ASTHMA IS AN INFLAMMATORY disease of the airways. Prominent effector cells of this inflammatory response include mast cells, eosinophils, and neutrophils, and, as such, understanding the contribution of secreted mediators from these cells to the pathophysiology of asthma has been the subject of intense investigation. Human mast cell tryptases (EC 3.4.21.59) comprise a family of trypsin-like neutral serine proteinases that are predominantly expressed in mast cells (31). There are α- and β-forms of the enzyme, but the present discussion will concern itself with the β-isoenzymes (βI, βII, and βIII), which appear to be activated intracellularly and stored in secretory granules (34). Tryptase is enzymatically active in the form of a non-covalently linked tetramer. Upon dissociation, the monomers lose activity. Tryptase is stabilized by heparin and by other negatively charged proteoglycans, and this mechanism is thought to govern tryptase activity in vivo (30, 34). Upon mast cell activation, the β-tryptases, bound to heparin, are secreted in parallel with histamine. Thus, historically, tryptase has often been used as a marker of mast cell activation. More recent in vivo and in vitro studies have, however, suggested a far more prominent role for this enzyme in both the acute and chronic inflammatory processes that contribute to the pathophysiology of asthma.

Tryptase has effects on peptides, proteins, cells, and tissues, and many of these actions can ultimately contribute to asthma symptoms. Tryptase degrades vaso-active intestinal peptide, an endogenous bronchodilator (35); activates prekallikrein and generates kinins (19, 21, 29), important mediators in the development of bronchoconstriction and airway hyperresponsiveness (13, 32); and activates and regulates mast cell secretion both in vivo (25, 26) and in vitro (15, 17). Injection of tryptase into the skin causes an acute wheal and flare in sheep (25) and a late inflammatory cell response in guinea pigs (16). While these late inflammatory events could naturally progress from mast cell activation, a second mechanism may involve the release of the potent granulocyte chemotaxin interleukin-8 (7, 10). Finally, tryptase can increase airway smooth muscle responsiveness to histamine in vitro (33) and airway hyperresponsiveness in vivo (26), although this may not occur in all species (18). The concept that tryptase is a major contributor to allergic airway disease is supported further by in vivo studies in experimental animals and in humans that show that small molecule tryptase inhibitors block allergen-induced early- and late-phase bronchoconstriction and airway hyperresponsiveness (9, 22, 36). These results indicate that inhibiting tryptase activity in the airways may have an acute beneficial effect on asthma.

In addition to these acute inflammatory processes, tryptase may induce more long-term effects. Tryptase is a known mitogen for dog tracheal smooth muscle cells (6), human smooth muscle cells (2), and human lung and dermal fibroblasts (1, 14). Both smooth muscle hyperplasia and fibrotic changes contribute to thickening of the airway wall that can lead to a permanent reduction in airway caliber. These structural changes are part of the process referred to as airway remodeling (4). Although the pathogenetic mechanisms of airway remodeling are poorly understood, the clinical consequences of this process include an irreversible component of airway obstruction and heightened airway hyperresponsiveness, both of which contribute to increased severity and frequency of asthma exacerbations. Part of this phenomenon is a consequence of smooth muscle shortening due to the thickened airway wall. Thus compared with normal airway smooth muscle, asthmatic airway smooth muscle needs to shorten by only 40% of its resting length to occlude the airway lumen (20).

The biological actions of tryptase outlined above seem to require an active catalytic site since tryptase-induced responses can be blocked by inactivating the enzyme by heating or by use of reversible tryptase inhibitors such as N-(1-hydroxy-2-naphthoyl)-1-arginyl-1-prolinamide hydrochloride (APC-366), leupeptin, benzamidine, or bis(5-amidino-2-benzimidazolyl)methane (8). One putative mechanism by which tryptase is
thought to stimulate cellular responses is through activation of protease-activated receptors (PARs). PARs are G protein-coupled receptors that are self activated by specific enzymes or by synthetic peptides (24). In the case of enzymatic activation, the NH₂ terminus of the extracellular domain of the receptor is cleaved, and the remaining peptide then binds to itself, thereby initiating transmembrane-signaling events. PAR stimulation can also be mimicked by synthetic peptides that bind to the appropriate domain of the receptor, but, in this case, activation occurs without proteolytic cleavage. Of the four PARs identified (PAR-1 through PAR-4), tryptase (and trypsin) activates PAR-2 (27). In the lung, immunolocalization of PAR-2 expression has been found on mast cells (12) and human airway smooth muscle cells (11).

The localization of PAR-2 on mast cells is consistent with the aforementioned physiological actions of tryptase in the airways, and, in a recent study, Berger and coworkers (2) provide evidence that tryptase-induced mitogenic activity in human airway smooth muscle cells is dependent on PAR-2 activation. In their study, tryptase-induced mitogenic activity was measured by thymidine incorporation. The maximum response was seen 24 h after incubating the cells with tryptase (30 mU/ml), recombinant trypsin, or PAR-2-activating peptide and trypsin (which also activates PAR-2). Incubation of tryptase with the protease inhibitors leupeptin, benzamidine, and APC-366 reduced tryptase enzymatic activity by 98%, 45%, and 44%, respectively, and reduced the tryptase-induced mitogenic activity in the smooth muscle cell cultures by 113%, 81%, and 79% compared with the response seen with no inhibitor present. Heat inactivation of the enzyme also inhibited the mitogenic response, and there was no mitogenic response after incubation of the cultures with scrambled (inactive PAR-2) peptide. Tryptase-induced thymidine incorporation was abolished by treating the cells with pertussis toxin, which suggests that a pertussis toxin-sensitive G protein is involved in the proliferative response. Collectively, these findings indicate that the mitogenic effects of tryptase on human airway smooth muscle cells require PAR-2 stimulation by the enzyme with an active catalytic site. Berger et al. (3) also arrived at similar conclusions after examining the effects of tryptase on cytoplasmic calcium concentrations in human airway smooth muscle.

In the study by Brown et al., one of this issue’s articles in focus (Ref. 5, see p. L197), Brown and colleagues provide evidence to the contrary. These investigators confirm that tryptase, at physiologically relevant concentrations, acts as a mitogen for human airway smooth muscle cells. Muscle cell mitogenic activity was measured by bromodeoxyuridine incorporation 24–48 h after exposure to mitogens. In this preparation, tryptase’s mitogenic activity is comparable to classic peptide growth factors such as recombinant platelet-derived growth factor-BB and recombinant human basic fibroblast growth factor. The tryptase response, however, is much greater than other serine protease mitogens, including thrombin, factor Xa, and factor XII. Comparable experiments were done in dog cells with similar results, except that in the dog cells recombinant human insulin growth factor 1, which had no effect on human cells, had a marked proliferative effect. The unique and now controversial finding is that inhibiting tryptase’s proteolytic activity did not block its mitogenic effects in human airway smooth muscle cells, even though these cells appeared to express PAR-2 by PCR. For this study, Brown and colleagues used amidino phenylmethanesulfonyl fluoride (p-APMSF), an irreversible inhibitor, rather than the reversible inhibitors used by Berger et al. (2). The p-APMSF reduced tryptase enzymatic activity by 99%, similar to the degree of inhibition seen with leupeptin in the study by Berger et al., but the tryptase-induced mitogenic effect was unaffected. Interestingly, the results were different in the dog tracheal smooth muscle cell cultures in which inhibiting tryptase activity with p-APMSF significantly reduced, but did not completely block, the tryptase-induced mitogenic response. These results indicate that tryptase catalytic activity is not required for mitogenic activity in human cells, but it is required to achieve a complete mitogenic response in dog cells. Thus Brown and coworkers demonstrate a proliferative response to inactivated tryptase in cells from two species, although to different degrees. These results suggest that the tryptase-induced mitogenic effect in human airway smooth muscle cells is, for the most part, nonproteolytic and therefore most likely not mediated through PAR-2 activation. One putative mechanism suggested by the investigators is the binding of glycosylated residues on the tryptase molecule to cell surface mannose receptors (28) since these receptors were shown to mediate bovine smooth muscle cell proliferative responses (23).

It is difficult to explain the divergent results of these two studies. Obviously, the differences can be attributed, in part, to experimental conditions and methodology. Could the differences in the tryptase inhibitors used explain the findings? Possibly. However, differences in inhibitors cannot explain the mitogenic effects produced by the PAR-2-activating peptide seen by Berger and colleagues (2). Brown and coworkers demonstrated that their cultures expressed PAR-2 but did not test the effect of the PAR-2-activating peptide in their system. This could prove to be an interesting experiment in light of the different findings from the two groups. Could it be that the tryptase-p-APMSF complex activates PAR-2 directly or somehow generates a PAR-2-activating peptide?

Barring these potential explanations, the possibility that tryptase acts via PAR-2-dependent and -independent mechanisms is an intriguing hypothesis. As indicated by Brown and coworkers (5), such findings may impact the development of tryptase antagonists designed to treat the various disorders in which this enzyme plays a role. Thus the present inhibitors that modulate the more acute tryptase-mediated responses...
to allergen exposure (e.g., bronchoconstriction and airway hyperresponsiveness) may fail to control aspects of airway remodeling not completely dependent on PAR-2 activation. Future studies will be required to determine the relative importance of tryptase-induced PAR-2-dependent and -independent mechanisms as they relate to smooth muscle cell hyperplasia and increased collagen deposition in the asthmatic airway. Such studies will be important for determining the contribution of tryptase to the remodeling process. Does tryptase continue to have a role as remodeling progresses, and if it does, can we block or reverse this effect? Answers to such questions will be important not only for understanding pathophysiology of asthma but possibly other mast cell-mediated diseases.

REFERENCES


