Isolated human bronchi serve as an in vitro model for the investigation of airway smooth muscle physiology and pharmacology, and studies with preparations of isolated human bronchi had already appeared in the literature since the early 1950s (1, 2). From these studies it is evident that under in vitro conditions human isolated airways from nonatopic individuals exhibit inherent smooth muscle tone that can be quantified, i.e., as the relaxant response after addition of catecholamines (1). Various other bronchodilating drugs, such as β-adrenoceptor agonists (3) and phosphodiesterase inhibitors, as well as stable analogs of cyclic adenosine monophosphate (cAMP) (4) and leukotriene and histamine receptor antagonists (5) have also been demonstrated to relax human bronchus in vitro. More detailed analysis revealed that inherent airway smooth muscle tone results mainly from a balance of continual production of contractile cysteinyl-leukotrienes (Cys-LTs) and—to a lesser extent—histamine on one side (5) and inherent tone results mainly from a balance of continual production of contractile cysteinyl-leukotrienes (Cys-LTs) and—to a lesser extent—histamine on one side (5) and bronchodilator prostanoids, such as prostaglandin E₂ on the other. While the source of the prostanoids is believed to be the airway epithelial cell (6), histamine and leukotrienes are likely to be produced by resident inflammatory cells such as mast cells and also eosinophils in the airway wall (7, 8). Cysteinyl-leukotrienes are powerful constrictors of human airway smooth muscle in vitro (9, 10) through direct interaction with Cys-LT₁ receptors (11), and with a potency at least 1,000-fold greater than histamine.

In vivo cysteinyl-leukotrienes cause bronchconstriction both in healthy individuals and in patients with asthma (12) and these responses are inhibited by Cys-LT₁ receptor antagonists (13, 14). At present, however, it is not fully elucidated whether inherent tone as described under various experimental conditions is also present under in vivo conditions in normal individuals, although it has been demonstrated that β₂-adrenoceptor agonists cause bronchodilation in healthy subjects with normal lung function (15, 16). Interestingly, however, leukotriene receptor antagonists and 5-lipoxygenase (5-LO) inhibitors do not affect baseline airway caliber in normal subjects but have a small and varying effect on baseline lung function in patients with mild asthma (17, 18) while they significantly increase baseline FEV₁ in patients with mild to moderate disease (19–24).

HUMAN AIRWAYS IN VITRO: METHODOLOGY

For in vitro studies human airways are usually obtained from patients who must undergo surgery for lung cancer or from donor lungs for transplantation. After resection, peripheral airways with internal diameters ranging between 1 and 5 mm are dissected free of alveolar tissue and cut into rings or strips. Thereafter, preparations may be stored in Krebs-Henseleit or related buffer solutions for several hours before use.

Most often changes in isometric tension of bronchial preparations are measured in an immersion organ bath; superfusion systems are also available for this purpose (25, 26). The resting tension which is applied to a smooth muscle preparation at the start of an isometric in vitro experiment is particularly critical for the subsequent relaxant responsiveness, while responses to contractile agonists are tension independent over a wide range of resting loads (27). Since resting tension may be causally related to the detection of inherent tone, methods have been sought to circumvent isometric conditions with an imposed a priori load. These efforts have resulted in alternative ways to quantify bronchoconstriction or changes in airway tone by optical means consisting of simple magnifying lenses (28), or with more complex algorithms using computerized videomicroscopy to allow online measurements of airway constriction.

Here changes in airway diameter are expressed as a function of pixel number, changes in auxotonic conditions are then expressed as percent area reduction (29, 30). From these data it is evident that inherent tone in human isolated airways is detectable also under auxotonic conditions and does not exclusively depend on a preexisting load.

A airway responsiveness in isolated human bronchi is assessed by pharmacological (e.g., isoprenaline, carbachol) and immunological means (i.e., allergen, anti-IgE) as well as electrical field stimulation. However, in analogy to increased airway responsiveness that is demonstrated in patients with asthma, in vitro airway responsiveness can also be altered. Passive sensitization of isolated human airways has been demonstrated to alter airway smooth muscle reactivity (31) and to induce specific allergen responses in vitro (32).

INHERENT TONE

Under the in vitro conditions described above resting tension represents a combination of passive properties of the tissue on the one hand, and active or inherent tone of the smooth muscle on the other. Curiously, the ability of airway smooth muscle to spontaneously develop tone in vitro varies among species. The guinea pig exhibits a high degree of intrinsic tone, whereas there is no inherent tone in airways isolated from the cat (33). The spontaneous tone in guinea pig airways, however, is quite distinct from the human situation: while inherent tone in human airways is believed to result from a balance of contractile cysteinyl-leukotrienes, and, to a lesser extent, histamine (5) and bronchodilating prostanoids, such as prostaglandin E₂, tone in the guinea pig is exclusively dependent on cyclooxygenase products, is abolished by indomethacin, and is epithelium dependent (34, 35). In the human airway the bronchodilating prostanoid(s) are believed to originate from the epithelium (6), histamine and leukotrienes are likely to be produced by inflammatory cells found in close proximity to the airway smooth muscle (7, 8).

While inherent tone can be modulated by agents that increase cAMP such as β-adrenoceptor agonists (3, 36), forskolin, phosphodiesterase (PDE) inhibitors, and stable analogs of...
cAMP (4, 37) it is also susceptible to inhibition of 5-lipoxygenase (38) as well as antagonism of receptors for cysteinyl-leukotrienes and histamine (5). It may be speculated whether the effects of cAMP elevation on smooth muscle tone in isolated human airways is exclusively due to protein kinase A (PKA)-mediated smooth muscle relaxation or whether inhibition of local eicosanoid production also contributes to this effect (39).

Studies that specifically addressed the role of mediators of inherent tone suggest that this tone is due to the continual production and release of both cysteinyl-leukotrienes and histamine (Figure 1) (5), but it is at present unclear what drives and stimulates their production and release. Although smooth muscle cells are capable of producing prostaglandins in vitro (40, 41) it seems unlikely that they are themselves the source of the contractile eicosanoids since the genetic ability of cells to generate cysteinyl-leukotrienes resides with cells originating from the bone marrow (42, 43).

Products of the cyclooxygenase pathway do not seem to be of major importance in the generation of inherent tone in isolated human airways, since indomethacin—in contrast to the guinea pig—does not significantly decrease resting tension in human bronchial preparations (5, 38, 44). Furthermore, pretreatment with indomethacin was shown to lead to a substantial increase in resting tension under conditions of superfusion (33) and in the immersion organ bath (6).

Role of Airway Epithelium
The influence of the bronchial epithelium or involvement of cyclooxygenase products appears limited in the human airway, again contrasting with the findings in the guinea pig. In the study of Watson and coworkers (6), inhibition of the 5-lipoxygenase pathway by zileuton uncovered a subtle role of the bronchial epithelium in the production of tone and an influence of relaxant cyclooxygenase products. Under these exper-
imental conditions epithelial removal did not significantly change the amount of baseline inherent tone. Zileuton decreased tension in tissues independent of epithelial removal while indomethacin showed a tendency to increase tone, again independent of epithelial integrity. However, in the presence of 5-LO inhibition, indomethacin significantly increased tone only when the epithelium was intact. This suggests that epithelial cells produce eicosanoids that are not derived from 5-lipooxygenase metabolism, but cause contraction of bronchial smooth muscle (Figures 2A and 2B) (6).

**INDUCED TONE AND RESPONSIVENESS**

Exogenously applied cysteinyl-leukotrienes result in a concentration-dependent contraction of isolated human airways (9, 10, 45–47) most likely through direct interaction with Cys-LT$_1$ receptors on airway smooth muscle cells (11), which so far have been characterized only functionally. Maximal contractions induced by leukotrienes in vitro are within the same magnitude as maximal contractions induced by histamine; however, they are approximately 1,000-fold more potent than histamine in nonsensitized (45–47) as well as passively sensitized (47) human airways.

**Eosinophils and Airway Tone In Vitro**

Human eosinophils can be stimulated by platelet-activating factor (PAF) to generate cysteinyl-leukotrienes, predominantly LTC$_4$. The incubation of human airway preparations with PAF-stimulated eosinophils leads to bronchoconstriction while coincubation with unstimulated eosinophils or PAF alone has no effect on airway caliber (28). Preincubation of isolated human airways with a 5-lipoxygenase inhibitor, A63162, leads to a concentration-dependent reduction of airway narrowing induced by activated eosinophils; inhibition of cyclooxygenase by indomethacin had a small and variable effect. These data suggest that PAF-activated eosinophils cause lumenal narrowing of isolated human airways predominantly through activation of the 5-lipoxygenase pathway in eosinophils and to a lesser degree through cyclooxygenase activation (Figures 3A and 3B) (28).

**Figure 3.** Effect of 5-lipoxygenase inhibition (A63162) and cyclooxygenase inhibition (Indomethacin, INDO) on airway narrowing and wall thickness caused by platelet-activating factor (PAF)-activated eosinophils (28). (A) In comparison with unstimulated eosinophils, PAF-activated eosinophils caused a significant decrease in lumenal diameter of isolated human bronchi. Lipoxigenase inhibition prevented concentration dependently the decrease in lumenal diameter; indomethacin had a lesser, but also significant, effect on lumenal diameter. (B) In comparison with unstimulated eosinophils, PAF-activated eosinophils caused a significant increase in wall thickness of isolated human bronchi. This effect was significantly reduced by lipoxigenase inhibition, but not by cyclooxygenase inhibition. **p < 0.001 in comparison with untreated eosinophils (cells only); *p < 0.05 in comparison with PAF-activated eosinophils (cells + PAF).

**Figure 4.** Effect of protein kinase C (PKC) inhibition on cysteinyl-leukotriene release by activated eosinophils (49). Platelet-activating factor stimulated concentration dependently leukotriene C$_4$ release by human eosinophils (open circles). Preincubation of the cells with a 1 μM concentration of the selective PKC inhibitor bis-indoylmaleimide I (Bis I) for 10 min before addition of PAF at the indicated concentrations enhanced the release of LTC$_4$ (closed circles) as compared with eosinophils that were only PAF stimulated. Leukotriene production is expressed as the accumulation of LTC$_4$ in the cell supernatant per 10$^6$ cells in 5 min. *p < 0.05; **p < 0.001, compared with cells preincubated without Bis I.
unstimulated cells with fibronectin has no effect on airway narrowing and does not in itself cause release of leukotriene C\(_4\). Therefore, eosinophil ligation via VLA-4 to fibronectin primes rather than simulates augmented release of contractile leukotrienes from eosinophils to an extent that is sufficient to increase significantly the capacity of the cells to contract human airway smooth muscle.

Although the signal transduction pathways mediating eosinophil responses in clinical asthma are incompletely understood, data have demonstrated that PKC inhibition significantly enhances leukotriene C\(_4\) generation by PAF-activated human eosinophils (Figure 4) (49). This supports the assumption obtained earlier in eosinophils from guinea pigs (50) that protein kinase C together with phospholipase C and cytosolic free calcium plays an important role in the generation from eosinophils of contractile mediators such as LTC\(_4\). Furthermore, it has been shown in vivo that in patients with aspirin-intolerant asthma the bronchial expression of LTC\(_4\) synthase, which converts LTA\(_4\) to LTC\(_4\), is increased, thus allowing marked overproduction of cysteinyl-leukotrienes (51).

The altered regulation of eicosanoid production through genetic factors, matrix interactions, or through so far incompletely understood priming mechanisms may play an important role in obstructive airways disease, given the importance of LTC\(_4\) in the maintenance and generation of tone in human isolated airways.

**Allergic Reaction**

In sensitized individuals allergen responses are mediated through interaction of allergen with IgE antibodies bound to the surface of mast cells, resulting in the increased release of leukotrienes and histamine, which are also known to be the most important mediators of allergen-induced bronchoconstriction in isolated human airways (52). A allergen-induced bronchoconstriction is one characteristic feature of bronchial asthma, which can almost be abolished by combined pretreatment with leukotriene and histamine receptor antagonists in patients with asthma (53). In close analogy to these in vivo findings, allergen-induced contractions of isolated airways obtained from patients with extrinsic asthma are also abolished.
after pretreatment with the combination of leukotriene and histamine receptor antagonists in vitro (54). Therefore, leukotrienes and histamine are considered to be the most important mediators of allergen responses in human airways under in vivo as well as in vitro conditions.

In addition, adenosine, which is produced as a result of inflammatory cell activation (55), can cause bronchoconstriction in isolated asthmatic human airways but not in those from nonasthmatic individuals, in close analogy to the in vivo situation. These in vitro responses have been characterized pharmacologically and are mediated through an increased, endogenous liberation of leukotrienes and histamine (56).

Leukotriene Responsiveness

Passive sensitization—the incubation of tissue from normal individuals with IgE-rich serum—induces specific IgE-dependent responsiveness to allergen (32) and increases nonspecific responsiveness to histamine (Figure 5A) (47, 57, 58), K CI (57), and neuropeptides (59). The induction of allergen responses by passive sensitization depends primarily on the presence of specific IgE antibodies in the sensitizing serum (32, 60) and is most likely effected through the liberation of mediators such as histamine and leukotrienes from mast cells. However, the contribution of histamine to allergen responses in vitro appears to be of minor importance, whereas leukotrienes are believed to be the single most important class of mediators (54). In this context it appears relevant that passive sensitization of isolated human airways also increases the responsiveness to leukotriene C4 (Figure 5B) (47), resulting in an airway response to allergen that is the combined result of increased leukotriene release and increased smooth muscle sensitivity to this inflammatory mediator.

A iway responsiveness of isolated human airways to LTC4 and histamine are closely related to each other. The shift in potency as well as in maximal contractions caused by passive sensitization in similar in magnitude to the shift observed in histamine responsiveness (Figure 5C) (47), with a potency ratio between LTC4 and histamine of about 1,000:1 in nonsensitized and sensitized airways. This potency ratio is in accordance with earlier studies in isolated human bronchi, which demonstrated that understanding the regulation of human airway tone and airway reactivity are closely linked to the understanding of baseline and stimulated production of and smooth muscle responsiveness to leukotrienes in vitro and in vivo.

References


