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Localization of immunoreactive tissue kallikrein in the seromucous glands of the human and guinea-pig respiratory tree

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Summary

An immunocytochemical study focused on the cellular localization of tissue kallikrein along the human and guinea-pig respiratory tracts is reported. A strong immunoreactivity for tissue kallikrein was observed in the seromucous glands of the nasal mucosa, trachea, and bronchi. In these glands, the immunostaining was restricted to the serous component of the acinus whereas mucous cells showed no staining. Since no immunoreactivity to kininogen was observed in any of the tissue constituents of the human and guinea-pig respiratory tree, transudation of the substrate from plasma was considered to be the preferred mode of delivery of the kininogen into the bronchopulmonary interstitium and lumen. Our results provide morphological evidence for the well documented presence of tissue kallikrein in bronchial lavage fluids and support the hypothesis that kinins may be one of the more important mediators involved during acute episodes of asthma and rhinitis.

Introduction

Tissue kallikreins are serine proteases that are widely distributed in a range of mammalian tissues (see Bhoola *et al.*, 1992). Their cell specific distribution has led to the suggestion that tissue kallikreins perform different functional roles depending upon their site of origin (Schachter, 1980).

The first report on the presence of a kininogenase in the respiratory tissue was provided by Meier *et al.* (1979) who found kinin-generating activity after antigen challenge of passively sensitized human lung fragments. Generation of this activity in response to antigen challenge focussed attention on the mast cell as a possible source of this enzyme. Later studies confirmed the presence of tryptase, an enzyme which occurs in high concentrations in these cells but has weak kininogenase activity that generates bradykinin optimally at pH 5.5 (Proud *et al.*, 1985, 1987a). Such a lack of potency in releasing kinins suggested that tryptase was not the enzyme described earlier by Meier *et al.* (1979), thereby raising the possibility of another kininogenase source in the respiratory tree.

So far in only one study, by Christiansen *et al.* (1987), kinin levels and kininogenase activity have been demonstrated in bronchial lavage fluids obtained from asthmatic patients but not from control subjects. The same authors had previously shown the formation of kallidin during an immediate response to nasal airway challenge of allergic individuals (Proud *et al.*, 1983). The finding of kallidin strongly suggested the presence of a tissue kallikrein in nasal secretions. Subsequent analysis demonstrated that this enzyme possessed the functional and immunological characteristics of a tissue kallikrein (Christiansen *et al.*, 1987).

In this morphological study we have extended our previous preliminary investigation on the guinea-pig (Bhoola *et al.*, 1989) and human (Figueroa *et al.*, 1992) trachea, and performed comprehensive immunocytochemical experiments to localize tissue kallikrein along the human and guinea-pig respiratory tree. The enzyme is localized in the serous cells of seromucous glands of the nasal mucosa, trachea, and bronchi. In addition we discuss

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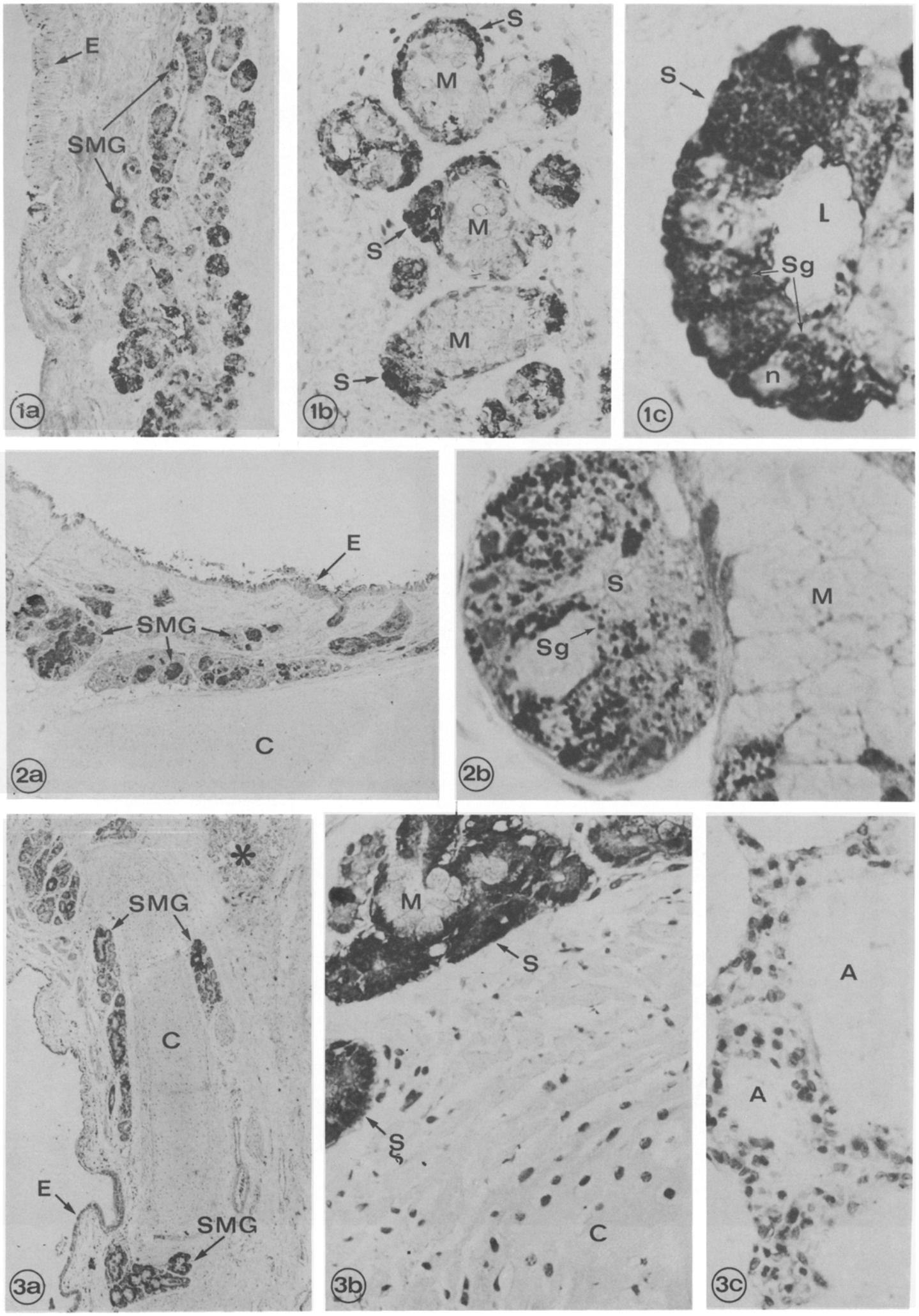
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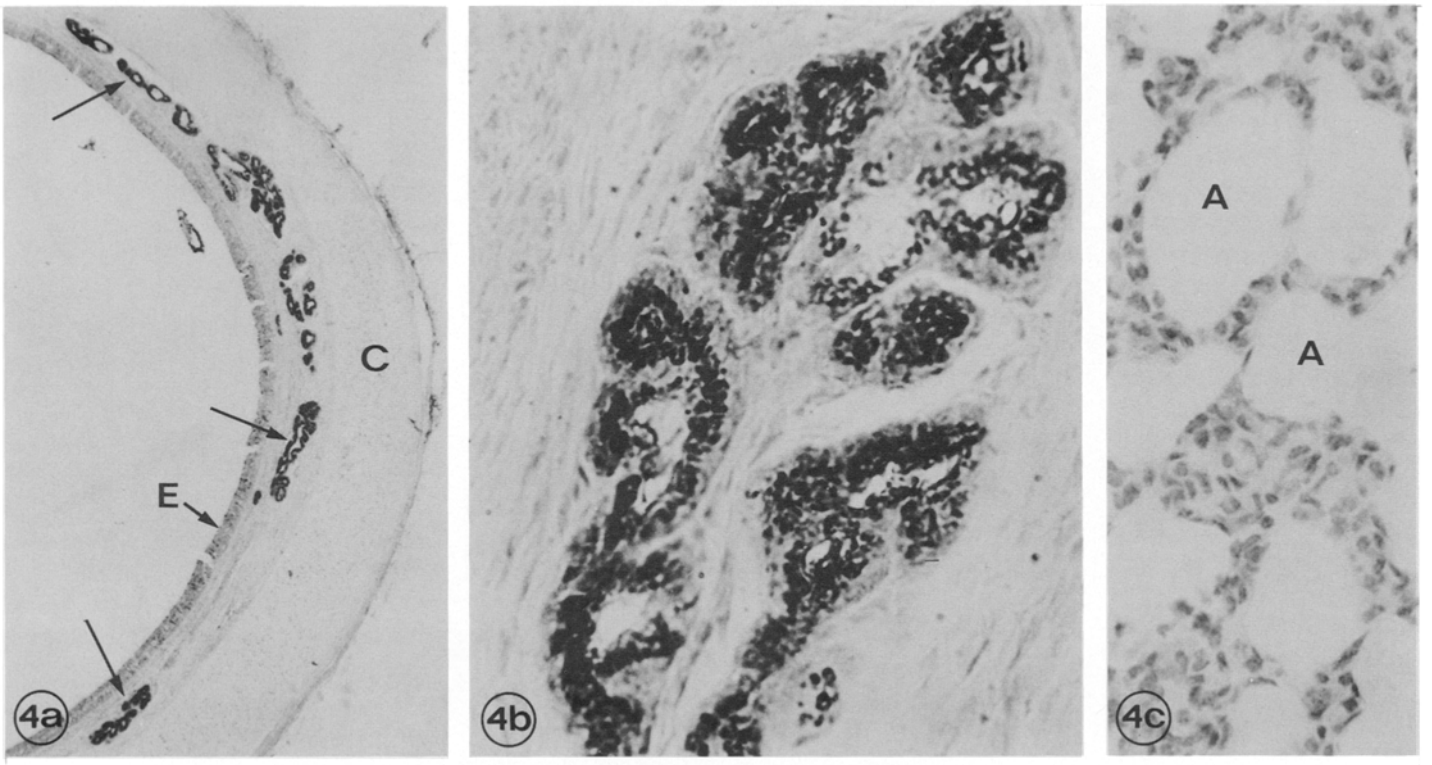

Fig. 4. Guinea pig trachea (a, b) and lung (c). (a) An intense immunostaining for tissue kallikrein is observed in the serous tracheal glands (arrows) (Ab2). ×40. E = epithelium; C = cartilage. (b) Higher magnification of the tissue kallikrein-containing glands shown in (a). ×400. (c) Lung parenchyma showing no staining for tissue kallikrein. ×500. A = alveoli.

the importance and involvement of kinins released by kallikreins in the causation of bronchial hyper-reactivity.

Materials and methods

Tissue processing

Human and guinea-pig tissues were fixed in 10% formal-saline for 24–28 h at room temperature, dehydrated in ethanol and embedded in paraffin wax as previously described (Figueroa *et al.*, 1988). The human respiratory tissues used in this study corresponded to conserved areas of tissues surgically removed due to the presence of adenomas or carcinomas (turbinate nasal mucosa and trachea). Autopsy material was also used especially to study the distribution of tissue kallikrein in the human lung and trachea; the human tissues were obtained between 4 to 6 h after death. At least five or six separate autopsy samples were used for each type of tissue in order to confirm the initial localization.

Immunocytochemistry

Tissue sections (5 μm thick) were de-waxed, rehydrated and incubated with four different polyclonal antibodies (Ab) (1:200 to 1:1000) raised against human urinary tissue kallikrein: Ab1, rabbit antiserum (Bagshaw *et al.*, 1984); Ab2, rabbit antiserum (Vio *et al.*, 1988); Ab3, goat antiserum (Protogen, Switzerland); Ab4, rabbit antiserum kindly supplied by Dr David Proud of the Division of Clinical Immunology, Johns Hopkins University School of Medicine, Baltimore, USA, its characterization and specificity have been previously described (Baumgarten *et al.*, 1986). One of the antisera (Ab2), strongly cross-reacted with guinea-pig tissue kallikrein and was used to trace the presence

Fig. 1. Human nasal mucosa. (a) Low-power view of immunoreactive seromucous glands (SMG) (Ab3). ×110. E = epithelium. (b) The immunoreactive tissue kallikrein is restricted to the serous component (S) of the acini, while mucous cells (M) remain unstained (Ab3). ×370. (c) High-power view of the serous portion (S) of an acinus. The immunoreactive tissue kallikrein localizes in the granules of the serous cells (Sg) (Ab4). ×1800. n = nucleus.

Fig. 2. Human trachea. (a) Low-power view of a human trachea section immunostained for tissue kallikrein (Ab2). ×60. SMG = seromucous glands; E = epithelium; C = cartilage. (b) Higher magnification of a serous (S) and a mucous (M) acinus. Immunoreactive tissue kallikrein appears in the granules of the serous cells (Sg) (Ab2). ×1000.

Fig. 3. Human lung. (a) Immunoreactive tissue kallikrein is observed in the seromucous glands (SMG) of an intrapulmonary bronchus (Ab4). ×50. Asterisk = the lung parenchyma is devoid of staining; E = epithelium. (b) Higher magnification of the glands showed in (a). Tissue kallikrein localizes in the serous (S) component of the acini (Ab2). ×450. M = mucous portion of the acini; C = cartilage. (c) Lung parenchyma showing no staining for the enzyme (Ab2). ×460. A = alveoli.

of the enzyme in the guinea-pig trachea and lung. In addition, rabbit anti-guinea-pig, anti-porcine and anti-rat tissue kallikrein were used to immunolocalize the enzyme in the guinea-pig tissues (Bhoola *et al.*, 1989).

Bound antibodies were detected by the peroxidase/anti-peroxidase method as previously described (Figueroa *et al.*, 1984, 1988). Visualization of peroxidase was achieved by incubation in a diaminobenzidine (0.1%)–hydrogen peroxide (0.03%) solution. After immunostaining, tissue sections were lightly counterstained with Harris Haematoxylin, dehydrated and mounted in Canada balsam.

Immunocytochemical controls

Controls included omission of the first antibody or replacement of each of the tissue kallikrein antisera by non-immune rabbit serum or an unrelated IgG of the same species. Controls also included pre-absorption of the diluted specific antibody with 20 $\mu g\ ml^{-1}$ of commercially purified human urinary kallikrein (Protogen AG, Switzerland). Preabsorption controls for the guinea pig tissues were performed using the same human urinary kallikrein and the anti-human tissue kallikrein sera (Ab2). The diluted antiserum and tissue kallikrein were preincubated overnight at 4°C and then used to incubate the tissue sections.

Results

Immunocytochemistry performed on both guinea-pig and human respiratory tissues showed a similar localization for tissue kallikrein (Figs 1–4). All the antibodies used in this study revealed the same pattern of immunolocalization for tissue kallikrein in the two species examined. Control experiments in which the specific anti-tissue kallikrein serum was either omitted, or replaced by non-immune serum or pre-absorbed with purified tissue kallikrein, showed no immunolocalization of the enzyme in both guinea-pig and human airway tissues.

Human nasal mucosa

A strong immunostaining was observed in the seromucous glands of the nasal mucosa. In these glands the immunoreactivity was restricted to the serous component of the acini whereas mucous cells showed no staining (Fig. 1, a and b). Identification of the mucous cells was based on their morphological features and on their histochemical reactivity to periodic acid–Schiff (PAS) staining. Intracellularly, tissue kallikrein was localized in the granules of the serous cells (Fig. 1c). In addition, a thin immunostaining was observed on the surface of nasal epithelium. Other tissue constituents showed no presence of the enzyme.

Human and guinea-pig trachea

As in the human nasal mucosa, tissue kallikrein immunoreactivity specifically localized in the seromucous tracheal glands (Fig. 2, a and b). Immunolocalization of the enzyme in human trachea was identical to that described for nasal mucosa. On the other hand, guinea-pig tracheal glands consisted of serous cells only, which showed an intense staining with all antisera used (see Fig. 4, a and b). In both species the surface of the tracheal epithelium showed a thin layer of immunoreactive material. Structures such as cartilage, submucosa and epithelium displayed no reactivity to tissue kallikrein antibodies (see Figs 2a, 3a, 4a).

Human and guinea-pig lung

Immunoreactive tissue kallikrein was detected only in the seromucous glands of extrapulmonary and intrapulmonary bronchi (Fig. 3, a and b). At this site the immunoreactive glands were observed as far out in the bronchial tree as the cartilage extended. The tissue kallikrein-containing glands were usually situated deeper than the muscular layer, through which their ducts penetrate to open on the free epithelial surface. Other respiratory structures of the lung such as bronchioles, alveolar ducts and alveoli (see Figs 3c, 4c) were all devoid of immunostaining.

Tissue kallikrein immunovisualization was abolished when the specific antisera were omitted, replaced by non-immune serum or when it was used after pre-absorption with purified urinary tissue kallikrein.

Discussion

Various studies have strongly suggested that kinins may be involved in several inflammatory airway disorders such as asthma and rhinitis, especially airway hyperreactivity secondary to rhinovirus infection. Thus, immunoreactive tissue kallikrein, kininogens and both bradykinin and kallidin have been found in bronchial lavage fluids obtained from these patients (see Proud & Kaplan, 1988). However, the specific segments of the respiratory tree and the cells that contain tissue kallikrein or the kinin-forming substrates had not been clearly identified.

The present study is the first to report on the immunolocalization of tissue kallikrein in each of the human and guinea-pig airway segments, unlike only one other study that was restricted to the human trachea and published while our localization experiments were in progress (Proud & Vio, 1993). Our study clearly demonstrates the existence of immunoreactive tissue kallikrein in the serous cell granules of the seromucous glands of nasal mucosa, trachea and bronchi. These results support the early description of a tissue kallikrein in bronchoalveolar fluid of asthmatic patients (Christiansen *et al.*, 1987), and provide further support for the involvement of tissue kallikrein and kallidin in asthma and rhinitis.

Because no immunoreactive kininogen (the substrate for tissue kallikrein) containing cells have so far been found in the human respiratory tract (Figueroa *et al.*, 1992), the question as to the source of the substrate for tissue kallikrein has to be addressed. Our considered view is that kininogen reaches the bronchopulmonary interstitium and lumen in transudates of plasma. In agreement

with this concept is the finding of Baumgarten *et al.* (1985) who have shown that during an allergic response both low-molecular-weight kininogen and plasma prekallikrein–high-molecular-weight kininogen complexes enter nasal secretions from plasma.

Another kininogenase found to be involved in kinin formation during airway inflammation is plasma kallikrein. This enzyme circulates complexed to high-molecular-weight kininogen and during the immediate phase of the challenge response, transudation of prekallikrein–high-molecular-weight kininogen complexes and Hageman factor occurs (see Proud & Kaplan, 1988). The precise mechanism of contact activation involved is not known, but participation of negatively charged mucous macromolecules or mast cell heparin has been suggested (Hojima *et al.*, 1984; Proud & Kaplan, 1988). Although plasma kallikrein is present in approximately 10-fold higher concentrations than tissue kallikrein, the latter is responsible for most of the kinin-forming activity in post-challenge lavages (Baumgarten *et al.*, 1986). In addition, post-challenge nasal lavages contain high levels of an aminopeptidase able to convert kallidin (lys-bradykinin) to bradykinin (Proud *et al.*, 1987b), suggesting that the bronchial lavage kallidin levels may initially be greater than those detected by Proud *et al.* (1983).

It is clear that subjects who do not manifest symptoms of rhinitis have no kinins in their lavages (Proud *et al.*, 1983; Proud & Kaplan, 1988). On the other hand, intranasal administration of bradykinin in normal subjects induces nasal obstruction and rhinorrhea as well as sore throat, all common accompaniments of rhinovirus infection (Proud *et al.*, 1988). The presence of kinins in nasal lavages of subjects with symptomatic rhinovirus infection together with the fact that bradykinin administration can elicit the relevant symptoms, suggest that these peptides are one of the major mediators of rhinovirus symptoms. Similar observations have been made in asthmatic subjects who have an increase in bronchoalveolar kinin levels after allergen challenge (Christiansen *et al.*, 1992). Moreover, bradykinin causes severe bronchospasm in asthmatic but not in normal subjects (Herxheimer & Stesemann, 1961; Varonier & Panzani, 1968).

The mechanisms involved in the release of tissue kallikrein from seromucous glands sited along the respiratory tract require to be elucidated. Furthermore, the precise contribution of kinins to the pathogenesis of inflammatory airway conditions remains an important experimental challenge. The clinical use of specific kinin antagonists, such as Hoe 140 (Wirth *et al.*, 1991) or CP0127 (Whalley *et al.*, 1992), will certainly establish the precise spasmogenic role played by kinins in patients with airway hyper-reactivity, especially asthmatics.

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