Airway hyperresponsiveness is an important feature of clinical asthma. This means that asthmatic patients will develop bronchoconstriction after inhaling a smaller concentration of a bronchoconstrictor agonist (usually 10-100 times less) than is needed to induce the same degree of bronchoconstriction in non-asthmatic subjects. Airway hyperresponsiveness is not only an objective measurement which can distinguish asthmatic from normal subjects, but also the degree of airway hyperresponsiveness is related to the severity of asthma, and to the amount of treatment needed to optimally control symptoms.

In many asthmatic subjects, airway hyperresponsiveness is a stable phenomenon when measured over several years; however, in some subjects, airway responsiveness can be increased after exposure to inhaled allergens, ozone, upper respiratory tract infections or occupational sensitizing agents. This increase in airway responsiveness is associated with increased symptoms of asthma. All of these stimuli are naturally occurring stimuli; however, allergen, ozone and occupational sensitizing agents such as toluene diisocyanate (TDI) have been used in both human and animal preparations to study the pathogenesis of airway hyperresponsiveness in the research laboratory.

Each of the stimuli known to induce airway hyperresponsiveness has been thought, in addition, to cause acute airway inflammation. The first direct evidence, however, that airway hyperresponsiveness and airway inflammation may be causally related came from studies examining the pathogenesis of airway hyperresponsiveness which can be induced by inhalation of ozone in dogs. When dogs inhale ozone (2-3ppm for 2h), airway hyperresponsiveness to inhaled acetylcholine develops 1h after the exposure, is still present 24h later, and is returned to baseline values by one week. Initial studies by Holtzman et al documented a temporal association between the development of airway hyperresponsiveness and an acute inflammatory response in the central airways of dogs, as measured by an influx of neutrophils into the epithelial and sub-epithelial layers of the airway. These observations were confirmed by Fabbri et al from the same laboratory, who demonstrated a large influx of neutrophils, as well as desquamated epithelial cells in bronchoalveolar lavage fluid from dogs with airway hyperresponsiveness after inhaling ozone. Subsequently, other investigators using other animal preparations confirmed that airway inflammation had occurred at the same time as airway hyperresponsiveness had developed. For example, Marsh et al demonstrated an influx of polymorphonuclear cells and macrophages in lavage fluid when rabbits were made hyperresponsive after allergen. Also, Murias and Room have shown that airway mucosal injury and mast cell infiltration has occurred when guinea pigs are hyperresponsive after ozone inhalation. Lastly, and most importantly, Stelzer et al demonstrated an influx of neutrophils into lavage fluid of human subjects made hyperresponsive after ozone exposure. Thus, while the type of inflammatory response may differ because of the different species studied or different stimulus used to induce airway hyperresponsiveness, each of these studies was consistent with the hypothesis that some component of an acute inflammatory response was responsible for the development of airway hyperresponsiveness.

**CELLULAR COMPONENTS OF INFLAMMATION**

The initial attempts made to determine the important component of the inflammatory response concentrated on the neutrophil, as this was the most prominent cell seen in both dogs and human subjects who had developed airway hyperresponsiveness after inhalation of ozone. Dogs were initially depleted of circulating neutrophils, using intravenous hydroxyurea, and were then exposed to ozone. In the neu-
tropicen dogs, ozone exposure did not lead to airway hyperresponsiveness. When the circulation neutrophil counts had returned to normal levels (six weeks later), all dogs developed airway hyperresponsiveness after ozone exposure. This suggested that neutrophils were playing a central role in the pathogenesis of airway hyperresponsiveness in dogs. These observations were supported by studies from Murphy et al in neutropenic rabbits. However, Murlas et al could not demonstrate inhibition of airway hyperresponsiveness in neutropenic guinea pigs after inhalation of ozone. Each of these studies must be interpreted cautiously, however, as they all have used drugs which do not specifically deplete neutrophils and which may be having other actions on cells in the airway. To attempt to circumvent this problem, workers in Nadel's laboratory at the University of California at San Francisco have isolated human neutrophils (>95 percent pure), which were stimulated with the calcium ionophore A23187 and arachidonic acid. The supernatant from these stimulated cells was subsequently nebulized into six dogs, each of which developed airway hyperresponsiveness 1h after the inhalation of the supernatant. Inhalation of A23187 and arachidonic acid without the neutrophils caused bronchoconstriction in each dog, but no dog developed airway hyperresponsiveness. These data suggest that human neutrophils have the capacity to release substances when stimulated, which can increase airway responsiveness in dogs.

Studies in human subjects have examined cellular inflammation as measured by numbers of cells in bronchoalveolar lavage fluid in subjects exposed to ozone, allergen, and occupational sensitizing agents such as Western red cedar. While neutrophils were increased in lavage fluid after ozone inhalation, eosinophils were increased in lavage fluid after allergen and in Western red cedar-induced asthma. Thus, it would appear that while the cellular response to an inflammatory stimulus may differ with different stimuli, or may depend on the way that the stimulus initiates the inflammatory response, the pathophysiologic consequence, i.e., airway hyperresponsiveness, is the same.

Chemical Mediators of Inflammation

Early studies of ozone-induced airway hyperresponsiveness in dogs had demonstrated that the predominant influx of neutrophils was into the epithelial layer of the trachea. This suggested that the epithelium may be the source of the chemotactic factor for neutrophils. Subsequently, Holtzman et al demonstrated that isolated epithelial cells from canine airways could convert arachidonic acid to leukotriene B4 (LTB4), which is known to be a potent neutrophil chemotactic factor. When LTB4 is nebulized into dogs' airways, neutrophil influx occurs which is associated with a progressive increase in airway responsiveness up to 3h after the LTB4 has been inhaled. Therefore, LTB4, by itself can mimic the pathophysiologic changes induced by ozone and is likely to be, at least in part, responsible for the neutrophil influx induced by ozone in dogs.

The mediators released during the inflammatory response which cause airway hyperresponsiveness largely have been investigated using inhibitors of mediator production. Airway hyperresponsiveness induced by ozone in dogs appears to be mediated by cyclooxygenase products of arachidonate metabolism. The evidence for this is based on studies which have shown that pre-treatment with BW 755c, an inhibitor of both cyclooxygenase and lipoxygenase, inhibited airway hyperresponsiveness after ozone. In addition, indomethacin, an inhibitor of cyclooxygenase, prevented airway hyperresponsiveness, but not the neutrophil influx after ozone. Lastly, when cyclooxygenase products were measured in lavage fluid during LTB4-induced airway hyperresponsiveness, a ten-fold increase in thromboxane B2 was demonstrated. Also, a thromboxane synthetase inhibitor, OKY-046, prevented airway hyperresponsiveness after LTB4 and after ozone exposure. Thus, in the dog, thromboxane appears to be necessary for the development of airway hyperresponsiveness. However, this does not appear to be true in the guinea pig. Lee et al have reported that BW 755c, but not indomethacin, inhibits airway hyperresponsiveness in guinea pigs after ozone exposure and have interpreted this as indicating that lipoxygenase rather than cyclooxygenase products are important in this response.

The mediators responsible for the development of airway hyperresponsiveness in human subjects after ozone or allergen are not yet known. However, Fairfax et al and Joubert et al have shown that the late asthmatic response after inhaled allergen is inhibited by indomethacin and other cyclooxygenase inhibitors. Also Shephard et al have measured a rise in plasma thromboxane B2 levels during the late asthmatic response. The late asthmatic response clearly has been shown to be closely associated with the development of airway hyperresponsiveness after inhaled allergen; it is therefore tempting to speculate that inhibition of the late response by indomethacin may also inhibit the subsequent onset of airway hyperresponsiveness; however, these studies have not yet been reported.

When all of these studies are considered together, it is clear that an acute inflammatory response is involved in the pathogenesis of airway hyperresponsiveness after stimuli such as ozone. The relative importance of the variety of cells which make up the inflammatory response or of different mediators in the pathogenesis of this response is not yet resolved, particularly in human subjects. In addition, the importance of airway inflammation in the maintenance of persisting airway
hyperresponsiveness that is characteristic of asthma is unknown. However, studies that are currently ongoing in a number of research laboratories will hopefully improve our understanding of the importance of airway inflammation in both stable and transiently increased airway hyperresponsiveness.

ACKNOWLEDGMENT: I am grateful to Mrs. L. Rogers for typing this manuscript and to Dr. H. Ramsdale for helpful advice in its preparation.

REFERENCES
27. Fairfax AJ. Inhibition of the late asthmatic response to house dust mite by non-steroidal anti-inflammatory drugs. Prostagland Leuk Med 1982; 8:239-48
29. Shephard EG, Malan L, MacFarlane CM, Mouton W, Joubert JR. Lung function and plasma levels of thromboxane B_2, 6-ketoprostaglandin F_1α and B-thromboglobulin in antigen-induced asthma before and after indomethacin treatment. Br J Clin Pharmac 1985; 19:459-70

CHEST / 90 / 4 / OCTOBER, 1986 577