghake who has a great deal of experience with this technique.

PRECAUTIONS
Abstain from food or liquids for 6 h before BAL
Premedicate with atropine, metaproterenol, morphine
Anesthetize with topical lidocaine
Soft endotracheal tube in place
Monitor with ear oximetry and EKG
Saline warmed to 37°C
Observe closely until gag reflex returns and FEV_1 returns to baseline
Supplemental oxygen postchallenge during observation

Table I

On average a transient decrease of FEV_1 (averaging 13%) below the prelavage baseline that returned to the prelavage baseline within 15 minutes. Figure 16 indicates the FEV_1 of patients challenged by antigen exposure in the presence or absence of subsequent

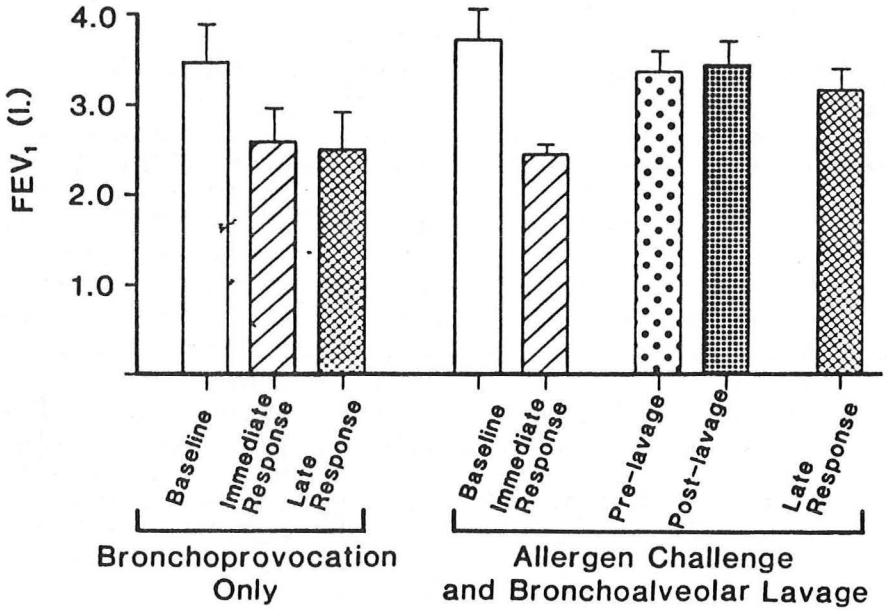

Figure 16

bronchoalveolar lavage. These data demonstrate that there was no significant bronchoconstriction as a result of bronchoalveolar lavage (BAL) after resolution of the transient reduction in airflow. Although frequent cough and occasional wheezing occur, both respond quickly to beta agonist therapy and only one of 63 patients with well controlled asthma was not able to complete the procedure (due to discomfort).

Two approaches to antigen challenge have been utilized in the setting of BAL. In the first, the patient inhales aerosolized antigen which is then deposited in the airways in a manner analagous to natural antigen exposure (although the dose is much greater). BAL is then performed in one or more airways by the instillation of 20 ml of saline that is withdrawn and the lavage procedure repeated 4 additional times. In the second approach, antigen is deposited locally into a segmental airway in a total of 5 ml in a dose that represents 10% of that required for a positive skin test. The dose is increased by 5 fold increments until investigators see a visual decrease of 30% of the airway diameter. Following this observation, cells and mediators are obtained by washing the airway 5 times with 20 ml of saline as before. Although the former approach better approximates physiologic exposure to antigen, the latter permits the patient to serve as his own control because unstimulated contralateral airway can be used in a control challenge

Recovery of Cells in BAL Fluid in Patients with Asthma

When normal individuals or patients with mild or well controlled asthma are subjected to BAL in the absence of antigen challenge, there is very little difference in the nature of the cells that are observed - principally alveolar macrophages with a few lymphocytes, epithelial cells and a few eosinophils in patients with asthma. After allergen inhalation a brisk accumulation of neutrophils is observed 2-4 hours after challenge in patients with appropriate allergic sensitivity. The absolute increase in neutrophils declines rapidly and is back to baseline within 24 hours (Figure 17). Subsequent to the rise to neutrophils is an influx of eosinophils 3 hours or more hours after initial antigen stimulation. When individuals are challenged with histamine alone there is no increased in presence of any cell type, suggesting that bronchoconstriction alone is not responsble for the inflammatory response but is more likely an outgrowth of it. The kinetics of the inflammatory response after local allergen deposition (compared to inhalation challenge) are different. The onset is delayed and differs from inhalation challenge not only the time course, but also by the presence of an impressive accumulation of lymphocytes and activated macrophages 48 and 96 hours after stimulation (Summarized in Table II). Electron microscopic evaluation of eosinophils and the few mast cells obtained by BAL after antigen challenge demonstrate a relative paucity of granules - an expected finding as a result of antigen-induced mast cells secretion which together with other cells release mediators that cause activation of eosinophils (see section on eosinophils). Although the data obtained from humans do not conclusively prove any theory, parallelism with data obtained in

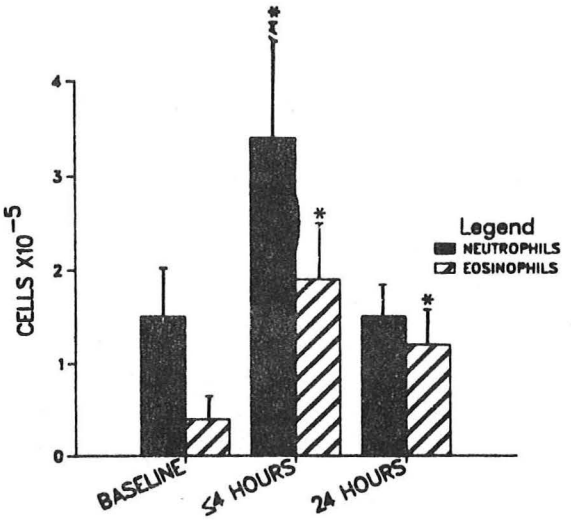

FIGURE 2. Comparison of neutrophils and eosinophils (cells $\times 10^{-5}$) in BAL from asthmatic patients at baseline and ≤4 hr or 24 hour after BPC. Asterisk indicates statistical significance. Bars equal standard error of the means.

Figure 17

animal models (described subsequently) is gratifying and suggests that inflammation is a critical event in the development of increased NSBH and symptomatic asthma after exposure to antigen.

CELLULAR COMPOSITION OF ASTHMATIC BAL AT BASELINE AND IN RESPONSE TO ALLERGEN

Condition	Time* (h)	Total Cells	Macrophages	Lymphocytes	PMN	Eosinophils
Baseline asthmatics†	–	0‡	0	+/0	0	+/0
Aeroallergen challenge§	< 4	0	0	0	+ +	+ + +
	24	0	0	+/0	0	+ +
Local allergen deposition	48	+ + + +	+	+	+ + + +	+ + + +
	96	+ + +	+ +	+	0	+ + +

* Time is given in hours after allergen challenge.
† Qualitative changes compared to values in BAL of normal volunteers.
‡ In these comparisons, numbers of total cells, macrophages, lymphocytes, neutrophils, and eosinophils are compared.
§ Qualitative changes relative to baseline asthma values.

Table II

Animal Studies Using BAL to Study Airway Inflammation

Several studies in animal models have examined the in inflammation that might occur in asthma. In experiments examining the response to a simple irritant exposure (rather than antigen challenge), Nadel and coworkers have demonstrated that exposure of

dogs to ozone results in a brisk increase in neutrophils and epithelial cells several hours after exposure. After granulocyte depletion using hydroxyurea, exposure to ozone causes no infiltration by neutrophils and only a modest, but statistically significant, decrease in shedding of epithelial cells (Figure 4). Perhaps most importantly there was a loss of the ozone-induced increase in NSBH by granulocyte depletion.

Allergic Mediator Detected Using BAL in Humans

In a recent study it was shown that histamine could reliably be measured in BAL fluid. Three groups of patients were examined - allergic asthmatics, allergic rhinitics, and normal individuals. As illustrated in Figure 18, allergic asthmatics had higher levels of BAL fluid histamine compared to patients with allergic rhinitis alone although the majority of allergic rhinitics had levels that were below the level of detection (100 pg/ml). Only one normal patient had a detectable BAL histamine level (significant at $p < .05$). This confirms the previously accepted, but unproven assumption that

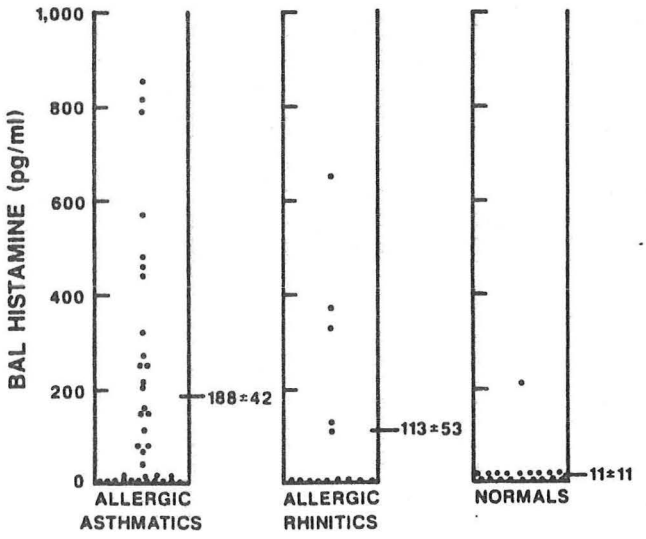

Figure 3. BAL histamine levels in three groups of subjects. Values are expressed as the mean±SE. Allergic asthmatics had a significantly higher mean histamine level versus the normal subjects ($P < 0.05$ by the Tukey-Kramer Test).

Figure 18

histamine is a mediator present in asthma. Although these results fail to demonstrate large increases in histamine levels in patients with asthma compared to rhinitis, it is important to keep in mind that these asthmatics had relatively mild and well controlled disease and were not challenged with antigen. When individuals with allergic asthma with low BAL histamine levels (< 100 picograms/ml) were compared to those with higher histamine, the former group demonstated a seven fold lower PD_{20} (indicative of **greater** NSBH) suggesting that

the processes resulting in increased histamine production resulted in markedly increased airway reactivity (Table III).

Table II. Comparison of Allergic Asthmatics with High and Low BAL Histamine

	Histamine < 100 pg/ml	Histamine > 100 pg/ml	
Number of subjects	19	17	
Histamine (*pg/ml*)	15±7	382±60	$P < 0.001$
FEV_1 (ml)	3,504±234	3,735±245	P = NS
Methacholine PD_{20} (breath units)	49±19	7±2	$P < 0.05$
Lavage cells/ml	116,739±12,931	104,066±11,596	P = NS
% Macrophages	89.4±2.2	92.1±1.6	P = NS
% Lymphocytes	8.1±1.9	5.7±1.4	P = NS
% Neutrophils	1.6±0.4	1.1±0.3	P = NS
% Eosinophils	0.9±0.2	1.1±0.5	P = NS
% Mast Cells	0.04±0.01	0.06±0.02	P = NS

Statistical comparisons calculated using Student's *t* test.

Table III

Another second mediator that may be involved in asthma that has been examined by BAL is the eosinophil cationic protein (ECP). Although only modest data are available, it appears that this eosinophil derived granule mediator is present in much greater quantities in the BAL from patients with dual reactions vs isolated early reactions.

Although not a mediator, it has been shown that there is a marked increase in plasma protein exudation in BAL fluids after antigen challenge examined by direct quantitation or accumulation of radiolabeled albumin. This indicates that there is a physiologically relevant increase in vascular permeability and edema as a result of allergic inflammation. Importance of the presence of plasma proteins may relate to the increased presence of complement which may become activated as a result of exposure to a variety of proteases released by inflammatory cells with subsequent production of anaphylatoxins which are able to alter neutrophil and mast cell function. The possible importance of altered vascular permeability to the development of clinical asthma is described in more detail in a separate section.

Allergen challenge studies support the existence of edema in allergic inflammation. As a result of local instillation of antigen, bronchoscopy reveals brief blanching followed by hyperemia and narrowing of the airway by a combination of bronchospasm and wheal-like blebs in the airway mucosa. Importance of this latter observation is that while airway obstruction in asthma has been thought to be principally the result of smooth muscle contraction, it seems entirely possible that airway edema may be very important. If true, this would suggest that therapy of moderately severe asthma may need to address the recovery of vascular integrity.

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INCREASED VASCULAR PERMEABILITY/EDEMA IN ASTHMA

Pathologic studies have demonstrated that biopsy of airways of patients with asthma demonstrate fairly impressive epithelial loss and edema of the surrounding mucosa and submucosa. While the contribution of edema to the development of asthma is uncertain, its presence is unequivocal. Figure 19 illustrates this process schematically. Although not a mediator, it has been shown that there

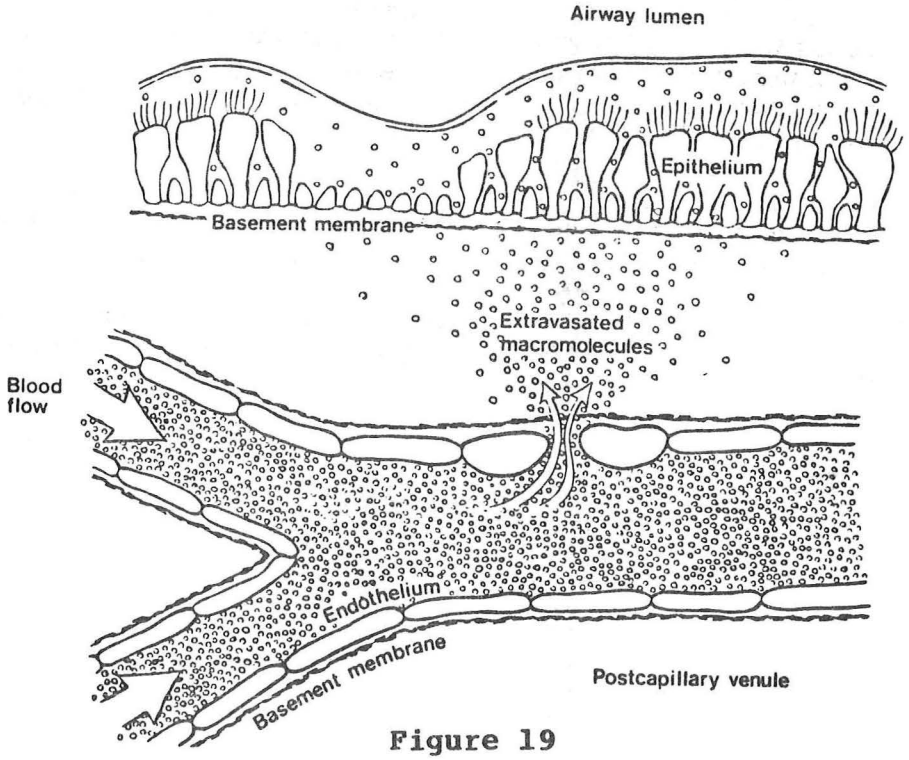

Figure 19

is a marked increase in plasma protein exudation in BAL fluids after antigen challenge examined by direct quantitation or accumulation of radiolabeled albumin. This indicates that there is a physiologically relevant increase in vascular permeability and edema as a result of allergic inflammation. Importance of the presence of plasma proteins may relate to the increased presence of complement which may become activated as a result of exposure to a variety of proteases released by inflammatory cells with subsequent production of anaphylatoxins which are able to alter neutrophil and mast cell function.

In animal and human systems treatment with inhaled mediators such as histamine, PAF, Substance P and leukotrienes results in leakage into the airways of radiolabeled albumin or dextran - compounds normally retained intravascularly. It appears that the vascular leak occurs principally at the level of the postcapillary venule as a result of contraction of adjacent vascular endothelial cells, thereby permitting escape of fluid out of vessels and into the interstitium. Because most of the mediators described above have relatively short biologic half lives, it is uncertain which (if any) of the mediators might be produced on an continuing basis in asthma. Alternatively,

other compounds might be responsible for the vascular leak that is observed chronically in patients with asthma. Besides cell-derived mediators, other systems (such as complement) may contribute to increased vascular permeability and edema.

Allergen challenge studies support the existence of edema in allergic inflammation. As a result of local instillation of antigen, bronchoscopy reveals brief blanching followed by hyperemia and narrowing of the airway by a combination of bronchospasm and wheal-like blebs in the airway mucosa. Importance of this latter observation is that while airway obstruction in asthma has been thought to be principally the result of smooth muscle contraction, it seems entirely possible that airway edema may be very important. If true, this would suggest that therapy of moderately severe asthma may need to address the recovery of vascular integrity.

The effect of the accumulation of edema fluid in the lumen and airway wall is uncertain. It is proposed that intraluminal edema fluid may result in cilliary dysfunction by elevating the mucus blanket. The mechanical presence of edema may facilitate the loss of epithelial cells (which are shed in models of irritant inhalation in the absence cellular infiltration) by mechanical forces. Along a different line, theoretical work described by Hogg and associates suggests that the edema of the walls of airways increases airway resistance may help to explain bronchial hyperreactivity because smooth muscle contraction on the background of a partially reduced lumen would result in proportionally greater increases in resistance of airways by challenge with histamine or cholinergic agents. Unfortunately, little direct data exists to support this hypothesis.

The ability of systemic glucocorticoids to exert a relatively rapid partial improvement in pulmonary function in patients with asthma may relate to the ability of these agents to stabilize endothelial cells and reduce vascular leakage and secondary edema. Other agents able to reduce vascular leakage induced by a variety of mediators and mechanisms include theophylline preparations, beta-agonists and cromolyn but their contribution to improving asthma by this mechanism as opposed to others is uncertain.

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NEUROPEPTIDES AND ASTHMA

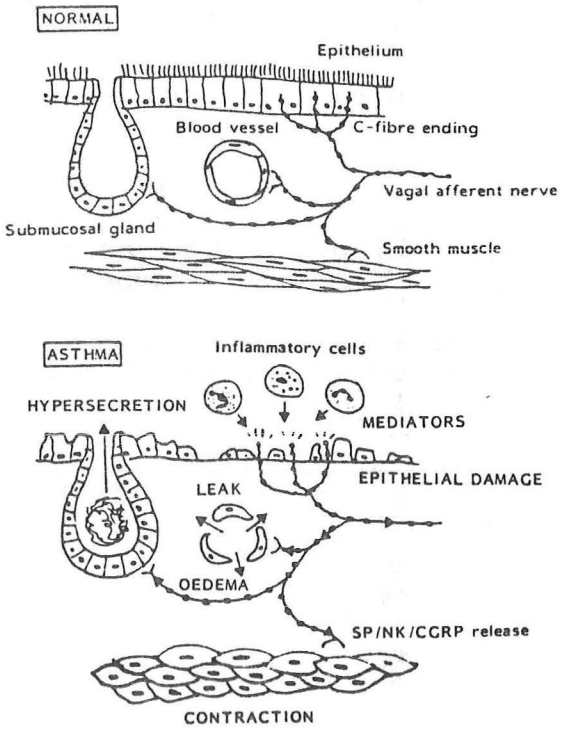
Introduction

The involvement of the central and peripheral nervous systems in provoking and/or perpetuating asthma has long been suspected - the result of observations that emotional factors may be contributes to the evolution of an asthmatic exacerbation. While the clinical data were convincing, a mechanism that would provide an adequate explaination was lacking. Cholinergic bronchoconstriction occurring as a result of increased activity of vagal efferents would be expected to be transient.

Increasing recent evidence suggests that asthma may in part be a result of the activity of locally released and regulated neuropeptides. Neuropeptides of interest in the lung include substance P (SP), neurokinin A (NKA), the calcitonin gene related peptide (CGRP) and vasoactive intestinal peptide (VIP). These peptides have 10-12 amino acids and are structurally very similar with certain invariant portions. These mediators are released as a part of what is termed the "nonadrenergic noncholinergic nervous system."

Release of these materials may occur by either of two mechanisms: 1) increased CNS activity causes increased vagal outflow resulting in classical orthodromic conduction to postganglionic fibers located near blood vessels and airways that result in the release of neuropeptides and 2) stimulation of type C fibers located in the bronchial epithelium by a variety of irritants results in antidromic (retrograde) conduction of action potentials and local release of neuropeptides (Figure 20).

Figure 20



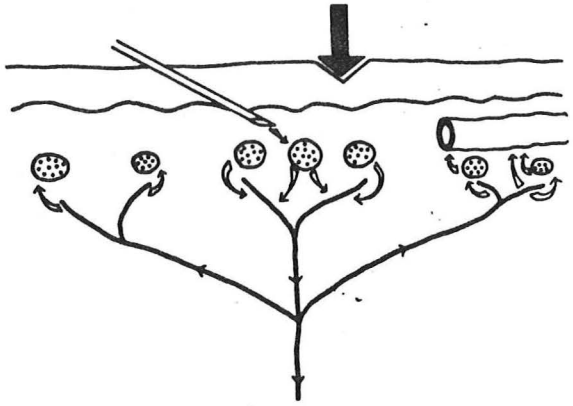


FIG. 1. Model for the interaction of mast cells and sensory neurones in the neurogenic response to injury and inflammatory stimuli. See text for explanation.

Figure 21

Antidromic conduction and release of neuropeptides is felt to cause the vascular responses that are involved in the cutaneous flare (in the wheal and flare response). Loss of peripheral neuronal tissue after denervation explains the long recognized clinical observation that the flare, but not the wheal is markedly attenuated after cutaneous antigen challenge.

Neuropeptides in the Lung

Figure 22 illustrates the possible role that neuropeptides may play in the evolution of inflammatory responses that contribute to asthma. The release of substance P and neurokinin A are able to induce a number of important physiologic responses. In a number of model systems they have been shown to cause vasodilation and increased vascular permeability resulting in edema formation at concentrations well within expected physiologic concentrations (10^{-9} M). In addition, substance P is able to cause smooth muscle contraction that may contribute to bronchospasm. These effects are mediated both by direct action of neuropeptides on appropriate receptors as well as by responses that presumably involve neurologic intermediates (mediated by release of acetylcholine) since a portion of the physiologic effects of substance P are inhibited by anticholinergic agents. Data suggests that substance P may augment the activity of other mediators in the lung. Of note is that immunofluorescent studies demonstrate that substance P receptors seem to be present preferentially in larger airways and become absent distally.

In addition to direct and indirect effects of neuropeptides, on vessels and smooth muscle, Substance P and neurokinin A have profound effects upon a variety of inflammatory cells. In particular, the mast cell is highly sensitive to the presence of these compounds and approximately half maximal secretion occurs at low micromolar

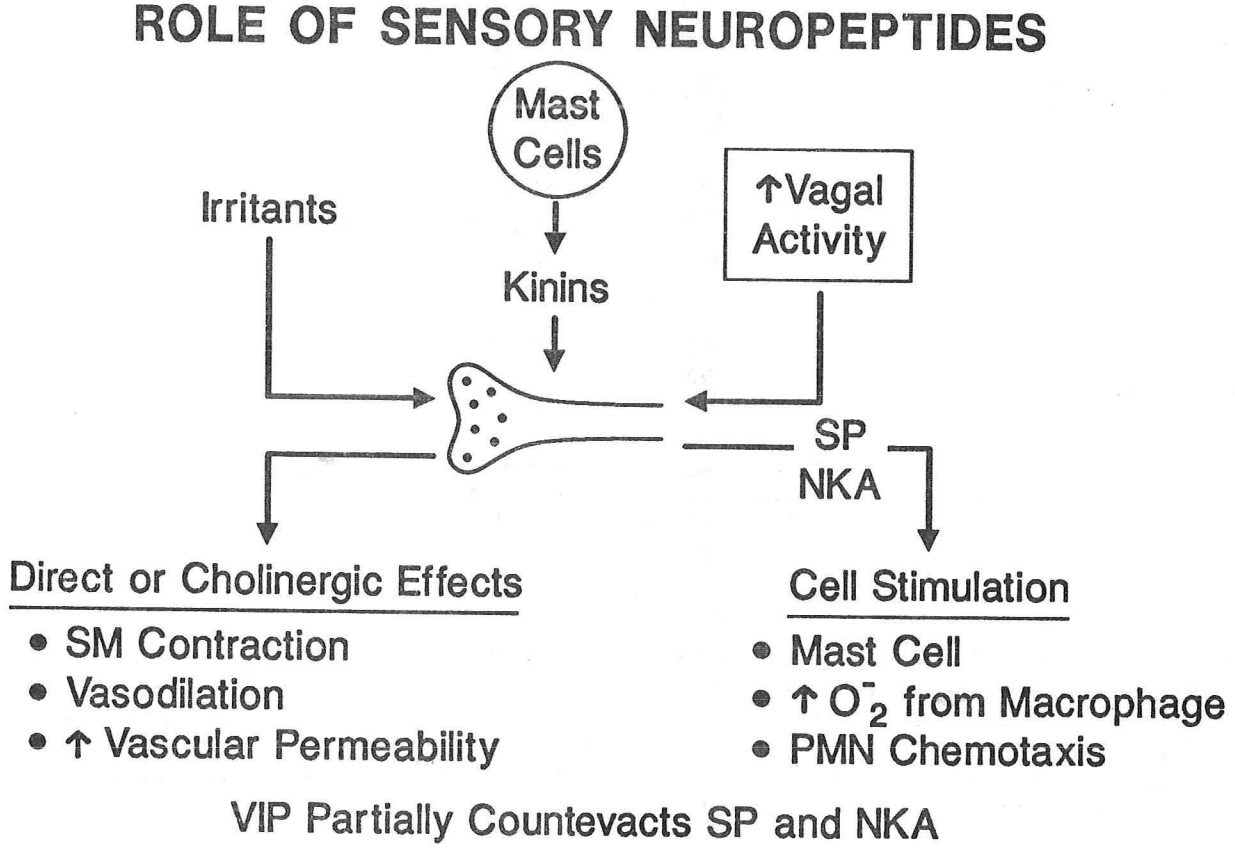


Figure 22

concentrations. Of interest is the observation that the mucosal mast cell, rather than the connective tissue mast cell, seems to be principally effected. [Mast cells have been shown to be heterogenous both from histologic and functional perspectives and at least 2 forms exist. The principal mast cell in the human lung is the mucosal mast cell. These issues will not be addressed in detail in these Grand Rounds, but references for further study are provided in the section on mast cells.] Thus, the release of Substance P can result an impressive array of physiologically relevant enpoints (bronchospasm, increased mucus production and edema) not only by its direct effects, but by its ability to recruit cholinergic neurons and mast cells to release relevant bronchoconstrictive, inflammatory and chemotactic mediators. Substance P also increases superoxide generation from macrophages and is a potent chemotactic agent for neutrophils. In addition, substance P increases the phagocytic capacity of human neutrophils and mouse macrophages. Interestingly, substance P also causes smooth muscle proliferation <u>in vitro</u> at concentrations in the nanomolar to micromolar range - a finding that suggests that smooth muscle hypertrophy may in part occur as a result of substance P release.

VIP, on the other hand, exerts an action that partially counters that of substance P in that inhalation of VIP causes bronchodilation by mechansism that are not clear.

Clinical Role of Neuropeptides

A number of studies lend support to the concept that neuropeptides are involved in pulmonary responses to inflammatory stimuli. Inhalation of substance P causes cough, chest tightness and increased airway resistance. Electical stimulation of the vagus nerve in experimental animals results in markedly increased vascular permeability and edema. Capsaisin (the active ingredient in red peppers) causes neuropeptides to leak out of neuronal tissue thereby causing acute proinflammatory responses, but after chronic exposure depletion of neuropeptides occurs and neuropeptide-mediated processes are markedly attenuated. In humans, inhalation of capsaisin results in cough, chest tightness and briefly increased airway resistance. In animal models where exposure has been chronic there is marked reduction of irritant responses to chemicals or inhalation challenge to either bradykinin or histamine.

The importance of the sensory neuropeptides in the evolution of asthma may most importantly relate to several aspects of the pathophysiology of this disorder. First, as discussed previously, SP may amplify mast cell responses and result in a positive feedback loop by the SP-induced release by mast cells of kallikreins which result in the formation of kinins that in turn are able to stimulate the release of SP augmenting mast cell mediator release, etc.. Secondly, neuropeptides may be important in the evolution of exacerbations of asthma that come about as a result of emotional stress presumably as a result of increased vagal activity. Lastly, the loss of bronchial epithelium as a result of edema and cellular inflammation results in exposure of C fiber terminals and increased sensitivity to irritants, mast cell secretion, smooth muscle contraction, vasodilation and edema by the local release of substance P and/or neurokinin A.

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THE ROLE OF MAST CELLS

Introduction

The role of pulmonary mast cells in the evolution of allergic asthma has been known for quite some time and was reviewed extensively in Dr. Sullivan's Grand Rounds protocol several years ago. Over the years since that review, several interesting aspects of mast cell involvement have come to light and will be reviewed here. The reader is referred to Dr. Sullivan's review for more detail regarding well established mechanisms. Figure 23 illustrates a number of mechanisms that may result in mast cell secretion and additionally describes some of the principal ways in which mast cell mediators may result in pathophysiologically relevant processes in bronchial asthma.

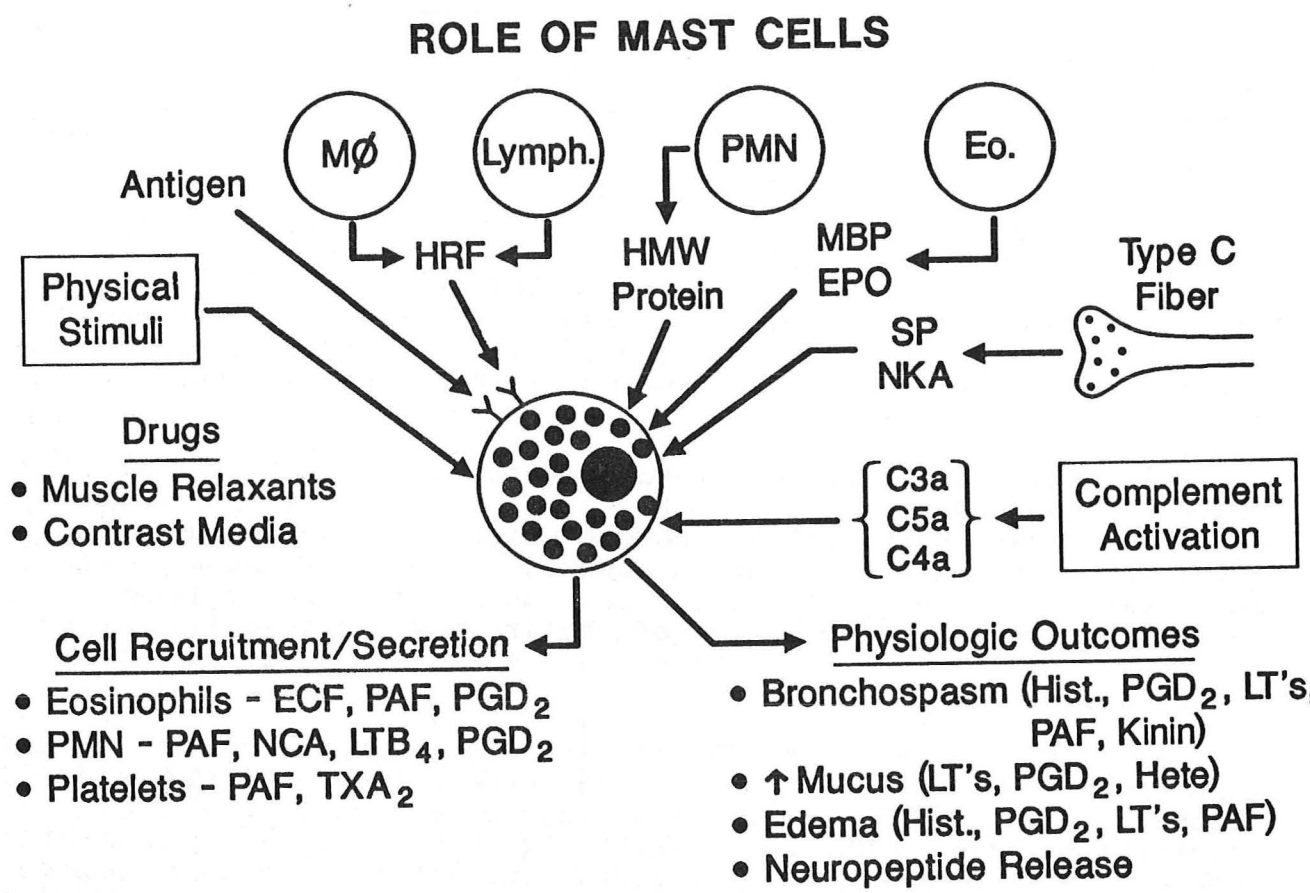

Figure 23

It has long been known that mast cells can be stimulated by crosslinking IgE receptors by appropriate antigen and surface bound IgE. In addition, anaphylatoxins produced as a result of complement

activation (C3a, C5a, C4a) are well known to cause mast cell secretion. Immunologically nonspecific and physiologically relevant compounds that may cause mast cell secretion include physical stimuli, kinins, and a variety of drugs such as muscle relaxants, opiates, iodinated radiocontrast media, dextran and some antibiotics. Further, a high molecular weight protein released by neutrophils after stimulation has been shown to cause mast cell mediator release.

Recent developments that contribute to our thinking about the pathophysiology of asthma include several pathways that may result in positive feedback loops. Specifically, two proteins secreted by activated eosinophils [major basic protein (MBP) and eosinophil peroxidase (EPO)] have been shown at physiologically relevant concentrations to result in noncytotoxic mast cell mediator release. Thus, mast cells can recruit and stimulate eosinophils which can cause mast cells secretion - a feedback loop that may contibute to the perpetuation of airway hyperreactivity in patients with intrinsic asthma. A second feedback mechanism relies on the ability of mast cells to be stimulated by the neuropeptides substance P and neurokinin A. The subsequent release by mast cells of granule-associated kallikrein results in the formation of kinins which are able to stimulate type C fibers which in turn release substance P, etc.

The concept that the mast cell may most importantly act to amplify inflammatory reactions in asthma has resulted in the therapeutic hypothesis that interventions that interfere with positive feedback loops might result in marked improvement of patients with both extrinsic and intrinsic asthma. These concepts are reviewed in more detail subsequently in the section dealing with the therapy of asthma.

Dr. Sullivan's Grand Rounds protocol as well as that of Dr. Tharp (on mastocytosis) reviews the roles of the large number of mediators that mast cells secrete which might induce a variety of physiologic effects important in asthma. Table IV summarizes the most important mediators from mast cells and other relevant cells while Table V indicates some of their phsysiologically relevant actions. Little new knowledge has emerged regarding mast cell mediators.

As described in more detail in other sections, the likely importance of other cells in the perpetuation of inflammation in asthma has diminished the role of the mast cell. In fact, the observation that alveolar macrophages bind IgE to low affinity receptors and then release a variety of mediators (including platelet activating factor) as a result of antigen exposure further undermines the concept that mast cells are solely responsible for the events that occur even in allergic asthma.

Although of uncertain importance in the current context, much work has developed the concept that there are is more than one type of mast cell. Although controversy exists as to their exact

Cellular sources of representative mediators of clinical significance.

	Baso	Endo	Eos	Epith	Lymph	Macro	Mast	Neut	Plt
HETEs			x	x		x		x	x
Histamine	x						x		
LTB_4		x	x	x		x		x	
LTC_4, D_4, E_4	x		x			x	x	x	
MBP	x		x						
PAF	x		x		x	x	x	x	x
PGD_2							x		x
PGE_2		x	x	x		x			
Proteases	x	x	x			x	x	x	
Proteoglycans	x						x		
Serotonin									x
Superoxide	x		x			x	x	x	
Thromboxanes					x	x		x	x

Legend: Baso = basophil; Endo = endothelial cell; Eos = eosinophil; Epith = epithelial cell; Lymph = lymphocyte; Macro = macrophage; Mast = mast cell: Neut = neutrophil; Plt = platelet, MBP = major basic protein.

Table IV

Inflammatory actions of mediators of clinical significance.

	BC	BD	CHEMO	MED	MUC	VP	VC	VD	HYPO
Bradykinin			x			x		x	x
HETE			x	x					
Histamine	x		x		x	x		x	x
LTB_4	x		x	x					
LTC_4	x				x	x	x		
PAF	x		x	x		x	x	x	x
PGD_2	x		x					x	
PGE_2		x				x			
PGF_2	x				x				
Serotonin	x						x	x	
Thromboxanes	x						x	x	

Legend: BC = bronchoconstriction; BD = bronchodilatation; CHEMO = chemotaxis; MED = enhances mediator release; MUC = increases mucus secretion; VP = increases vascular permeability; VC = vasoconstriction; VD = vasodilatation; HYPO = systemic hypotension.

Table V

definition, these distinctions are important because the two major classes (connective tissue and mucosal) have very different capacities for the secretion of histamine and leukotrienes as a result of stimulation. The pulmonary mast cells are principally of the mucosal type. Tables VI and VII list characteristics of both.

EFFECT OF DIFFERENT AGENTS ON MAST CELLS

Agent	Mast Cell Source	
	Peritoneum	Intestine
Antigen, anti-IgE	+ +	+ +
Neutrophil cationic protein C3a, C5a, Dextran, Polylysine	+ +	?
40/80, Bee Venom peptide 401	+ +	0
Ionophores	+ +	+/+ +
Substance P	+ +	+
VIP, Somatostatin, Bradykinin, Neurotensin	+ +	0
Dynorphin, B-Endorphin, Neoendorphin	+ +	0
Cortisone	0	+ + (depletion)
Cromoglycate	+ +	0
Quercetin, doxantrazole	+ +	+

Table VI

MAST CELL MEDIATORS

Mediator	Mast Cell Subtype	
	Mucosal	Connective Tissue
Spasmogenic-vasoactive		
Histamine	+ +	+ + +
Prostaglandin D_2	+	+ + + +
Leukotriene C_4	+ + +	½ +
Platelet-activating factor	+ + +	–
Adenosine	+ +	+ + +
Chemotactic (subtype distribution unknown)		
HMW-NCF*	+ + +	
ECF (3 families)†	+ + +	
Monocyte directed	+ +	
Lymphocyte directed	+ +	
Basophil directed	+	
Enzymatic		
Tryptase	+ + + +	+ + + +
Chymase	–	+ + +
Lysosomal hydrolases	+ + +	+ + +

* High molecular weight neutrophil chemotactic factor.
† Eosinophil chemotactic factors.

Table VII

IgE DEPENDENT RELEASING FACTORS FOR BASOPHILS AND MAST CELLS

Historical Aspects

The formation of soluble factors that result in IgE-dependent secretion of histamine from basophils and/or mast cells was first described in 1979 by Theuson and Grant when they examined the culture supernatants from mitogen or antigen stimulated mononuclear cells. The supernatants - able to cause the release of histamine from basophils - were shown to contain heterogenous materials that seemed to be responsible with molecular weight species of approximately 15-50 kD. This activity was subsequently confirmed in studies by a number of investigators, but efforts toward characterization resulted in divergent findings with a variety of molecular weights from 15-90kD. Certain of the activities have been shown to be active with mast cells although the focus of these studies has been on the circulating basophil. More recently, a similar factor obtained from ubiqitious sources has been the subject of more thorough investigation which has suggested that it might be of considerable physiologic importance in the amplification of inflammatory reactions in asthma.

IgE Dependent Histamine Releasing Factor

Recently studies by a number of investigators in Dr. Lichtenstein's laboratory have demonstrated the presence of an IgE-dependent histamine releasing factors (HRF's) that causes mediator secretion from certain basophils. HRF's are produced by nasal lavage, platelets, cultured endothelial cells, peripheral blood mononuclear cells and macrophages. Of particular note is the observation that HRF is spontaneously released from the epithelium of the upper airway. Its presence in the lower airway (in BAL fluids has not been addressed to date), but seems likely to be found there as a result of the finding that cultured macrophages synthesize and release HRF. Histamine releasing factors have apparent molecular weights (determined by gel filtration chromatography) of 15-30 kD with a smaller broad peak at the void volume (in excess of approximately 50 kD).

HRF is dependent upon the presence of IgE on the surface receptors of basophils and mast cells. Basophils treated to remove 90% of surface IgE (by lactic acid treatment) are unable to respond to HRF. Perhaps more important, however, is the observation that there seems to be functional heterogeneity of IgE molecules in their ability to passively sensitize the basophil for HRF-mediated exocytosis. Early work showed donor to donor variability in basophil responsiveness to HRF, but more recent studies have shown that the ability to respond is a characteristic of the donor's IgE rather than his/her basophils. Thus, IgE from donors with "responsive" basophils will passively sensitize basophils that are "non-responsive". Additional data suggesting that this factor binds to IgE is that it can be removed by "responder" IgE affinity columns. Other data that suggest that HRF acts through activation of

the classic IgE receptor is that prior treatment of cells with anti-IgE in the absence of calcium renders the basophil relatively unresponsive to HRF but not to non-IgE receptor dependent stimuli.

Potential Clinical Relevance - Speculation

The potential importance of these reactions is great although at present unproven. An attractive hypothesis would be that HRF may be important in the generation of late phase reactions (isolated late asthmatic responses and dual responses) by the release of HRF by platelets, macrophages, and leukocytes that are present in the inflammatory response occuring in asthma (Described in detail in previous sections). Even more attractive speculation is the possibility that the ability of an individual to manifest a late phase response may be determined by the nature of the IgE that an individual posesses that causes amplification of allergic inflammation by HRF-mediated augmentation of mast cell and basophil secretion. Supporting this view is the observation that the amount of HRF released from lyphoctes corellates positively with the degree of NSBH. The presence of IgE capable of interacting with HRF(s) may determine an individual's predisposition to the development of clinical asthma as a result of allergen exposure. Further, this concept may provide an explanation for the observation that certain individuals with equally positive skin test reactivity and titers of antigen-specific IgE have allergic rhinitis and conjunctivitis without asthma while other patients will have asthma with only modest rhinitis.

The nature of the differences in IgE that make it possible to interact with HRF are only recently receiving rigorous attention and data are preliminary. Thusfar, no data exists that supports the existence of IgE subclasses, but it has been suggested that there may be genetically determined differences in glycosylation of IgE. Thus, it is proposed that HRF may act as a lectin by binding to specific carbohydrate moieties on "responder" IgE.

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THE ROLE OF EOSINOPHILS

Historical Considerations

It has long been known that eosinophils frequent allergic reactions but their role has undergone significant change particularly during the last few years. Shortly after Erlich's recognition of eosinophils by histologic staining in the 19th century, it was observed that they were present in the sputum produced by patients with asthma but not in patient with other pulmonary disorders with sputum production. Further, it has been recognized for many years that severe asthma is often accompanied by impressive peripheral eosinophillia. Only during the last decade have there been dramatic strides in the delineation of the role of eosinophils and systematic investigation of the mediators that are synthesized or secreted when eosinophils are stimulated.

Until quite recently, the prevailing view was that eosinophils antagonized the inflammatory response initiated by the release of mast cell-derived mediators. This hypothesis was supported largely by the observations that a number of mast cell mediators were either destroyed or antagonized by products released from eosinophils. Examples of this antigonism include: the destruction of histamine by an eosinophil granule enzyme, histaminase; the destruction of platelet activating factor by phospholipase D; the inactivation of sulfidopeptide leukotrienes (LTC, LTD, LTE) by the action of arylsulfatase; and the binding of heparin by ionic interaction with eosinophil major basic protein (MBP).

Although this concept was widely popularized for a number of years, it has become increasingly clear that this view is inaccurate. Not only was it difficult to rationalize that eosinophils were able to both kill very hardy parasites and also dampen allergic reactions in asthma. More worrisome data to this troubled hypothesis came when it was determined that the activity of histaminase was quite modest compared to the quantitative presence of histamine in areas of vigorous mast cell mediator release (local concentrations likely reach the low millimolar level). Along the same lines, it was shown that arylsulfatase from eosinophils had very modest activity towards the sulfidopeptide leukotrienes and was therefore able to degrade a rather modest quantitative proportion of leukotrienes. Further undermining this concept that the esosinophilmight be responsible for destroying leukotrienes is the observation that they synthesize significant quantities of the 4 and 15 series leukotrienes themselves after physiologically relevant stimulation. Eosinophil phospholipase D has been subsequently shown to be inactive against PAF completing the destruction of the concept that eosinophils are principally important in down regulating allergic inflammation by mediator inactivation.

Currently, eosinophils are viewed as having a proinflammatory relationship in the evolution of asthma (Figure 24). The ability of this cell to produce a variety of toxic and inflammatory mediators has grown out of the work of several major investigators in this

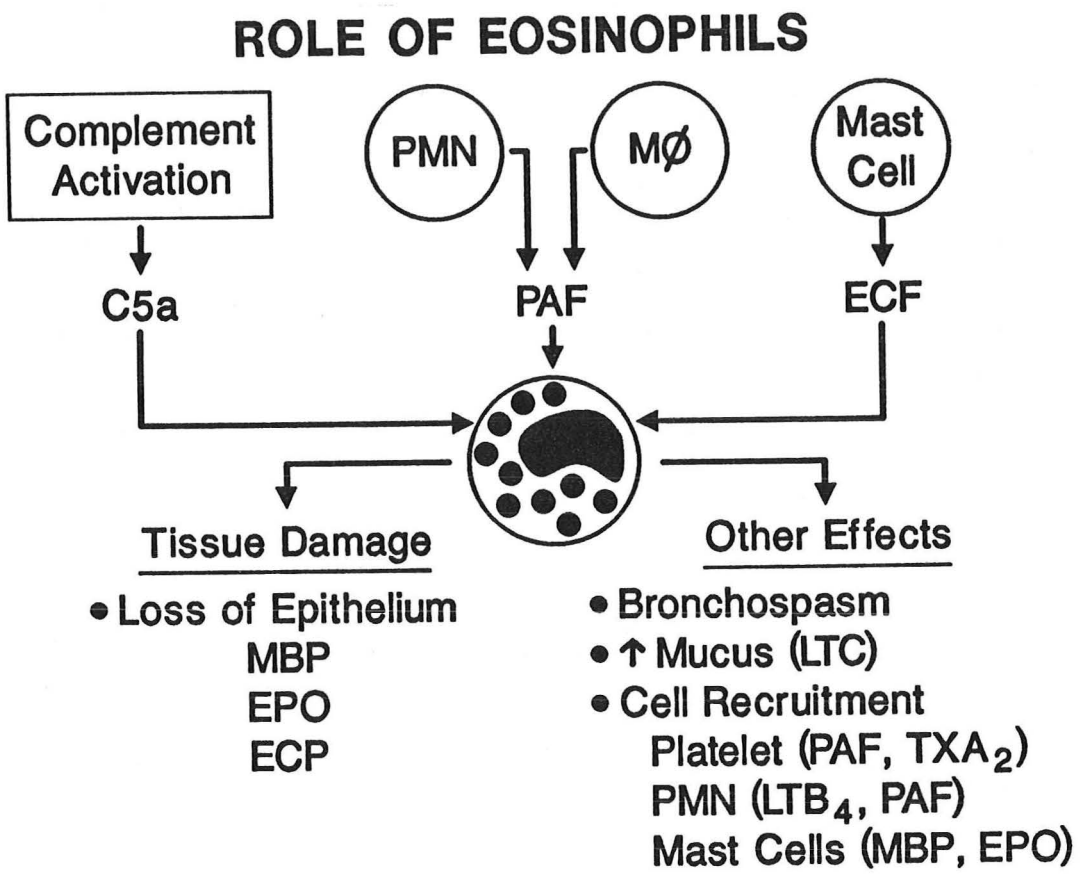

Figure 24

field. The remainder of this section will deal with the likely important contribution that eosinophils have in the evolution of increased airway reactivity, epithelial damage, bronchospasm and increased mucus production that is characteristic of patients with bronchial asthma.

Eosinophil Granule Proteins

Major Basic Protein (MBP) - This low molecular weight (9.3 KD) protein represents more than half of the mass of eosinophil granule proteins. MBP is extremely basic with a PI that exceeds 10. The importance of MBP from a teleologic perspective is that it is very effective in the destruction of parasites - organisms that are resistant to attack by other inflammatory cells (which do not contain significant quantities of MBP). MBP has been shown in vitro to have toxic properties against a number of parasitic agents.

The importance of considering MBP in the current context is that it is able to cause significant effects on airway epithelium. In studies examining the effects of purified MBP on guinea pig tracheal

rings in culture, it was found that over a concentration range of 10 to 100 ug/ml, a dose related increase in epithelial cells detachment from the underlying basement membrane as well as a marked reduction in activity of ciliary activity and presence were noted (Table VII). The importance of epithelial toxicity, shedding and loss of cilliary activity is described in a subsequent section but it is noteworthy that these effects likely amplify the proinflammatory effects of other cells in this regard. In addition to toxic effects, major basic protein has been shown to be able to cause mast cell and basophil secretion. It is attractive to hypothesize that a vicious cycle exists in chronic asthma that involves the release by mast cells of agents that are chemotactic for eosinophils which in turn release MBP that causes further mast cell activation. MBP has been purified to homogeneity and interestingly has been shown to have sulfhydryl groups which must be in a reduced state for full activity.

EFFECT OF MBP ON EPITHELIUM

	3 hour		48 hour	
Cone MBP (ug/ml)	Exfoliation	Cilial Activity	Exfoliation	Cilial Activity
10	-	-	+	partially
50	-	-	++	complete
100	+	_	++	complete
250	++	_	++	complete

Cultured guinea pig tracheal ring exposed to purified guinea pig MBP.

Table VIII

Clinical Studies of MBP - In several clinical studies major basic protein has been shown to be present in the sputum patients with bronchial asthma at levels that suggest it contributes the epithelial loss seen in patients with asthma. Gleich and coworkers developed a radioimmunoassay able to quantitate the presence of MBP levels as low as 0.1 micrograms/ml (approximately 10 nM) in human sputum. Figure 25 shows the results of a study performed that examined the MBP levels in the sputum of patients with asthma or other respiratory diseases. Only one patient with asthma had a level of MBP below 0.1 ug/ml and only a small fraction of patients with other disorders had MBP levels above this. The geometric mean of MBP levels in sputum of patients with athma was 0.34 ug/ml while nonasthmatic patients with

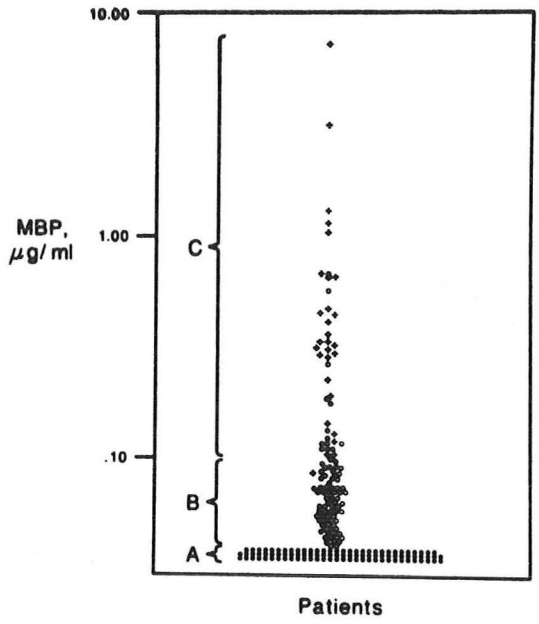

FIG. 6. Sputum MBP levels in 206 patients with various respiratory diseases including asthma. Each symbol represents one patient. The *crosses* identify the patients with asthma; patients without measurable MBP and without asthma (●); patients with measurable MBP but without asthma (○).

Figure 25

other pulmonary diseases had MBP levels averaging Ø.Ø5 ug/ml (at the detection level of the assay in most). Of interest is the observation that the sputum MBP levels in patients hospitalized for exacerbations of asthma were even greater (geometric mean of 7.1 ug/ml) with levels that ranged as high as 92.9 ug/ml. Figure 26 illustrates that the levels of MBP declined as a result of therapeutic intervention during hospitalization. In addition to quantitating the presence of MBP in sputum by radioimmunoassay, the availability of this antibody has permitted immunofluorescence detection of areas of eosinophil activity in which no eosinophils can be seen histologically. In addition to asthma, increased tissue levels of MBP have been demonstrated to occur in atopic dermatitis and urticaria.

Eosinophil Peroxidase (EPO) - Eosinophil peroxidase is a modest component of the eosinophil granule but is quite active in several processes. It has a molecular weight of 75 kD and like MBP, EPO is highly positively charged with a PI that exceeds 1Ø. EPO is similarly disposed toward killing parasites by the generation of toxic oxygen metabolites such as superoxide and is able to kill bacteria through a superoxide/halide system. As with MBP, EPO has some degree of pulmonary toxicity although this the contribution to pathologic changes in asthma by EPO are not. Of note is that EPO is also able to induce mast cell secretion in a non-cytotoxic fashion independent of the generation of toxic oxygen metabolites. Levels of EPO have not been examined in detail in clinical disorders.

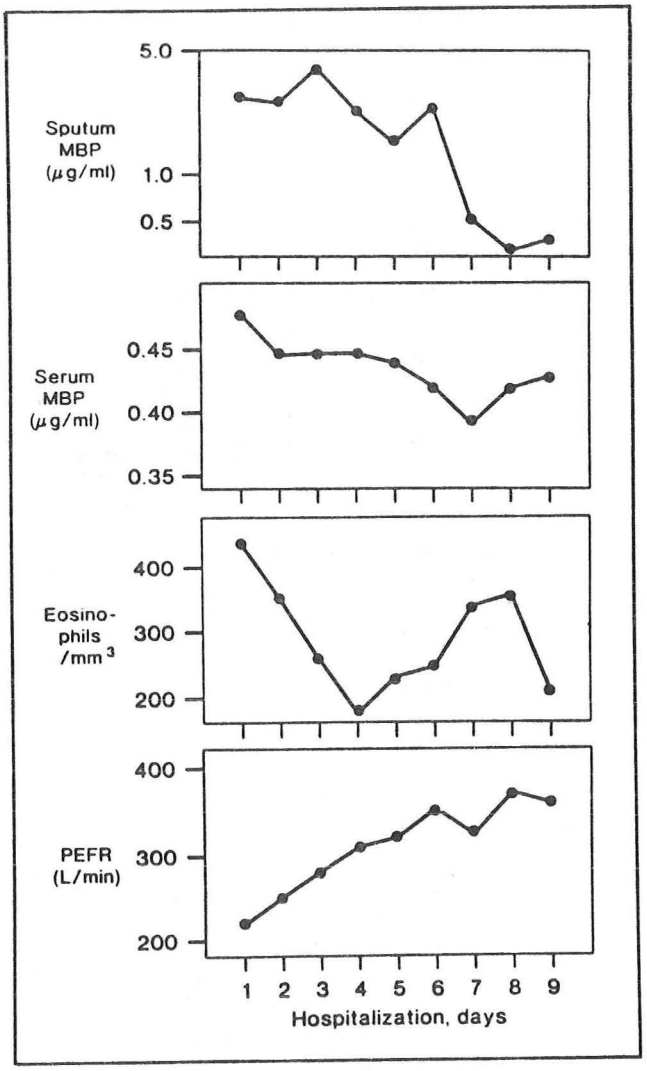

Fig. 3. Changes in peak expiratory flow rates (PEFR) blood eosinophil levels, and serum and sputum MBP levels in 15 patients hospitalized with asthma. Points for the peak expiratory flow rates, blood eosinophils, and serum MBP levels are arithmetic means of daily observations. Sputum MBP levels are geometric means of values shown in Table 1.

Figure 26

Other Eosinophil Granule Proteins - Eosinophil cationic protein (ECP) represents fully 30% of the eosinophil granule protein and possesses a molecular weight of 21-28 kD and is positively charged with PI greater than 10. ECP is able to effect parasite killing and has uncertain effects at relevant concentrations on pulmonary epithelium. Another highly basic protein termed eosinophil derived neurotoxin (EDN) has a molecular weight of 18.9 and like ECP is able to induce parasite killing. EDN derives its name because of its ability to induce a fatal demyelinatng syndrome when injected in

small quantities into the CSF of experimental animals (a similar reaction occurs with ECP). This effect may be of some importance in the central nervous system disorders seen in patients with hypereosinophil syndrome.

Other Eosinophil-Derived Mediators

Eosinophils produce several other compounds as a result of stimulation that likely contribute to inflammatory responses. Arachidonic acid is liberated and converted to LTC_4 and LTD_4 as well as 15 series leukotrienes. These mediators are able to induce bronchospasm and can cause enhanced mucus production and increased vascular permeability. Eosinophils have also been shown to form platelet activating factor (PAF) which can induce a host of important responses in the lung (described in detail in another section) that result in bronchoconstriction, increased nonspecific bronchial hyperreactivity, increased vascular permeability and recruitment of eosinophils and neutrophils as well as platelets.

Eosinophils also generate phospholipase D which is capable of removing the hydrophillic portion of phospholipid head groups (producing phosphatidic acid). As described above, the initial view that eosinophil phospholipase D was important in the detoxification of PAF has been discarded and its role is currently uncertain. The eosinophil produces large amounts of lysophospholipase which often crystalizes forming the Charcot Leyden crystals seen in the sputum of patients with asthma. The role of this enzyme is speculative as it is unclear that lysophospholipids have a major pathophysiologic role in the evolution of asthma.

Eosinophils in Patients with Asthma

In addition to being recruited into the airways and sputum of patients with asthma, there is often a concomitant increase in circulating eosinophils. Eosinophils are formed in the bone marrow and after spending approximately 5-6 days there, enter the circulation for less than 12 hours, and reside in tissues for 2-7. Only 0.2 to 1% of the eosinophils are circulating. Circulating eosinophil counts can increase 40-50% after antigen challenge, but do not increase after methacholine-induced bronchospasm of similar magnitude. Acute increases in circulating eosinophils is by release from the bone marrow while chronic eosinophillia occurs as a result of the production of a 50kD T cell-derived lymphokine which increases eosinophilopoiesis. The lung contains approximately 10% of the body's eosinophils.

Eosinophils present in the sputum and blood of patients with asthma appear to be activated. In the sputum, electron microscopic evaluation show a reduction in granules suggesting that exocytosis has been occurring <u>in vivo</u>. Recently it has been found that patients with asthma have an alteration in the density profile of their circulating eosinophils. Figure 27 demonstrates the profile of normal compared to asthmatic patients. The lower mean density is

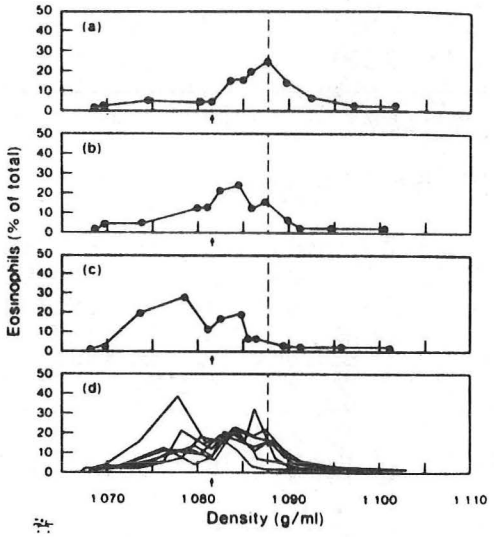

Fig. 3. Density distribution profiles of eosinophils from patients with asthma. a to c. Profiles from 3 representative patients. d. Profiles from 7 additional patients. Results are shown as percent of the total eosinophils recovered from the gradients. Each point represents 1 fraction. The arrows indicate 1.082 g/ml. The broken vertical lines indicate the mean peak density of eosinophils from the 10 normal subjects.

Figure 27

reflective of the presence of "hypodense eosinophils." Active exocytosis of granules as a result of pathologic stimulation in asthma causes this reduction in density.

Eosinophils are recruited into areas of inflammation by a variety of mechanisms. Pertinent to the discussion of bronchial asthma is the observation that a number of mast cell mediators result in eosinophil chemotaxis including: two mast cell-derived tetrapeptides, a high molecular weight mast cell factor, and both histamine and PGD_2 which are chemokinetic. In addition to these mast cell products, eosinophils are vigorously chemotactically responsive to the anaphylatoxins formed as a result of complement activation (C3a and C5a). More recently it has been shown that eosinophils are highly chemotactic (as are neutrophils) to PAF which also results in activation of eosinophils (Figure 28). Because PAF is so highly effective in eosinophil chemotaxis, it seems quite possible that cells which vigorously produce it (neutrophils and macrophages) may be more important in recruiting eosinophils into areas of allergic inflammation. The relative chemotactic activity of a number of eosinophil products is shown in Figure 29.

Summary

The previous discussion strongly suggests that eosinophils produce a variety of proinflammatory substances which likely result

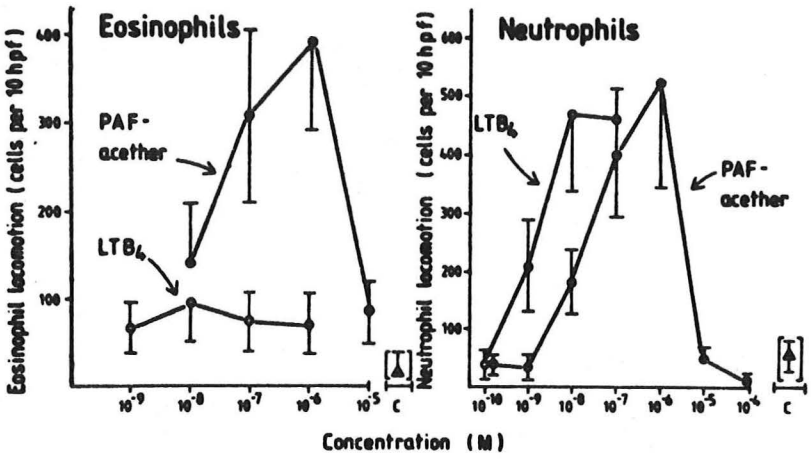

Figure 28

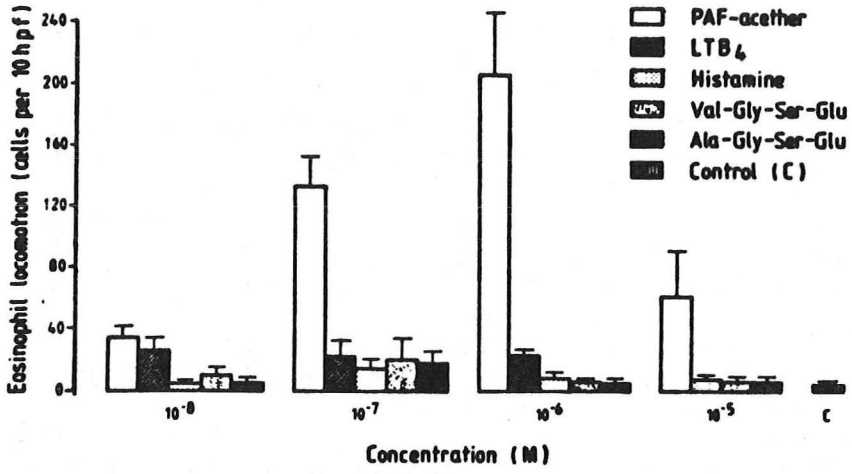

Figure 29

in damage to the bronchial epithelium, bronchospasm and increased mucus production either directly or by their ability to elicit mast cell secretion. The compounds produced by eosinophils that were thought to inactivate proinflammatory mast cell mediators are effete and the hypothesis that grew from these early observations must now be discarded. The finding of significant quantities of MBP in the sputum of asthmatics and that levels rise and decline inversely with clinical status suggests that directing a greater proportion of our scientific as well as therapeutic engergies toward the eosinophil will result in enhanced care of patients with asthma.

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ROLE OF PLATELET ACTIVATING FACTOR (PAF)

General Considerations and Early Observations

The structure of platelet activating factor (1-alkyl,2-acetylglycerophosphorylcholine or PAF) is illustrated in Figure 30. This compound is formed from 1-ether linked species of phosphatidylcholine molecules after deacylation of the 2 position fatty acid followed by enzymatic acetylation. One major degradative reaction is deacetylation to form lyso-PAF. This compound was named PAF as a result of its potent ability to cause platelet aggregation before it's structure or the diversity of its effects on a wide range of cells and tissues were recognized. Cells that are capable of making significant amounts of PAF in sites of allergic inflammation include alveolar macrophages, neutrophils, platelets and mast cells.

Platelet Activating Factor

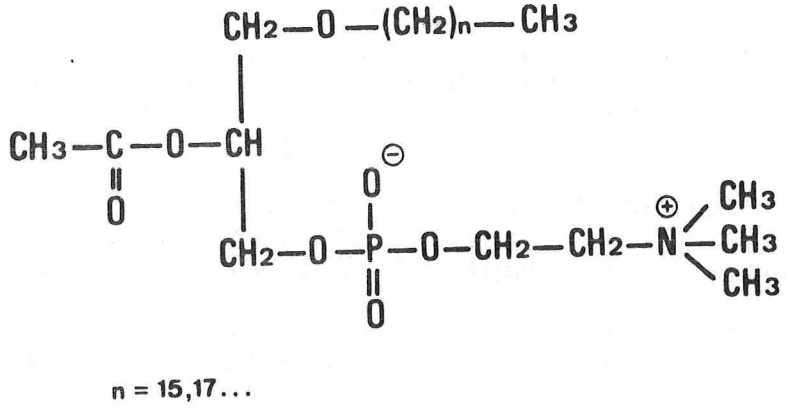


Figure 30

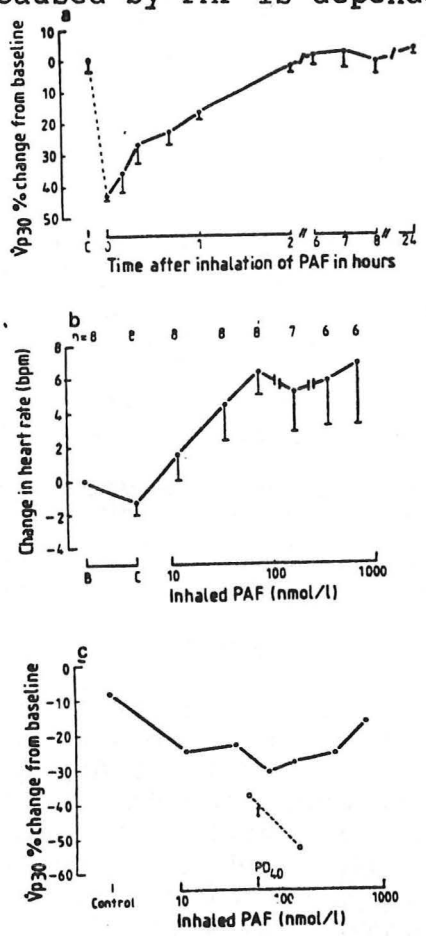
Involvement of PAF in asthma responses was suggested by clinical observations before direct data became available. A comparison between atopic patients with a variety of disorders and nonatopic individuals shows a statistically significant increase in bleeding time despite a statistically significantly increased platelet count. A more recent and notable finding is that asthmatic patients show a seasonal reduction in responsiveness of platelets to PAF that returns to normal at the end of allergen exposure. In these studies, however, there was no alteration in collagen-induced platelet aggregation. Although indirect, these studies suggest that exposure to allergen causes an increase in PAF that causes impaired <u>in vitro</u> responsiveness to PAF, but not other, physiologic agonists.

Physiologic Responses to PAF

After determining the structure of PAF, large amounts were

easily synthesized and a host of experiments in animals to determine how this ubiquitous mediator might participate in a variety of pathologic processes were begun. That PAF may be an important mediator in asthma, particularly of late phase reactions, was first strongly suggested when cutaneous injection of PAF resulted in a delayed reaction more characteristic of a late phase cutaneous reaction to allergen challenge than an immediate reaction.

PAF has been found to cause bronchospasm and flushing when administered either systemically or by inhalation a very low concentration (Figure 31). In some model systems the bronchoconstriction caused by PAF is dependent on the presence of



Acute effect of platelet activating factor (PAF) on pulmonary and cardiovascular function in normal subjects.

a. Duration of PAF-induced bronchoconstriction (●——●) and subsequent pulmonary function up to 24 h after inhalation (mean ± SEM, n = 6). Pulmonary function is measured by determination of expiratory flow at 30% of vital capacity during a partial flow-volume loop ($\dot{V}p_{30}$). The effect of the control (C) solution (ethanol, saline, and human serum albumin) on pulmonary function is also shown.

b. Change in heart rate from baseline (B) following inhalation of control solution (C) and cumulative doses of PAF (mean ± SEM). In two patients $\dot{V}p_{30}$ dropped by >40% after low doses of PAF so cardiovascular responses could not be studied at the highest doses.

c. Change in $\dot{V}p_{30}$ in one subject following inhalation of increasing doses of PAF (●——●) with tachyphylaxis being shown at higher doses. PD_{40} is calculated from drop in $\dot{V}p_{30}$ following higher initial dose of PAF (○- - -○).

Figure 31

circulating platelets. It is notable that this response is partially blocked by pretreatment with cromolyn, ketotifen, glucocorticoids and theophylline.

PAF has been shown in animal models to bring about long lasting increases in NSBH - a finding that is confirmed in preliminary data in humans (Figure 32). In both human and animal studies, the increased NSBH that is induced by a single exposure to PAF lasts 3-14 days (Figure 32). PAF-induced increases of NSBH are attenuated by pretreatment with cromolyn, ketotifen, glucocorticoids or theophylline. Platelet depletion, but not neutrophil depletion, results in a similar reduction in platelet activating factor-mediated increased NSBH in selected animal models, but extrapolation of this finding to the human may not be appropriate.

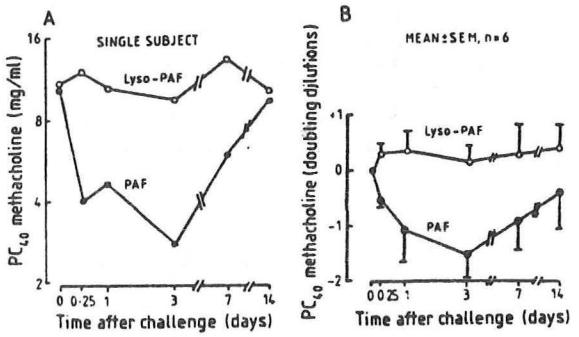


Fig 2—Effect of PAF (●) and lyso-PAF (○) on bronchial responsiveness to methacholine in normal subject.

A. Change in PC_{40} (mg/ml of methacholine) in a single subject.
B. Change in PC_{40} (doubling dilutions of methacholine—see text for details) in six subjects (mean ± SEM).

Figure 32

Recent work demonstrates that PAF is an excellent chemokinetic and chemotactic agent for both neutrophils and eosinophils (Figures 28. In fact, on a molar basis it appears to be the most potent eosinophil chemotactic mediator thus far described (Figure 29). That PAF may be physiologically relevant in the chemotaxis of eosinophils in asthma is supported by the observation that inhalation challenge using PAF results in a brisk influx of eosinophils detected by bronchial alveolar lavage. Lyso-PAF (its degradative product) was inactive.

Preliminary and unconfirmed studies have examined plasma levels of lyso-PAF (PAF is rapidly degraded in plasma and thus can not be measured). Lyso-PAF increased over a 1 to 6 hour period after antigen inhalation challenge in patients with dual asthmatic response, but rose less impressively and in a delayed fashion in patients with isolated early asthmatic reactions to antigen challenge. This was taken to suggest that the brisk increases in lyso-PAF seen in patients with dual responses might represent increased PAF synthesis and spillover into the circulation with

subsequent degradation, while the delayed rise in patients with isolated early asthmatic responses might represent reduced production by these patients of the synthesis of PAF or its effective destruction by other pathways.

Source of PAF

PAF is synthesized by a variety of cells in response to divergent stimuli that are relevant to allergic and nonallergic asthma (Table IV). Alveolar macrophages isolated from patients with relatively poorly controlled asthma released greater quantities of PAF spontaneously than do macrophages from patients without asthma or well controlled asthma. Alveolar macrophages isolated from patients with allergic asthma and are exposure to appropriate antigen or stimulated to synthesize more PAF (IgE-dependent macrophage stimulation is discussed in a separate section). PAF is also made vigorously by neutrophils in response to exposure to LTB_4 and anaphylatoxins, while eosinophils mast cells and platelets synthesize considerably less.

PAF Antagonists

There has been considerable activity in the pharmaceutical industry with regard to the production to PAF antagonists in an effort to produce an agent which might be more useful in asthma than other single mediator inhibitors. Several compounds have been isolated from natural sources including the Ginko tree (a source of herbal medication for asthma) and have impressive antagonist activity in platelet aggregation assays. More relevant is the finding that a purified Ginkgolide (BN 52021) blocks antigen-iduced pulmonary anaphylaxis in guinea pigs. Clinical trials of these agents are underway, but results are thusfar not available. In addition to the effect of BN 52021 on pulmonary anaphylaxis and PAF-induced bronchospasm, it has been shown that this compound is able to reduce endotoxin-induced hypotension, immune complex induced vascular permeability, and cardiac allograft rejection in animal model systems. Thus, this family of compounds shows considerable therapeutic promise for the future, not only in asthma, but in a variety of disorders in which platelet aggregation and inflammation may be pathological.

Of interest is an unconfirmed preliminary study that allergen-induced airway obstruction in an experimental model system is reduced by a lyophilized preparation of onion oils (Figure 33). Perhaps an onion a day will "keep the doctor away."

These data suggest that PAF may be of pivotal importance in the evolution of inflammatory responses in asthma. That it induces the NSBH that typifies chronic asthma in normal individuals is particularly exciting. This raises the question as to whether the defect that permits the chonic presence of NSBH in patients with asthma resides with PAF formation and/or its degradation or whether some other mechanism is involved. In any case, these questions will likely be ammenable to scientific investigation. Efforts toward the

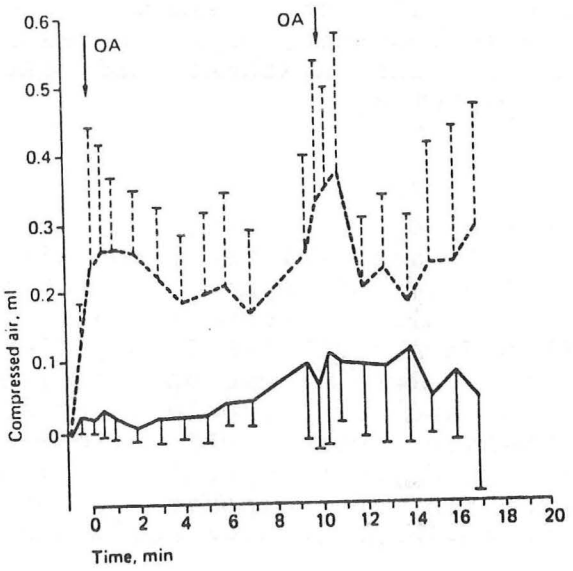


Fig. 1. Inhibition of allergen-induced bronchial obstruction by LOE, 100 mg/kg (——). Each animal inhaled twice a 1% ovalbumin solution (OA, arrows, time 0 and 10 min). The controls without LOE (- - -) show a distinct rise in obstruction at OA challenge. In both groups n = 9.

Figure 33

development of PAF antagonists are great and hold considerable promise for the future treatment of asthma.

Role of Platelets

As is illustrated in Figure 34, platelets may play a significant role in the bronchospasm of allergic and nonallergic asthma. There are a variety of mechanisms that may result in platelet aggregation as are illustrated, but data supporting a proposed mechamism whereby platelets produce a mediator of central importance in the evolution of asthma are lacking.

PAF certainly seems important in its capacity to affect a variety of cells and its direct effect in producing late phase reactions and increased bronchial hyperreactivity are compelling. Although several model systems suggest that these reactions depend on the presence of platelets, generalizing these observations to the human may not be appropriate (particularly in light of differences in the mediators present in the platelets in a number of different animal models). The release by platelets of growth factors may promote some of the hypertropic changes seen in asthma although this attractive hypothesis is unproven. The key limitation to presuming an important role for platelets in human asthma is the lack of an appropriate mediator released by platelets that might be responsible for the myriad of inflammatory changes seen in asthma. Platelets synthesize significant amounts of HETE's (hydroxylated

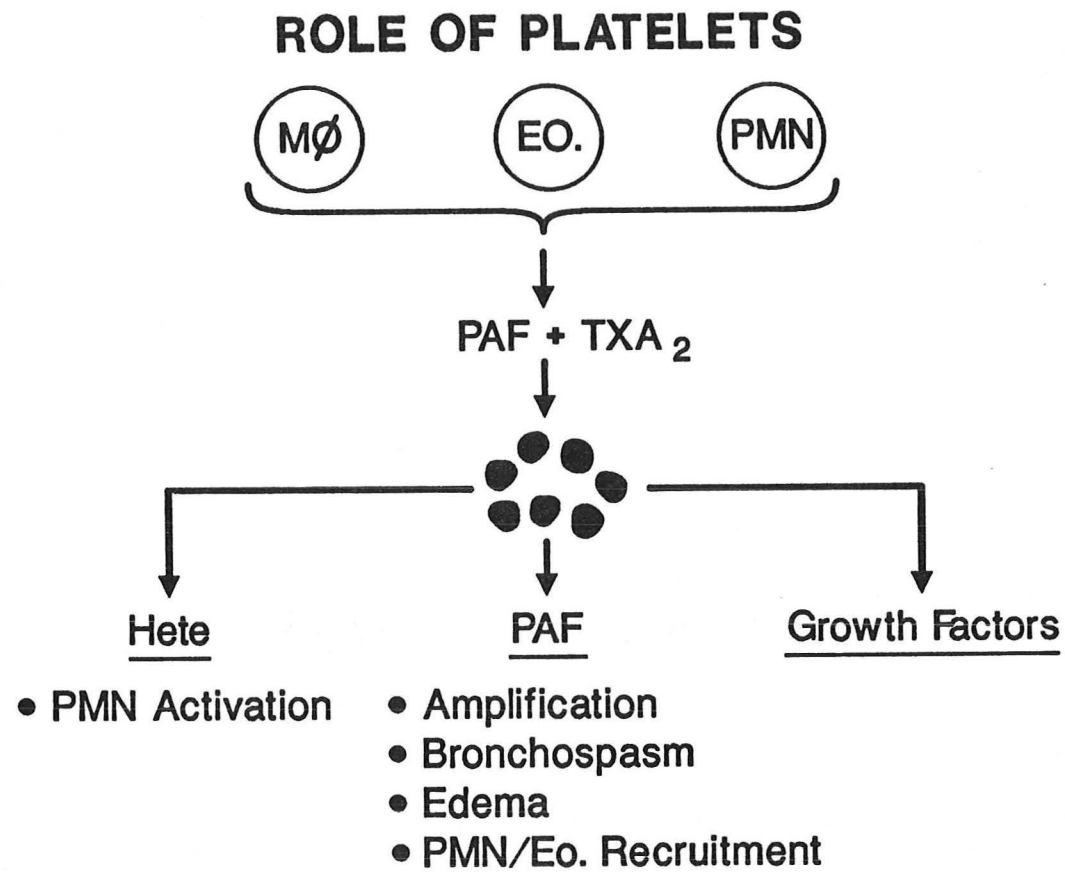


Figure 34

eicosatetraenoic acids) which alone do not cause these reactions, but which may augment neutrophil and mast cell activity. Thus, PAF-induced production of HETE by platelets is likely not responsible for the reactions that PAF causes in pulmonary tissues. Considerably greater study is warranted in order to support the contention that a significant fraction of the effects that PAF causes are mediated by platelets.

A tantalizing but unproven hypothesis relates to aspirin sensitive asthmatics where preliminary data suggest that platelets from aspirin sensitive platelets produce toxic oxygen molecules in response to ASA or NSAID's while normal platelets do not.

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ROLE OF ALVEOLAR MACROPHAGES

Despite the historical impression that alveolar macrophages have a minor and passive role in the evolution of asthma, increasing evidence suggests an important and perhaps key role in certain aspects of the development of airway inflammation (Illustrated schematically in Figure 35).

Interesting recent studies have demonstrated that macrophages have low affinity IgE receptors and exposure to relevant antigen results in the IgE-dependent release of proinflammatory mediators. In patients with allergic sensitivity, alveolar macrophages obtained by BAL have been shown to spontaneously release lysosomal enzymes, chemotactic factors, PAF and leukotrienes in greater amounts than normal individuals. Specificity for antigen-mediated responses has been shown to reside in the IgE since unresponsive alveolar macrophages can be passively sensitized with IgE from sensitive individuals (those possess antigen specific IgE).

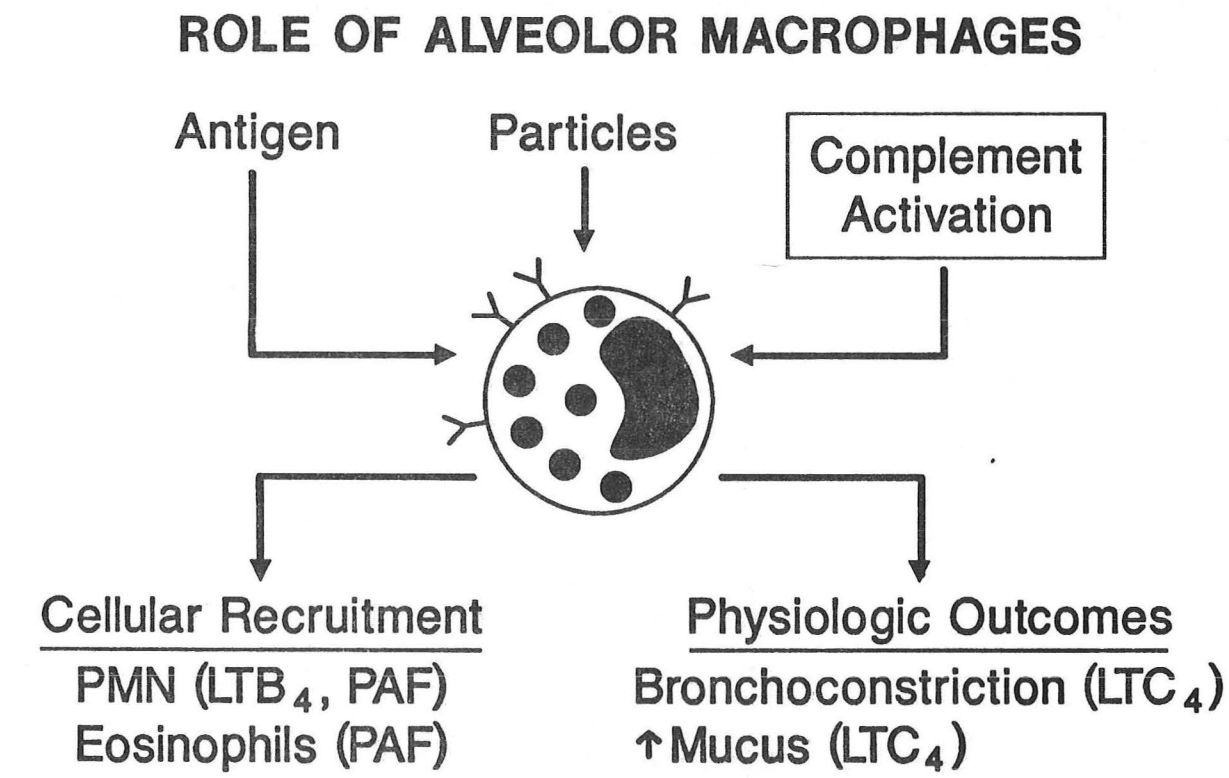

Figure 35

The ability of macrophages to release proinflammatory mediators (particularly PAF) as a result of exposure to appropriate antigens has important and almost certainly physiologically relevant implications. Although the actions of PAF are discussed in detail elsewhere in this protocol, it is useful to reiterate that it seems

likely that PAF is likely to be important in the evolution of late phase asthmatic reactions (that in turn seem to predict clinically significant chronic asthma).

Other data that support the possible importance of macrophages in the evolution of clinically relevant asthma include the ability of beta agonists to markedly reduce the early asthmatic response, but be lacking in its ability to reduce late phase responses. In this phenomenon, it seems possible that antigen exposure leads to parallel stimulation of mast cells and macrophages by IgE-dependent mechanisms and that the activation of the former is responsible for the early asthmatic response while the both contribute to the late asthmatic response through cellular recruitment. Thus, beta agonist-induced inhibition of mast cell release and early asthmatic reactions with minimal impact on the late asthmatic response is not unexpected because of the lack of beta agonist responsiveness of macrophages. Supporting this and the wider view of cromolyn's effectiveness are the observations that cromolyn: 1) reduces the activation of macrophages as well as attenuating responsiveness to PAF, 2) blocks mast cell activation and 3) reduces both early and late phase asthmatic reactions after antigen challenge.

While anaphylactic release of mediators during the 15 to 30 minute period after antigen challenge has been the principal model for asthma during the last 10 to 15 years, it may be more appropriate to view the role of mast cell as being a cell that contributes mediators during the late phase phase reactions as a result of its stimulation by the products of other inflammatory cells as well as its ability to release spasmogenic and chemotactic mediators as a result of antigen exposure. It is possible even that the alveolar macrophage has greater quantitative importance in the evolution of clinically meaningful late phase responses as a result of antigen exposure than does the mast cell, although quantitative data have not been generated to permit a clear view of their relative contributions in antigen-mediated late responses.

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ROLE OF EPITHELIAL CELLS

Although initially felt to be of relatively little importance in the evolution of asthma, increasing evidence suggests that epithelial cells may participate in amplify immune inflammation and contributing very significantly to the evolution of exacerbations of asthma. Figure 36 illustrates mechanisms that involve epithelial cells that may contribute significantly to the pathophysiology of asthma.

Recent work demonstrates that epithelial cells exposed to a variety of commonly inhaled irritants release arachidonatic acid from membrane lipids which is converted to LTB_4. LTB_4 is particularly important in its ability to vigorously recruit neutrophils into sites of irritation or toxic damage. This response is almost certainly a general phenomenon that facilitates the evolution of appropriate nonspecific inflammatory reactions inorder to prevent infections and enhance tissue repair after exposure to toxic materials.

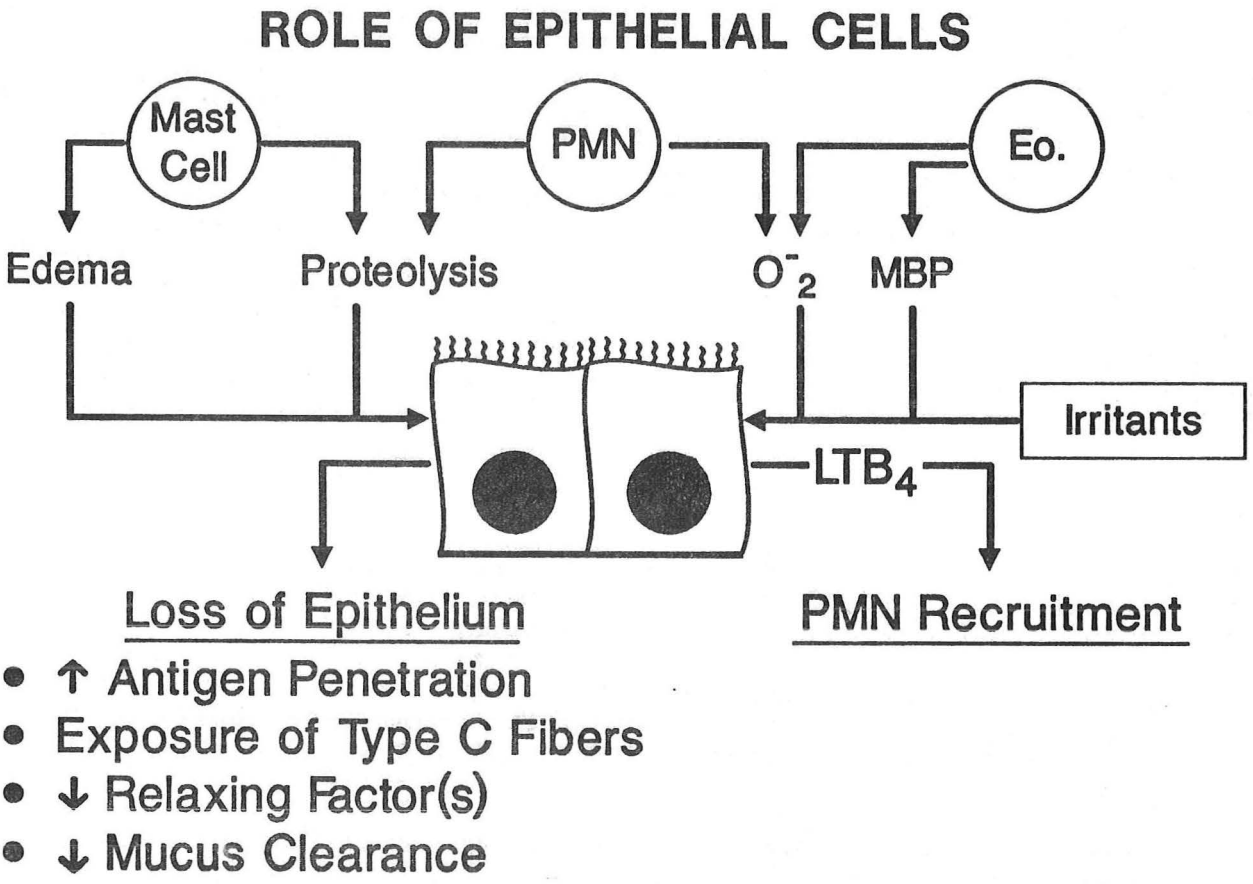

Figure 36

As described in more detail in a separate section, epithelial cells respond in a dose dependent fashion to eosinophil-derived mediators including major basic protein (MBP) and superoxide by a loss of attachment to the basement membrane and reduction of ciliary activity. The ability of damaged epithelial cells to release LTB_4 and recruit neutrophils may result in a vicious cycle by the release from neutrophils of a high molecular weight protein able to cause mast cell secretion which would further enhance both eosinophil and neutrophil chemotaxis. An additional vicious cycle may exist in the ability of recruited neutrophils to generate PAF which causes further chemotaxis of both neutrophils and eosinophils which likely cause even greater epithelial damage.

Reactions that involve epithelial shedding may involve several other processes as well. In patients with allergic sensitivity, the loss of epithelium may increased subepithelial concentrations of antigens resulting in greater mast cell secretion. The loss of ciliated epithelial cells reduces mucocilliary function which in turn causes an impairment of clearance of aeroallergens (in the case of allergic asthmatics) and irritants as well as potential pathogens. More recently, increasing evidence points to the importance of neuropeptides (released as a result of stimulation of C fibers in the pulmonary epithelium) in the evolution of asthma. The loss of epithelial integrity results in greater exposure of C fiber terminals to both the atmosphere and to sputum containing a variety of mediators capable of stimulating action potentials. As described in detail in a separate section, stimulation of type C fibers results in the releease of substance P - a mediator that augments mast cell secretion and which directly causes bronchospasm and increased vascular permeability. Reducing the importance of this hypothesis is the finding that patients with reduced epithelial integrity from nonallergic causes (smokers) do not have increased NSBH, but this occurs on the background of much reduced levels of mast cell and eosinophil mediators.

Of considerable recent interest are studies suggesting the existence of smooth muscle relaxing factors made by epithelial cells. Although no characterization of these factors has been accomplished, several studies suggest their existence. Figure 37 illustrates the dose response curve of tension generated by trachea strips in response to stimulation by substancce P. Compared to intact airway, preparations that have been treated by gentle mechanial abbrasion (removing only the epithelial cells) 40 fold more sensitive. In these studies the existence of a relaxing factor is suggested by a 10 fold shift in the dose response curve of the rubbed airway if it is placed in an organ bath containing intact epithelium.

Thus, not only does the epithelium maintain a barrier against antigen and irritant penetration and by a cilliary activity promote clearance of inflammatory mucus and mediators, but also may serve an important role in the basal release of agents that

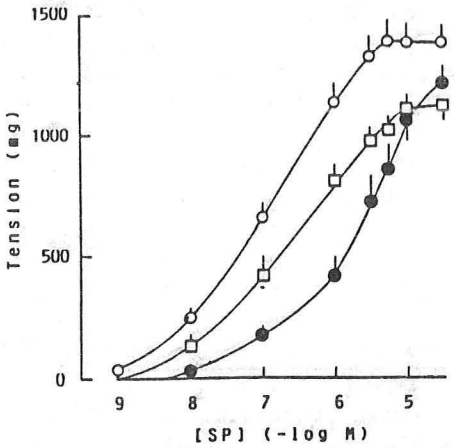

Fig. 1. Concentration-response curves for substance P-induced contractions of intact (●) and rubbed (○, □) tracheal pieces. Rubbed tracheal pieces were mounted alone in one organ bath (○) or according to a 'sandwich protocol' (□). In the latter case, two tracheal pieces, one intact and one without epithelium (□) were mounted in one organ bath with the two luminal surfaces facing. Values are means ± S.E.M. (bars). Results are given in mg of force of contraction.

Figure 37

promote relaxation of smooth muscle. Stimulation of epithelial cells by irritants or viruses results in the chemotaxis of neutrophils which in combination with epithlial shedding may be partially responsible for increased nonspecific bronchial hyperreactivity (NSBH) in patients with asthma.

Although somewhat speculative, it seems quite possible that many clinical exacerbations of asthma are induced by epithelial injury caused by exposure to irritants, viruses and bacteria and the resulting loss of epithelial function, generation of LTB_4 and subsequent neutrophil recruitment that together may cause the phenomenon of NSBH characteristic of asthma.

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THE ROLE OF NEUTROPHILS

Neutrophils interact with nearly all other cells involved in the development of inflammation in asthma either as a cell that generates a mediator of importance or as one that responds to a given mediator. Thus, their involvement in the evolution of asthma has been discussed prinicipally in the sections dealing with the roles that neutrophils play with respect to each of the other cells.

Neutrophils are recruited into the airway as a result of the local generation of PAF, LTB_4 and C5a. A number of model systems support the central role of neutrophils in the evolution of NSBH (discussed in more detail in the section on bronchial hyperreactivity). Thus, the ability of neutrophils to be called into the airway as a result of stimulation either by exposure to antigen and/or nonspecific irritants likely results in increased NSBH that characterizes clinical deterioration of asthma. The ability of the neutrophil to expand the inflammatory response has been less well studied in models of asthma. It seems quite likely that the release of toxic oxygen radicals, PAF, hydroxylated lipoxygenase products of arachidonate and lysosomal proteases either directly or indirectly results in recruitment and/or activation of platelets, mast cells, epithelial cells, basophils, eosinophils and macrophages or inflammatory protein systems such as complement which amplify the pathophysiologic reactions that culminate in airway hyperreactivity and asthma.

An exciting and important development is the emergence of studies that suggest that agents that alter neutrophil function may have benefit in patients with asthma. Specifically, methotrexate and gold have resulted in the reduction of morbidity in steroid-dependent patients and has in selected cases of moderate asthma induced what appears to be permanent remissions of disease (discussed in more detail in the section on therapy).

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THERAPEUTIC IMPLICATIONS

Overview

This section is not intended to be a complete review of the therapy of asthma, but rather an update of changes due to availability of new medications, new information regarding previously available agents and new approaches to combining medications. Experimental use of PAF antagonists and more vigorous antiinflammatory agents holds a great deal of promise for the future. Figure 38 illustrates the three major areas that contribute to asthmatic symptoms: IgE sensitization (for patients with allergic asthma), exposure either to antigens (in allergic asthma) or to irritating agents in patients with both nonallergic and allergic asthma, and constitutional factors that render the patient susceptible to these stimuli. These different contibutors to clinical asthma dictate independent approaches for the care of patients with asthma. These approaches are discussed in separate subsection below.

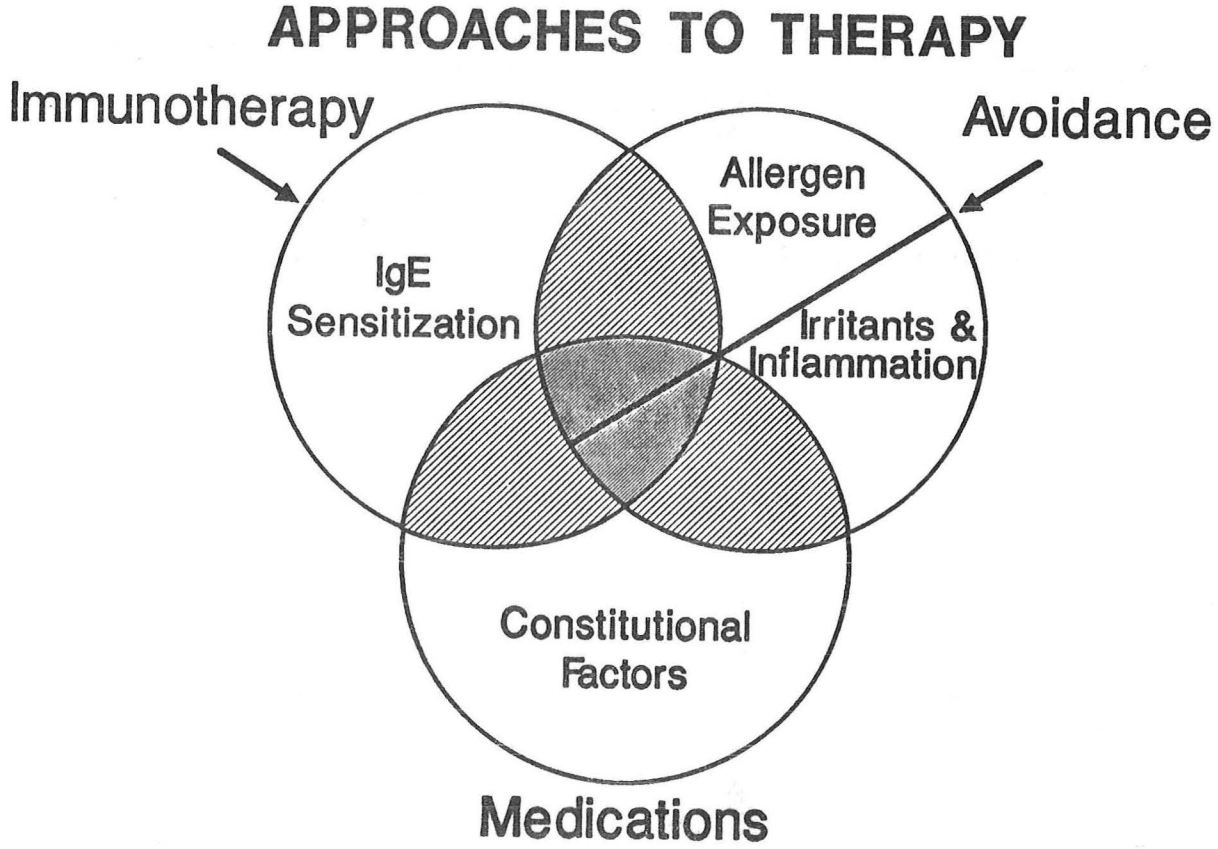

Figure 38

Avoidance

Antigen Avoidence - Platts-Mills has shown that if patients with dust mite induced asthma can avoid exposure to house dust by hospitalization for a prolonged period (several months), there is a marked improvement of symptoms, a reduction in medication, and perhaps most importantly a progressive normalization of NSBH (Figure 6). Although not of practical value, these data show that patients with allergic asthma can benefit significantly from antigen avoidance. Unfortunately, reduction of exposure to relevant antigens is nearly impossible except in certain circumstances - occupational exposure, animal sensitivity, and to a limited extent dust mite sensitivity. Not all patients with occupational asthma are benefited by avoidance therapy. In a recent study By Chan-Yeung examining occupational asthma induced by Western Red Cedar, the authors found that of the patients with complete avoidance, only 40% had complete resolution of their symptoms while 60% had ongoing asthma that appeared to be more "intrinsic" (Table IX). Further, in the patients who failed to improve it was found that NSBH to inhaled methacholine remained unchanged despite reduction in exposure. Additionally, data from this study suggested that patients diagnosed earlier who had less severe symptoms were more likely to have reversible bronchial hyperreactivity than those who were identified later. These findings have the very significant implication that earlier identification and specific treatment of of patients with allergic asthma might have a lifetime morbidity from asthma.

PC_{20} IN WESTERN RED CEDAR ASTHMA[1]

	No Exposure		Exposed	
	Recovered	Symptomic	Continuous	Intermittent
AtDx	1.46 ± 0.88	0.77 ± 0.79	0.86 ± 2.0	1.14 ± 1.17
F/U	4.35 ± 1.0	0.45 ± 1.8	0.61 ± 2.2	0.64 ± 1.02

A = B

1 = glometric mean ± SEM

A = $p < 0.01$

B = $p < 0.001$

Table IX

It is not practical for most patients with allergic asthma to significantly reduce their antigen exposure. In patients with occupational asthma, a change of occupation is essential. In the study mentioned above, those individuals who only had a partial reduction in their exposure failed to have any improvement of asthma or NSBH. Offending pets should be removed from the home environment of patients with allergic asthma. Of note are recent, but unpublished, studies that demonstrate that moving pets outside (as opposed to completely removing them) causes an insignificant decline in pet-associated antigen exposure apparently because a great deal of antigen is transported into the home on clothing. Avoidence of house dust mite in patients with house dust sensitivity is difficult and not often highly rewarding. Individuals in the home setting can only achieve approximately 50% reduction in dust mite levels. This is accomplished by avoiding the use of ceiling fans and other devices that disturb the settling process of house dust, the use of hypoallergenic pillows and matress covers as well as frequent cleaning and dusting of the room by a person other than the patient at a time when the patient is not in the environment. Of little value is attempting to reduce exposure to pollens and outdoor mold spores since these tend to be ubiquitous even in recently constructed homes that are fairly "tight." An exception to this is that in office buildings without exterior window ventilation, the industial heating and cooling systems are quite effective in the filtration of these particles from the air.

Irritants - In other sections of this Grand Rounds, data are presented that demonstrate that exposure to irritants causes damage to respiratory epithelium, influx of neutrophils and a transient increase in nonspecific bronchial hyperreactivity. These events occuring as a result of irritant exposure may accelerate the cycle shown in Figure 2 by increasing the detrimental positive feedback by increasing the presence and activity of inflammatory cells and reducing the presence and function of epithelial cells.

Although no data whatever bear on this subject, it seems rational that a significant and consistent reduction of exposure to nonallerggic irritants may benefit patients with either allergic or "intrinsic" asthma. A frequently unrecognized source of irritant exposure is gastroesophogeal reflux which tends to exacerbate asthma particularly at night. The mechanism by which this occurs is probably complex, but many patients have significant improvement of asthma after medical therapy of GE reflux.

Additional triggers that importantly contibute to the severity of asthma is sinusitis and/or allergic rhinitis. Recent studies by Reed and associates demonstrates that vigorous treatment of allergic rhinitis (without any change in the therapy of the patient's seasonal asthma) gratifyingly prevented the development of seasonal asthma while patients treated with placebo had the evolution of significant asthmatic symptoms (Figure 39). Nasal steroids were more effective than cromolyn in patients with either ragweed or mold spore sensitivity. Evaluating the paranasal sinuses for potential occult chronic sinus it is an important aspect of the search for reversible

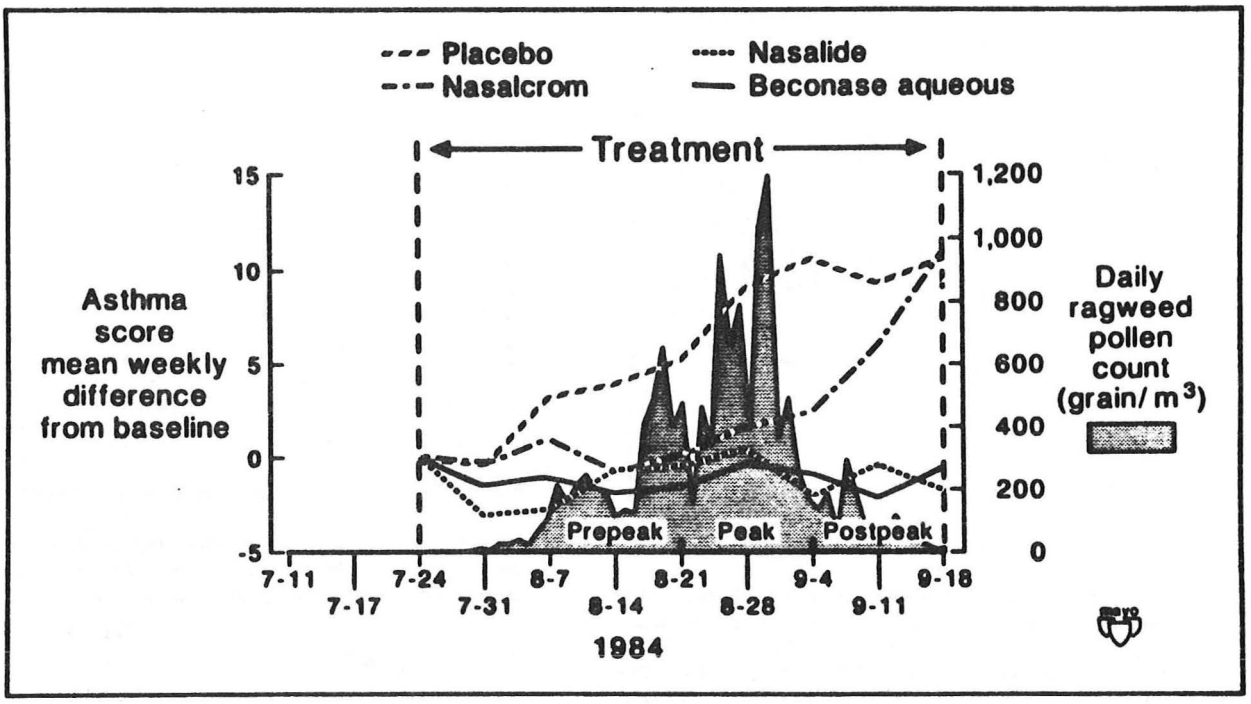

Figure 39

triggers for patients with chronic asthma. Although no controlled studies exist in adults, vigorous treatment of sinusitis often causes significant improvement in asthmatic symptoms. The more successful and less invasive sinus surgery now being performed using a rigid endoscope sugical intervention in patients with chronic sinusitis more attractive particularly if response to medical intervention is only partial.

IgE SENSITIZATION

Immunotherapy for patients with allergic asthma has been a modality of uncertain value that had not been rigorously studied by double blind placebo controlled trials until fairly recently. Although still somewhat controversial, a number of well conceived and executed studies have documented the utility of immunotherapy in the treatment of allergic asthma. Figure 40 illustrates a study that shows that antigen-induced bronchoconstriction was significantly reduced in patients treated with immunotherapy for cat allergy. In a study by Ortolani of patients who had seasonal grass pollen-induced asthma (Figure 41) there was a marked improvement in symptom scores in patients who were treated with immunotherapy compared to placebo in a double blind placebo controlled study. One potentially negative aspect to immunotherapy has been the observation by several groups that immunotherapy may cause an increase in NSBH suggesting that while responsiveness to antigen is reduced in patients who receive immunotherapy, a chronic low grade inflammatory response that may cause an increase in NSBH may offset some of these benefits. Despite these concerning observations, clear symptomatic improvement occurs

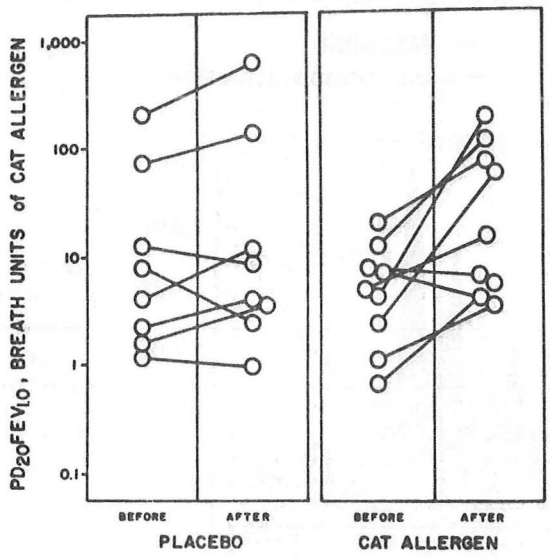

FIG. 1. Bronchial provocation with cat allergen. The geometric mean cumulative provocation dose of cat allergen in BU that resulted in a 20% drop in the $FEV_{1.0}$ ($PD_{20}FEV_{1.0}$, allergen) changed from 8.8 to 12.3 BU in the placebo-treatment group (NS) and from 4.27 to 20.7 BU in the active-treatment group ($p < 0.05$). A comparison of change from baseline between the placebo- and active-treatment groups revealed no significant difference.

Figure 40

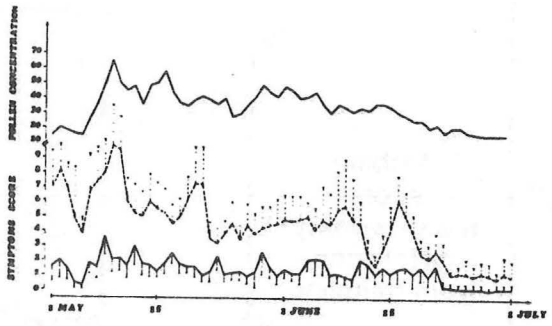

FIG. 1. Pollen concentration, *above*, in grains per cubic meter and symptom scores, *below* (mean ± SE), in actively treated subjects and subjects treated with placebo (*solid* and *dashed lines*, respectively) for the May to June grass pollen season during the study.

Figure 41

as a result of immunotherapy. Although reduced bronchospasm to antigens has been documented, a significant aspect of the benefit many patients derive from immunotherapy may stem from the more impressive and frequent improvement in associated allergic rhinitis since it appears that even medical treatment of rhinitis benefits asthma.

A number of exciting new avenues of therapy have developed within the framework of attenuating responsiveness to antigens. The discovery by the Ishizaka's laboratory of binding factors which have very impressive up- or down-regulation of the synthesis of the IgE isotype have potential therapeutic benefit and have recently been cloned and expressed. Studies of therapeutic value will likely be forthcoming. A second area of considerable interest involves relatively low molecular weight synthetic fragments of the Fc portion of the IgE molecule. These fragments have are able to displace intact IgE molecules from high affinity IgE Fc receptors on mast cells. Some of these fragments have been cloned and expressed and offer the theoretical disassociation of IgE <u>in vivo</u>. Thus, allergen exposure would be expected to fail to cause mast cell or basophil secretion as a result of the replacement of antigen specific IgE with inactive fragments.

USE OF MEDICATIONS

General Considerations

What follows will not attempt to review well known therapeutic agents in detail but rather seeks to provide an update regarding changes in indications, the use of new medications, new approaches to combining medications and experimental use of agents that have current and future utility in certain settings. An overview of the antiinflammatory effects of commonly used medications is presented in Table X.

Table—What drugs can—and cannot—do for airway inflammation in asthma

	Advantages	Disadvantages
Cromolyn sodium	Inhibits mediator release from activated mast cells, decreases airway hyperreactivity, attenuates eosinophil and neutrophil activation, inhibits neural reflexes, has a high therapeutic index	Given by inhalation only, has no direct effect on bronchial smooth muscle
β_2-Adrenergic agonists	Potent inhibitors of mast cell mediator release and bronchoconstriction, given both orally and by inhalation, have a high therapeutic index	Have no prophylactic effect on late asthmatic reaction or bronchial hyperresponsiveness
Methylxanthines	Inhibit activation of mediator-secreting cells and bronchoconstriction, attenuate late asthmatic reaction	Given orally and intravenously only, have a low therapeutic index
Corticosteroids	Inhibit late asthmatic reaction, acquired nonspecific bronchial hyperresponsiveness, and eosinophil, neutrophil, and monocyte chemotaxis	Have little effect on mast cell mediator release and early asthmatic reaction

Table X

Specific Reagents

Disodium cromoglycate (Intal) - Cromolyn was initially felt to act primarily as a mast cell stabilizing agent but recent evidence (summarized in Table X) suggests that it has antiinflammatory capabilities that are not limited to the mast cell. Its ability to reduce type C fiber stimulation, chemotaxis on the part of eosinophils and neutrophils, and the action of PAF on certain targets

supports this view. These findings motivated the use of cromolyn in nonallergic asthma in addition to its well accepted use in allergic asthma. Studies have shown that NSBH is reduced with the chronic use of cromolyn in both types of asthma. Because cromolyn has a high therapeutic index, its early use is encouraged in patients with chronic asthma. The principal limitation to its usefulness is that its potency is modest, but a related compound has been developed that appears to have greater effectiveness although release in this country will not occur soon (Nedocromil - Tilade®). Some of the actions of nedocromil are reviewed schematically in Figure 42. Cromolyn has recently become available in a metered dose inhaler in addition to solution for nebulization and the spinhaler. This delivery system should result in enhanced usefulness to patients by achieving greater compliance and better tissue delivery.

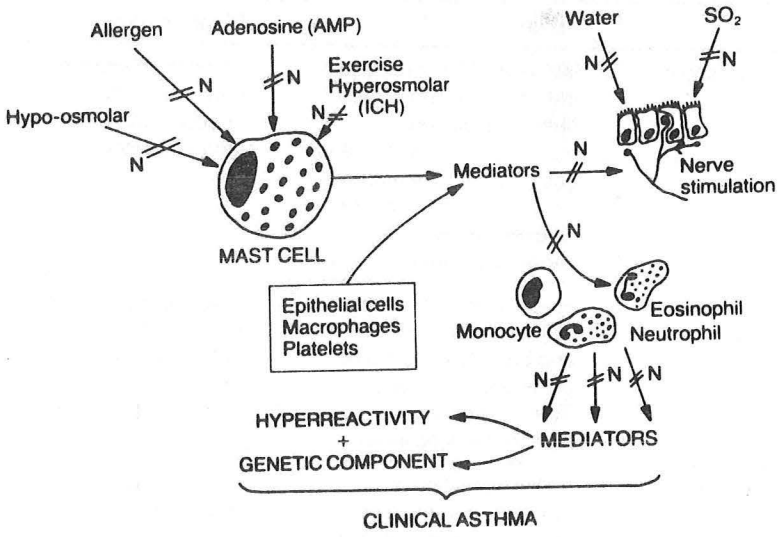

Figure 42

Beta Agonists - A variety of more or less selective beta agonists exist in both oral and inhaled forms and the use of these medications has not significantly changed in the last few years. With increasing recognition that these agents are less effective in reducing late phase reactions (Figure 43), their use should be for **symptomatic** relief rather than as a remittive medication likely to reduce the inflammation that will lead to long term improvement.

Theophylline - The usefulness of theophylline in asthma is quite clear but the mechanism of its action has been somewhat controversial. Initially felt to inhibit mast cell mediator release by increasing cAMP, it was found that this effect required very high concentrations. Subsequently it was found that at therapeutic levels theophylline affects adenosine-induced augmentation of mast cell stimulation. From a practical perspective, it reduces late asthmatic responses and therefore is somewhat more likely to be effective in remittive management of asthma than beta adrenergic agents.

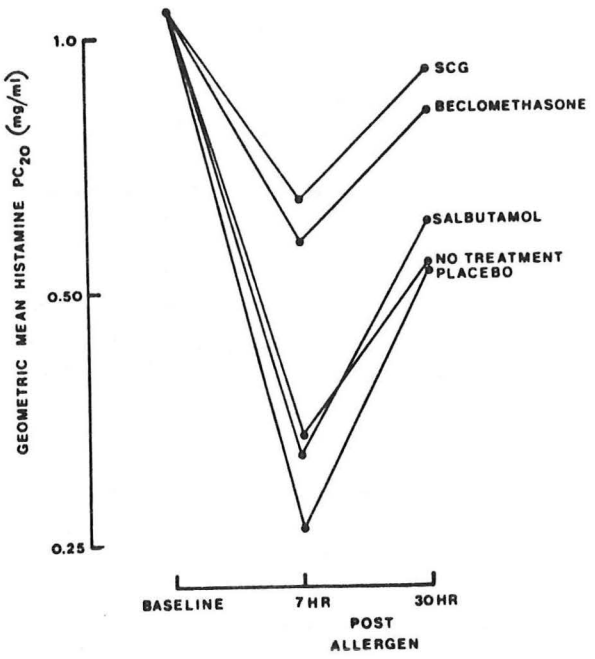

Figure 43

Unfortunately, it has a well known low therapeutic index which limits its use and is probably overused in this country. Several 24 hour preparations are available that not only permit once a day dosing and the increased compliance that ensues, but also is quite helpful in patients with nocturnal exacerbation since an HS dose will achieve consistently high levels during the night.

Glucocorticoids (inhaled and systemic) - These medications are very useful in attenuating late phase reactions by complex and only partially understood effects on secretion of mediators, chemotaxis and the generation arachidonic acid-derived inflammatory mediators. Both systemic and inhaled versions are effective in treating chronic asthma and while systemic corticosteroids have significant long term side effects, the inhaled preparations are quite effective with very little adrenal suppression until the recommended doses have been significantly exceeded. Inhaled glucocorticoids should be used more frequently than is typical of many phsyicians because of their effectiveness and remittive role in asthma. Further, the maximum recommended dose can be exceeded by several fold and while modest adrenal suppression may occur, the long term effects of this approach likely includes fewer complications than the chronic use of systemic steroids.

While systemic glucocorticoids have significant long term complications, their use by many physicians during exacerbations often results in less benefit to the patient than might be achieved by a modified approach to their use. Good patient-physician communication is essential. The discussions in other sections will hopefully have convinced the reader that reduction of inflammation

and preventing its evolution is essential to the proper management of patients with chronic asthma. Thus, it provides no benefit to patients who already use theophylline, cromolyn, inhaled steroids and beta agonists to wait until the exacerbation becomes severe before initiating systemic glucocorticoid therapy. Waiting almost certainly increases the total steroid use because higher doses are likely needed for longer peroids of time when the inflammation is allowed to intensify. Second, rapid tapering of systemic steroids in patients with moderate or severe asthma after resolution of an exacerbation may not be helpful because greater steroid-induced reduction of allergic inflammation will likely diminish the likelihood of an early recurrence of increased asthmatic symptoms. These approaches have not been studied in a rigorous fashion, but their use by the author and a number of others suggests that better control and a reduction in lifetime steroid use is likely to accrue by employing these strategies.

Ipratropium bromide (Atrovent) - Atrovent was released by the FDA within the last 6 months for the treatment of chronic bronchitis. It has been used with some effectiveness in patients with asthma but it appears to be no more effective to minimally more effective than beta agonists (depending on the study). The combined actions of beta agonists and atrovent may be of some benefit but the two are not additive. Atrovent is particularly helpful in situations in patients with significant cholinergic symptoms - significant cough associated with bronchospasm and bronchorrhea. Although it has not been a frequent problem patients with tenacious sputum, perhaps as a result of dehydration during an exacerbation, may be adversely affected by decreasing mucus production, increasing its thickness and reducing the patients ability to clear it. In a few patients significantly worse asthma has been observed with the use of anticholinergic medications particularly in the setting described above. Atrovent has particular future promise in the treatment of vasomotor rhinitis but has not been released for this indication.

Experimental Therapies

Methotrexate - Two recent studies (one published and one personal communication) have examined the use of methotrexate in patients with steroid dependent asthma (Table XI). An uncontrolled open study by Mullarkey demonstrated a statistically significant reduction in systemic steroid therapy in patients treated for 1Ø or more months with modest doses (5 mg po q 12 hr for 3 doses - 1 cycle per week). The results of this open study prompted the investigators to perform a double blind placebo controlled cross-over study which has been completed and which suggests that the conclusion that methotrexate is useful in steroid-dependent asthma is supported by their data (personal communication). No hepatic complications were observed, confirming experience in psoriasis.

Crysotherapy - Gold has been used in Japan for many years for the treatment of asthma after initial observations that patients treated in this manner for rheumatoid arthritis had occasional remissions of coexistent asthma. Limited experience in the United States with gold therapy prevents clear conslusions regarding its

Table 1. Average Prednisone Dose Before and During Methotrexate (MTX) Therapy

Case	Sex	Age	Before MTX		During MTX		MTX/wk, mg	Statistical Comparison	
			Avg. Prednisone dose/month, mg	No. Months Followed	Avg. Prednisone dose/mo, mg	No. Months Followed		Before MTX Z	After MTX
1	F	63	85.7	28	24.7	19	7.5	−2.2341	≤.012
2	M	63	438.0	49	148.8	12	7.5	−3.496	<.0006
3	M	59	487.7	22	145.5	10	15.0	−4.244	≤.0006
4	F	43	483.8	16	261.0	12	15.0	−2.337	≤.010
5	M	44	468.1	16	389.0	11	15.0	−2.07	≤.019
6	M	64	850.0	23	900.0	6	15.0	NS	NS

Table XI

effectiveness in asthma but results are likely to confirm the Japanese experience. In a recent US study examining patients with steroid dependent asthma, there was a statistically significant

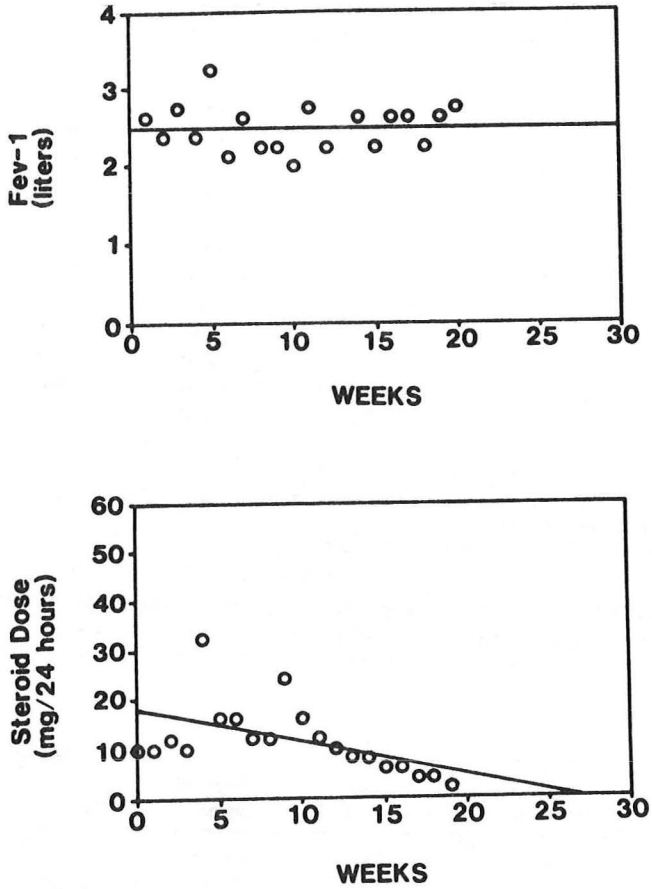

FIG. 2. Data for a responder to gold salt therapy. The **upper figure** displays measurements of FEV_1 through time during gold salt injections. The **lower figure** illustrates the corresponding steroid dose. The least squares regression line is plotted through each set of data. The slope for values FEV_1 through time was not significantly different from zero, whereas the steroid dose declined significantly at $p < 0.05$.

Figure 44

reduction in steroid dose in some of the patients who were treated by gold injection (Figure 44). Some data in the experimental model systems also suggest effectiveness in reducing basophil histamine release (by to auranofin with human cells) and attentuating NSBH (in a guinea pig model). In a long term randomized prospective study in Japan comparing the effectiveness of bronchodilator and steroids to gold + PRN bronchodilator steroids in patients with mild to moderate asthma, the investigators showed an impressive reduction in cholinergic bronchial hyperreactivity (NSBH) in patients treated with gold (Tables XII and XIII).

TABLE II. Bronchial responsiveness to acetylcholine and IgE levels in asthmatic subjects receiving gold therapy for more than 5 yr

No. of patient	Sex	Age at start of treatment (yr)	Age at onset of asthma (yr)	Type of asthma	IgE (IU/ml)		Respiratory threshold of acetylcholine (μg/ml)	
					Before	After*	Before	After*
A-1 (M. S.)	F	37	26	Extrinsic	730†	616	156	624
A-2 (T. I.)	F	45	39	Intrinsic	37‡	107	9	1,250
A-3 (H. K.)	M	16	13	Intrinsic	390‡	175	39	10,000
A-4 (S. H.)	M	17	13	Extrinsic	310†	110	1,250	5,120
A-5 (K. S.)	M	33	17	Extrinsic	100‡	670	312	2,500
A-6 (T. O.)	M	37	27	Intrinsic	60‡	110	78	10,000
A-7 (S. S.)	M	33	32	Intrinsic	500†	40	156	1,250
A-8 (M. S.)	M	21	19	Extrinsic	950‡	1,080	1,250	312
A-9 (Y. N.)	M	49	47	Intrinsic	360†	190	625	156
A-10 (M. K.)	M	48	39	Intrinsic	10†	99	39	156
A-11 (Y. K.)	M	44	26	Extrinsic	40‡	330	19	312
A-12 (H. S.)	M	40	26	Extrinsic	1,550†	770	625	625
A-13 (S. K.)	M	49	28	Unidentified	390‡	350	625	1,250
A-14 (Y. N.)	M	28	26	Unidentified	165†	165	156	625

*Bronchial responsiveness to acetylcholine or IgE levels were measured from 5 to 7 yr after onset of treatment.
†IgE levels were measured before treatment.
‡IgE levels were measured within 2 yr after onset of treatment.

Table XII

TABLE IV. Bronchial responsiveness to acetylcholine and IgE levels in asthmatic subjects treated symptomatically with bronchodilators or corticosteroids for more than 5 yr

No. of patient	Sex	Age at start of treatment (yr)	Age at onset of asthma (yr)	Type of asthma	IgE (IU/ml)		Respiratory threshold of acetylcholine (μg/ml)	
					Before	After*	Before	After*
C-1 (S. Y.)	F	40	33	Intrinsic	50†	142	9	312
C-2 (I. A.)	F	47	47	Intrinsic	25‡	70	1,250	625
C-3 (M. N.)	F	33	31	Unidentified	450‡	352	1,250	78
C-4 (F. G.)	F	48	24	Unidentified	340‡	580	312	625
C-5 (Y. K.)	F	40	30	Intrinsic	124‡	178	156	156
C-6 (K. H.)	F	26	20	Extrinsic	1,600†	163	78	156
C-7 (Y. S.)	M	39	5	Extrinsic	88†	100	1,250	2,500
C-8 (T. A.)	M	33	15	Intrinsic	80‡	105	156	2,500
C-9 (K. K.)	M	45	13	Extrinsic	3,400†	2,350	312	39
C-10 (K. T.)	M	31	23	Extrinsic	1,100‡	1,210	312	625

*Bronchial responsiveness to acetylcholine or IgE levels were measured from 5 to 7 yr after onset of treatment.
†IgE levels were measured before treatment.
‡IgE levels were measured within 2 yr after onset of treatment.

Table XIII

Thus, agents that are able to reduce inflammation (methotrexate and gold) as well as systemic steroids seem to hold considerable promise in the management of patients with asthma. These therapies have been principally used in patients with steroid dependent asthma (based on the rationale that significant morbidity will ensue by ongoing steroid use and potential morbidity using crysotherapy or methotrexate is offset by the potential benefit of the reduction of steroid use. Perhaps more optimistic might be the results of the use of these agents in patients with more moderate asthma as is the case in the Japaneese study in which **remissions** from asthma may be achieved although I am unaware of any studies in this country examining methotrexate in patients with moderate asthma (not steroid dependent). The initial success of methotrexate and gold would seem to warrant the use of these agents in controlled studies in patients with less significant asthma in a effort to interfere with the positive feedback loops that are (illustrated in Figure 2) that result in a perpetation of chronic asthma. It may well be possible to treat athmatic patients with inductive therapy (similar to chemotherapy in malignant disease) to result in remissions by vigorous application of antiinflammatory strategies followed by maintenance therapy using topical steroids, cromolyn and episodic systemic steroids and bronchodilators.

Summary and Personal View

A large number of therapeutic agents are now available for the treatment of bronchial asthma. Choosing from these agents is not simple. There is considerable national differences in results of this choice. In England, cromolyn has been quite popular for a long period of time and is used as a first line drug in patients with asthma. On the other hand, cromolyn has been much less regularly used in the US. A comparison of asthma treatment in the US to that in Canada indicates that there is much more frequent use of theophylline in this the US while inhaled and systemic beta agonists are used more vigorously in Canada. As described above, the use of gold is relatively common in Japan for patients with asthma while its use in the US is still experimental. What follows is a synthesis that largely reflects the author's personal view that has evolved out of the experience of myself and others as well as the published literature.

In the US we have tended to view asthma as a disorder strictly of excessive smooth muscle contraction using a mechanical model (Figure 45). As a result, our goals have been limited to the attempt to reverse the constriction of bronchial only smooth muscle by the use of bronchodilators. More attention has been focused by pharmaceutical firms on developing drugs that have longer duration of action rather than on new agents to deal with the cause of bronchoconstriction. This view is beginning to yield to the notion that airway obstruction is a combination of edema and broncoconstriction and that perhaps the most effective therapy of asthma involves not smooth muscle relaxation, but also the inflammation that causes it. The importance of inflammation in causing bronchial hyperreactivity suggests that antiinflammatory

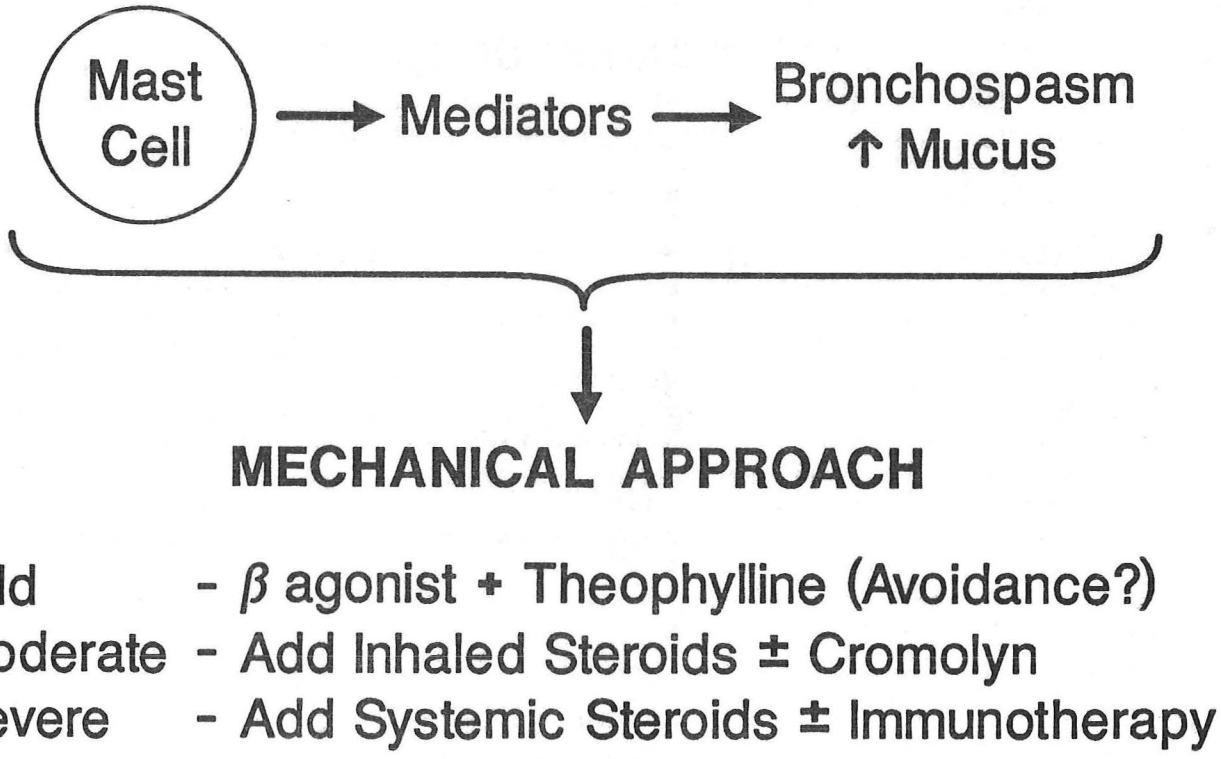

Figure 45

therapy should be instituted earlier and that bronchodilator therapy is principally for symptomatic relief. Thus, the long term therapy of patients with chronic asthma should include a vigorous and consistent attempt to chronically reduce inflammation by antigen or irritant avoidance, appropriate immunotherapy, and antiinflammatory medications (topical steroids, inhaled cromolyn, and to a lesser extent theophylline). The use of atrovent and beta agonists should principally be limited to PRN therapy for symptom reduction which occurs as a result of failure of antiinflammatory therapy.

A suggested approach to the management of patients with a variety of severity of asthma is illustrated in Table XIV. These represent the opinion of the author and have not been supported by rigorous experimentation. What is suggested in this Table largely stems from the goal of trying to institute more vigorous antiinflammatory therapy earlier in the evolution of asthma in the hope that perhaps that more severe and more difficult to reverse asthma might be avoided. Because effective immunotherapy and allergen avoidence may be important, early referral to an allergist for evaluation of potential allergic sensitivity is recommended.

STRATIFICATION OF THERAPY

Mild Episodic	-	PRN β agonist
Chronic Mild	-	Inhaled Steroids
		and/or
		Cromolyn
		PRN β agonists
		Rule Out Reversible Triggers
Moderate	-	Add Theophylline
		Allergy Referral → Allergen Avoidance ± IT
		Vigorous Systemic Steroids for Exacerbation
Severe	-	Add Specific IT
		Consider Increasing Steroids
		Consider Methotrexate/Crysotherapy

All Levels in Selected Patients - Ipratropium Bromide

Table XIV

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