

## Functions of proteins and lipids in airway secretions

J. Jacquot\*, A. Hayem\*\*, C. Galabert\*\*\*

*Functions of proteins and lipids in airway secretions. J. Jacquot, A. Hayem, C. Galabert.*

**ABSTRACT:** Proteins and lipids synthesized by airway secretory cells or transudated are active components in the protection of respiratory epithelium. Proteins and ions are involved in the control of mucus hydration. Secretory proteins, such as secretory immunoglobulin A (IgA), transferrin and lysozyme, participate in the airway antibacterial defence. Other biochemical components found in secretions, such as anti-inflammatory and antioxidant agents as well as antiproteases, contribute significantly to the protection of the underlying epithelium.

*Eur Respir J.*, 1992, 5, 343-358.

\* INSERM U 314 Université de Reims 51092 Reims Cédex, France. \*\* INSERM U 16 Place de Verdun 59045 Lille Cédex, France. \*\*\* Hôpital R. Sabran Giens, 83406 Hyeres Cédex, France.

Correspondence: J. Jacquot  
INSERM U 314  
CHR Maison Blanche  
45 rue Cognacq Jay  
51092 Reims Cédex, France.

Keywords: Airway secretions; antioxidants; antiproteases; lipids; proteins.

Human respiratory diseases, including acute and chronic bronchitis, asthma and cystic fibrosis, are often associated with excessive airway mucus production. In the conducting airways, the submucosal glands and surface epithelial (goblet) secretory cells are the major contributors to the mucus secretion [1, 2].

Because of the cellular heterogeneity of the airway secretory apparatus, the factors that govern secretion and composition of each secretory cell type and contribute to the biochemical pattern of mucus are not completely defined. To date, mechanisms possibly responsible for the regulation in the volume and quality of the respiratory tract secretions involve the transepithelial Cl<sup>-</sup> secretion across airway epithelium with passive diffusion of water [3, 4], secretory effects of a number of inflammatory mediators including arachidonic acid metabolites released either from airway epithelial cells [5-9] or activated inflammatory cells [10-12] and the increase in total number of mucus-producing cells [13].

The respiratory mucus is very complex and includes 95-98% of water, ions, sugars, amino acids, (1-3%) proteins, glycoproteins and lipids [14]. One of the main functions of mucus is to protect the mucosal surface from physical and chemical injuries and from inhaled microorganisms. Apart from its main participation in transport by mucociliary clearance, respiratory mucus also serves a variety of other protective purposes, including the control of airway hydration and lung tissue defence mechanisms. In this review, we will discuss recent data on potential roles of a number of ions, polypeptides, glycoproteins (other than mucins), proteases, antiproteases, plasma proteins, and lipids found in mucus and involved in: 1) the control of airway humidification and mucus hydration; and 2) the

airway epithelium defence by limiting both the adhesion of bacteria and viruses to respiratory epithelial surface and protecting the airways from injury produced by proteolytic and oxidant agents.

### Airway humidification and mucus hydration

The tracheobronchial epithelium is considered as an absorptive and secretory type epithelium [15]. It is now generally agreed that both trachea and bronchi are able to activate electrolyte absorption and secretion and, by osmotic coupling, water. The movement of electrolytes, more precisely the rate of transepithelial Cl<sup>-</sup> secretion and fluid across airways, appears to play an important role in the production and quality of airway gland secretions [3, 4, 15].

Airway surface epithelial cells are polarized anatomically and functionally, joined together by tight junctions, thereby delimiting an apical membrane with cilia and microvilli and a basolateral membrane facing the interstitial space. These two membrane domains have specialized functions for the transport of ions and water. The secretion of Cl<sup>-</sup> into the airway lumen, coupled or not with the absorption of Na<sup>+</sup>, occurs in the apical membrane and requires energy which is supplied by the Na<sup>+</sup>, K<sup>+</sup>-adenosine triphosphatase (ATPase) pump localized in the basal membrane. By generating local osmotic gradients, these ion transport processes regulate the water content and, thus, the depth of the periciliary sol layer [15]. It is thought that water movement occurs in intercellular spaces. An increased rate of Na<sup>+</sup> absorption, coupled with fluid movement, contributes to the relative dehydration of airway secretions that characterizes the cystic fibrosis disease [16, 17].

### *What are the physicochemical and physiological cellular mechanisms that modulate mucus hydration?*

The rate determining step in secretion, as for absorption, lies at the apical membrane where the activity of Cl<sup>-</sup> channels controls, in part, the transepithelial Cl<sup>-</sup> secretion [18]. Several intracellular second messenger pathways that activate apical membrane Cl<sup>-</sup> channels have recently been described in normal airway cells: 1) intracellular complementary adenosine monophosphate (cAMP) stimulates Cl<sup>-</sup> secretion and, therefore, the Cl<sup>-</sup> channel can be opened through phosphorylation brought about by protein kinase A (PKA), or at low intracellular Ca<sup>2+</sup> concentration, by protein kinase C (PKC). This activation (phosphorylation) by both kinases is defective in cystic fibrosis airway cells [19, 20]; 2) several secretory hormones, such as bradykinin, stimulate Cl<sup>-</sup> secretion in canine airway cells by activating phospholipase A2 and phospholipase C [21]; and 3) extracellular adenosine triphosphate (ATP) stimulates Cl<sup>-</sup> channels in human airway epithelial cells [22]. ANDERSON and WELSH [23] have recently shown that addition of arachidonic acid in cultured airway epithelial cells from normal and cystic fibrosis inhibits apical membrane Cl<sup>-</sup> channels. Fatty acids (arachidonic acid) would, therefore, directly interact with the Cl<sup>-</sup> channels or an associated protein to alter the Cl<sup>-</sup> channels.

### *Roles of proteins in mucus hydration*

Several proteins in respiratory mucus are considered to be active components in mucus hydration. According to TAM and VERDUGO [24], mucus hydration and swelling are governed by a Donnan equilibrium process and not by simple osmosis. Therefore, the concentration of free ions, pH, ionic strength and small polyionic proteins, such as proline-rich proteins, lysozyme and albumin, in the fluid on airway surfaces can modulate mucus hydration and its swelling rate [25, 26]. WIDDICOMBE [27] reported that airway surface fluid is hyperosmolar, and possesses an electrolyte composition different from that of interstitial fluid. He suggested that a homeostatic mechanism in mucosa may regulate the pH of the periciliary fluid layer. Recently, DEFFEBACK *et al.* [28] showed that different prostaglandins can selectively modify *in vitro* the volume and quality of secretions in the ferret trachea. They demonstrated that prostaglandin E<sub>1</sub> (PGE<sub>1</sub>) inhibited lysozyme release, a protein marker for serous cell secretion [29], whereas PGD<sub>2</sub> reduced secretion volume and PGF<sub>2α</sub> inhibited albumin transport. Conversely, PGE<sub>1</sub> was a potent, and PGD<sub>2</sub> a weaker, stimulant of albumin transport, whereas PGF<sub>2α</sub> increased the secretion volume, and was a potent stimulant of lysozyme release. The presence of albumin in airway and alveolar fluid has been attributed to either passive serum-transudation from local vessels [30] or active transport across tracheal epithelial cells to the airway lumen [28]. JOHNSON *et al.* [31] have also shown that

albumin may be actively absorbed from the lumen to interstitium by canine bronchial epithelium, probably involving specialized vesicular transport mechanisms. Using *in vitro* culture techniques, we have also demonstrated that an albumin-like protein can be synthesized *de novo* by bovine tracheal gland serous cells [32], and that the amounts of albumin-like protein released by these cells were dependent on the composition of culture medium [33]. Thus, the discovery that albumin can be actively transported bidirectionally through airways, and also synthesized by airway gland cells, suggests that the albumin protein may contribute to the regulation of lung fluid balance and, consequently, may be an important component in the control of mucus hydration. Other properties for albumin in the airways have been reported, including the increase of mucus viscosity [34], the binding of drugs, ions and a potential scavenger for free radicals [35].

### **Mucus components involved in the airway epithelium defence systems**

Apart from bronchial mucins, which by their heterogeneity of carbohydrate structures [36] are able to entrap, and thus eliminate, various bacteria and viruses by means of the mucociliary escalator, other components found in secretions (glycoproteins, peptides, lipids and protease inhibitors) also play important roles in the protection of the underlying epithelium.

### *Protective functions of lipids*

It has long been recognized that airway secretions contain noticeable amounts of lipids [37-42]. As reviewed extensively by WIDDICOMBE [43], different studies showed not only the variations in the overall amount of lipids, but also the variabilities in content of the different components, which include phospholipids, glycerides, cholesterol and its esters, glycosphingolipids, glyceroglycolipids [41], and free fatty acids. The major phospholipid component is phosphatidylcholine (PC), which generally contains less saturated fatty acids (palmitic acid) [39-42] than PC coming from the alveolar surfactant. Lipids are closely associated with the mucus glycoproteins [44-48] mostly *via* non-covalent binding [48, 49]. The amount of associated lipids may represent up to 49% of the weight of the solids in mucin preparations obtained from pathological secretions [47, 48]. In infected purulent secretions, such as in cystic fibrosis and bronchiectasis, the amount of lipids is largely increased [48].

It has generally been assumed that the lipids found in airway secretions originate from alveolar surfactant, from serum transudate, or from degradation products of shed epithelial cells, macrophages, inflammatory cells and bacteria [49]. In fact, the alveolar surfactant is unlikely to be the main source of lipids in the upper airways, since it was reported that only a very small

amount could reach the trachea [50, 51]. On the other hand, serum transudation and breakdown of membrane debris from various cells are certainly a major source of lipids in inflammatory and infected secretions. To date, it has become evident that lipids normally found in airway secretions are locally produced and do not simply represent the transport of alveolar surfactant from the periphery. Several reports [52-57] have demonstrated that phospholipids, glycosphingolipids, glycerides and sterols were associated to mucins isolated from supernatants of animal and human tracheal explants. According to these studies [46, 53], the amount of lipids ranges from 5-15% of the dry material. Recently, KIM and SINGH [58, 59] demonstrated that the lipids were synthesized and secreted in association with mucus glycoproteins in hamster cultured tracheal epithelial cells, which could explain the extreme hydrophobicity of the secreted mucins. Recently, GIROD *et al.* [60], using a cytochemical enzyme-gold technique, localized intragranular phospholipids in serous and mucous cells of human tracheobronchial gland. BARROW [61] compared the phospholipid secretion at the bronchoalveolar, bronchial and tracheal levels in sheep and suggested a local synthesis and release of phospholipids by tracheal epithelial cells. These convergent results suggest the production and secretion of surfactant material at the bronchiolar and tracheobronchial levels and support the hypothesis that mucosal airway secretory cells produce phospholipids which may be secreted in the airway lumen.

The first functional role of lipids in tracheobronchial secretions may be to participate in mucosal protection. It is well established that the protective function of the mucus lining the airway luminal surface is due mainly to the physicochemical properties of mucus glycoproteins (mucins). The fact that mucins are associated with a significant amount of lipids may increase their hydrophobicity and, consequently, modulate their physicochemical characteristics. Although experimental data are lacking for bronchial secretions in this field, phospholipids have been shown to influence ion diffusion in other mucous secretions: in gastric secretion, an adsorbed layer of surface active phospholipids may be an essential component to maintain the hydrophobicity of the mucosa [62, 63]. SAROSIEK *et al.* [64] and SLOMIANY *et al.* [65] have shown that lipids bound to gastric mucus contribute significantly to the retardation of hydrogen ion diffusion *in vitro*.

#### *Antibacterial components*

*Glycoproteins other than mucins.* The airways are protected by immunity mechanisms, including a combination of local mucosal immunity especially characteristic of the upper respiratory tract, and peripheral type immune reactions which characterize the alveolar level [66]. In humans, IgA represents about 10% of the total protein recoverable in bronchoalveolar lavage fluids [67]. The secretory IgA (sIgA), the

major immunoglobulin in bronchial secretions and other external body fluids [68-71] forms, along with the polymeric immunoglobins (IgG, IgM), a very early specific immunological defence against infection. Their synthesis and transport through the bronchial epithelium are mediated by specific transcytotic events [69, 72].

The IgA molecule is produced by submucosal plasma cells that are often localized in bronchus-associated lymphoid tissue (BALT). After secretion, two IgA molecules are covalently linked by a J-chain glycoprotein (15 kDa), also synthesized within the plasma cells. Then, a 70 kDa glycoprotein, called the secretory component, synthesized separately in gland epithelial cells and localized in their basolateral surfaces, acts as a membrane receptor for dimeric IgA (or polymeric IgM), now known as the polymeric immunoglobulin receptor (pIg-R). According to BRIETFFELD *et al.* [72], the pIg-R and ligand complex is then endocytosed in coated vesicles and transported by a variety of vesicles and tubules to the apical membrane surface, and extruded by exocytosis into the bronchial secretions. The extracellular portion of the pIg-R is proteolytically cleaved and remains associated with the IgA. By using electron microscopy, GOODMAN *et al.* [73] have shown that in human bronchial submucosal glands, more IgA was detected in mucous cells compared to serous cells. In ciliated cells, IgA was not identified, whereas the secretory component was present in the cell membrane. The role of secretory IgA in bronchial secretions is thought to prevent entry of antigens beyond the mucosal barrier by its antiviral properties and capacity to agglutinate and impair the adhesion of bacteria to the mucosal surface and, consequently, increase the antigen elimination by phagocytosis and clearance mechanisms. DANIELE [70] has recently reported that the combination of sIgA, lysozyme and, perhaps, components of complement in bronchial secretions may promote phagocytosis by alveolar macrophages of IgA-coated particles and bacteria.

Other immunoglobulins such as IgG and IgM are found in airway secretions. In cystic fibrosis (CF) patients, IgM concentrations in sputum are strongly associated with the degree of bronchial superinfection, evaluated by a quantitative cyto bacteriology in sputa [74]. The IgM concentration in CF sputa was shown to significantly increase with the severity of the disease assessed by the Schwachman score. Among the other main proteins with potential antibacterial activity (IgA, IgG, lysozyme and lactoferrin) measured in CF sputa, a significant negative correlation was observed between the Schwachman score and the concentration of lactoferrin [74]. Lactoferrin is an iron-binding glycoprotein (75-80 kDa) of the transferrin family that is common to exocrine secretions of mammals [75]. In human tracheobronchial tissue, Bowes *et al.* [76] showed that lactoferrin was localized in the secretory granules of submucosal gland serous cells. The biological functions of lactoferrin are still unclear, but it has been suggested that it is part of the primary system of nonspecific local secretory immunity with sIgA, myeloperoxidase and lysozyme against bacterial

infection [77] and, perhaps, as a regulator of human granulopoiesis [78]. Lactoferrin and transferrin exhibit very similar structure and biochemical properties, but differ by their affinity for ferric ions and their delivery process of iron to the cell. Transferrin transports ferric ions into cells by receptor mediated endocytosis, a unique process by which transferrin and its receptor are reutilized repeatedly in iron delivery. On the other hand, it has recently been shown that the lactoferrin mediates transfer of iron to HT 29-D4 cells, not by receptor-mediated endocytosis, but by releasing iron at the plasma membrane without itself being internalized [79].

Since the fundamental work of FLEMING [80], lysozyme (a 14.4 kDa protein) has been shown to exhibit a broad spectrum of antibacterial properties, serving in concert with IgA and lactoferrin to protect the mucous membrane. Both lysozyme, lactoferrin and IgA are detected in high concentrations in exocrine secretions such as saliva, tears, colostrum, gastroduodenal, middle ear and tracheobronchial secretions and only in small amounts in internal secretions, such as serum and pleural fluids [71]. In human tracheobronchial tissue, HINNRSKY *et al.* [81] recently demonstrated quantitative differences in the distribution of lysozyme in human airway secretory granule phenotypes. At the tracheal level, the density of lysozyme, evaluated by post embedding immunogold technique, did not vary significantly within the different secretory granule phenotypes, whereas, at the bronchial level, the differences were significant. Moreover, the lysozyme labelling density was much higher in the bronchial than in the tracheal secretory granules. In human airway secretions, KONSTAN *et al.* [82] estimated that 10–20 mg of lysozyme was secreted per day by the tracheobronchial tract, lysozyme being mainly synthesized in the submucosal layer containing glandular cells. Whether human tracheal surface epithelial cells participate in the synthesis of lysozyme has not yet been clearly demonstrated.

Lysozyme can also originate from leucocytes, present in high concentration when airways are infected by pathogenic bacteria [74]. Four enzymatically active lysozyme forms have been isolated and purified depending on whether lysozyme was purified from purulent secretions, non-purulent secretions from chronic bronchitic patients, or from normal tracheobronchial explants in organotypic culture [83]. Additional studies are needed to characterize and define the possible sources for these multiple forms of human airway lysozyme. The physiological role of human lysozyme remains unclear and several functions have been reported in recent years. It is generally claimed that lysozyme is an antibacterial agent, but there is no clear evidence to show whether this enzyme plays *in vivo* a direct or indirect role against pathogens present in airway secretions. It has been reported that most pathogenic bacteria are insensitive to lysozyme in the absence of antibodies, complement or other enzymes [84]. In fact, the major *in vitro* measurements of antibacterial activities of lysozyme have essentially been

conducted using hen egg-white lysozyme on oral or intestinal bacteria [85]. Nevertheless, it has been shown that exposure of pneumococci to purified human airway lysozyme results in a higher bactericidal activity than that obtained with hen egg-white lysozyme [86]. Apart from the restructuring effects of lysozyme in the gel network formation and, consequently, in the rheological and transport properties of airway mucus [87], GORDON *et al.* [88] have postulated that human lysozyme may also function in a negative feedback system to modulate the inflammatory response. They reported that *in vitro* human lysozyme inhibited the leucocyte chemotactic motility and the production of toxic oxygen radicals by stimulated leucocytes. Recently, PRIOR *et al.* [89] reported that serum lysozyme levels appear to be a useful marker both to assess disease activity in pulmonary sarcoidosis and to correlate clinical impairment and response to steroid therapy.

The cellular regulation of mucus production by the human airways and the mechanisms involved in mucus hypersecretion in chronic obstructive respiratory diseases are not completely elucidated. In the same way, whether the cell mechanisms involved in the excessive secretion are similar in all patients with obstructive airway diseases is not yet defined. Although the hypersecretion in patients with chronic bronchitis and cystic fibrosis follows a period of glandular hypertrophy, BHASKAR *et al.* [90], recently reported that in acute quadriplegic patients, the onset of mucus hypersecretion is sudden and due to disturbed neuronal control of bronchial mucous gland secretion. In humans, glandular secretions from tracheal and nasal airways are stimulated by exogenous acetylcholine, cholinergic and peptidergic analogues, leading to the secretion of mucous cell products such as glycoconjugates (mucins) and serous cell products such as lactoferrin, lysozyme and sIgA [91, 92]. Among airway neuropeptides, vasoactive intestinal peptide (VIP, a 28 amino acid peptide) is the most potent endogenous airway relaxant and has been demonstrated to have a protective effect against certain bronchoconstricting stimuli. VIP is released with acetylcholine by parasympathetic nerves surrounding the submucosal glands and may contribute to the physiological regulation of airway mucus secretion [92–95]. COLES *et al.* [92] have shown that in *in vitro* human bronchial cultures, VIP (10 ng to 1 mg·ml<sup>-1</sup>) causes a dose-dependent inhibition of baseline and methacholine-stimulated release of both glycoconjugates and lysozyme. On the other hand, VIP does not inhibit glycoconjugates and lysozyme in bronchial explants from chronic bronchitic patients. These authors suggest that this absence of sensitivity to VIP inhibition is one possible cause in the pathogenesis of the airway mucus hypersecretion. Recently, OLLERENSHAW *et al.* [94] showed that in airway tissues from patients with asthma, no immunoreactive VIP was found, compared to the abundance of VIP found in normal airways. Whether this loss of VIP and/or the absence of VIP sensitivity is a cause or a result of asthma and chronic bronchitis is unclear, but it is a matter for further investigation. BARANIUK *et al.* [95]

recently demonstrated that in normal human nasal mucosa, VIP-immunoreactive nerve fibres were found to be most concentrated in submucosal glands adjacent to serous and mucous cells. They also showed that VIP stimulated lactoferrin release from serous cells but did not affect glycoconjugate secretion from short-term nasal explant cultures.

**Lipids.** It is well known that carbohydrates can be specific receptors for bacterial lectins and can mediate the adhesion of bacteria to host tissue. The carbohydrate chains of glycosphingolipids have been shown to be specific adhesion receptors for different microorganisms in humans. This could be an important mechanism of bacterial colonization or, conversely, of bacterial clearance in the respiratory tract [96]. KRIVAN *et al.* [97], demonstrated that two pathogenic bacteria frequently isolated in CF respiratory secretions, *Pseudomonas aeruginosa* and *Pseudomonas cepacia*, specifically bind to gangliotetraosylceramide and gangliotriaosylceramide. These results suggest that the glycolipids associated to respiratory secretions as well as the carbohydrate chains of mucins are potential sites of binding for microorganisms. At the surface of the epithelia, as suggested by RAMPHAL and PYLE [98], the glycolipids present at the apical cell membrane could also represent receptors for bacterial adhesion. Gangliosides could also interact in bacterial adhesion by their ability to bind to fibronectin [99]. In some reports, high contents of free fatty acids have been observed in tracheobronchial secretions. It has been demonstrated by COONROD [100] that in alveolar surfactant, free fatty acids may have an antimicrobial activity. The same mechanism could be evoked in the upper respiratory tract.

**Enzymes.** Recent studies have clarified the previously described antibacterial properties of some neutrophil proteases [101], which will be described in a later chapter. Other antimicrobial polypeptides of human neutrophils (defensins, azurocidin, bactericidal permeability increasing factor (BPI) and cationic antimicrobial proteins (CAP)) possess the capacity to kill bacteria by a mechanism independent of their specific enzymatic activities [102, 103]. The most potent neutrophil protease possessing an antimicrobial activity is cathepsin G. The antimicrobial domain within the molecule is constituted by a heptapeptide [104] located at the surface of the molecule.

#### *Antioxidant and anti-inflammatory components*

**Antioxidant enzymes.** Three enzymes provide the main defence against oxygen-mediated tissue injury: catalase, superoxide dismutase and glutathione peroxidase [105].

Data on the "oxidant-antioxidant imbalance" in airway secretions can be obtained in the literature [105-110]. PEDEN *et al.* [111] recently reported that uric acid secreted by human nasal submucosal glands may play an important role in airway antioxidant

physiology. Because cigarette smoking is a most pertinent example of chronic oxidant stress of the lower respiratory tract, most measurements of these antioxidant enzymes were carried out in bronchoalveolar lavages from smokers: alveolar macrophages from smokers contain increased activities of catalase and superoxide dismutase [112]. Catalase present in peroxisomes is one of the enzymes which cleaves  $H_2O_2$ . In rats, catalase was found to be fundamental in protecting alveolar epithelial cells [113]. CANTIN *et al.* [114] recently demonstrated that, among antioxidant macromolecules in the epithelial lining fluid of the normal human lower respiratory tract, catalase played a major role in protecting lung parenchymal cells against oxidants present in the extracellular milieu. Superoxide dismutase is a metalloenzyme, with Cu and Zn, the gene of which is located on chromosome 21 [115]: unfortunately, no studies in humans have documented a clinical association between trisomy 21 and a possible protection from toxic effects of oxygen [116]. Superoxide dismutase is lacking in sputum samples from patients with cystic fibrosis [117].

Glutathione peroxidase has been indirectly evaluated by measuring the ratio of oxidized glutathione to total glutathione [118]. Epithelial lining fluids from patients with idiopathic pulmonary fibrosis are deficient in glutathione [119]. On the contrary, bronchoalveolar lavage fluids from smokers contain a higher glutathione concentration than those from nonsmokers [120].

**Anti-inflammatory agents.** Strategies to reduce inflammation in the airways may be considered for the treatment of mucus hypersecretion in patients with asthma, chronic bronchitis and cystic fibrosis. There is evidence that endogenous neuropeptides such as substance P and other tachykinins released from sensory nerves in human airway mucosa [121] are involved in the genesis of bronchial hyperresponsiveness and airway inflammation. It has been shown that tachykinins produce a series of effects, referred to as neurogenic inflammation, including increased vascular permeability [122], neutrophil adhesion and chemotaxis [123], submucosal gland secretion [124], and cough and smooth muscle contraction [125]. Studies have demonstrated that neutral endopeptidase (encephalinase, EC 3.4.24.11), a cell membrane binding peptidase found in many organs and tissues including human lung [126] can prevent the neurogenic inflammation effects [127-129]. Recently, it has been proposed that therapy using recombinant human enkephalinase may be useful in treating cough or bronchial narrowing associated with airway diseases such as asthma, chronic bronchitis and cystic fibrosis [129].

Glucocorticoids are effective drugs used for the treatment of chronic inflammatory diseases. The mode of action of glucocorticoids is very complex [130], as almost all known pro-inflammatory mediators are modulated in their synthesis or degradation [131, 132]. The anti-inflammatory actions of glucocorticoids include the inhibition of the release of arachidonic acid derivatives and the increase of lipocortin levels in

airways associated with a significant reduction in respiratory glycoconjugate secretion [133]. Lipocortins, also called annexins, belong to a family of calcium and phospholipid binding proteins, which have been proposed as mediators of the anti-inflammatory actions of glucocorticoids, possibly *via* inhibition of phospholipase A2 activity [134, 135].

In human lungs, lipocortin 1 (a 35–37 kDa protein) has been detected in bronchoalveolar lavage fluids [136] and has also been reported to be synthesized and released by human airway submucosal gland cells in culture [137]. Using cultured rat alveolar epithelial cells, it has been shown that corticosteroids increase the amounts of lipocortin 1 produced by these cells [138]. Human recombinant lipocortin 1 impairs the release of thromboxane A<sub>2</sub> and prostacyclin *in vivo* in perfused lung and isolated cells [139]. Two other reports have also described anti-inflammatory actions of recombinant lipocortins *in vivo* [140, 141], although their physiological importance has not been demonstrated directly. Lipocortin 1 also causes inhibition of superoxide anion release from activated guinea-pig alveolar macrophages [142] and decreases platelet-activating factor (PAF) production from stimulated human polymorphonuclear neutrophils (PMN) [143]. Since reports have demonstrated that messenger ribonucleic acid (mRNA) and protein amounts of lipocortin 1 in other cell types [144–146] were not altered by dexamethasone, lipocortins do not seem, to date, to have a central function in the mediation of glucocorticoid effects. It has also been proposed that lipocortins are involved in phospholipid cytoskeleton interaction, exocytosis and that some of them (annexins I and II) are able to promote aggregation of phospholipid vesicles in mediating contact between vesicle membranes [147].

#### *Protease and antiprotease components*

**Proteases: major destructive agents.** The proteolytic enzymes which have been identified in lung secretions seem to be mainly neutrophil-derived [148] and many papers have dealt with proteases from purulent sputum. In fact, some proteases have been described originating from cells in culture, either normal [149], transformed [150], or cancerous [151]. Testing for the presence of an elastolytic activity in bronchial secretions has been, for a long time, the major investigation carried out to determine a possible destruction of lung parenchyma. Up to now, it seems that neutrophil elastase could be mainly in charge of proteolytic events in lungs.

Neutrophil elastase is a serine protease found in azurophil granules of the mature neutrophil. In blood neutrophils, the content is about 0.5–3 pg·cell<sup>-1</sup>. Since neutrophils enter the lungs through the pulmonary vasculature [152], it is likely that lung neutrophils are identical to blood neutrophils in that way. However, some recent data have shown different amounts of neutrophil elastase in blood neutrophils from patients with moderate to severe emphysema [153]. The gene has been described and is located in chromosome 11

at q14 [154]. The primary structure of the molecule has been determined before by sequencing at the protein level [155] and corresponds to that derived from the nucleotide sequence.

Neutrophil elastase presents a broad substrate specificity against extracellular matrix components: several lines of evidence have shown its *in vitro* destructive ability not only against elastin but also against type III collagen [156, 157], type IV collagen [158], adhesion molecules such as fibronectin [159] or laminin [160] and proteoglycans [161]; its role *in vivo* was extrapolated from these results. If neutrophil elastase has a role to play in some bronchopulmonary pathologies ("protease-antiprotease imbalance hypothesis" [162]), evidence for uncontrolled elastolysis would have to be given. Different amounts of active neutrophil elastase have been noted between mucoid, mucopurulent or purulent sputum samples [117, 163–165]. The results of BRUCE *et al.* [166] showed a significant correlation between the excretion of elastolysis products and the severity of lung disease in cystic fibrosis patients. Localization of neutrophil elastase was investigated in normal and emphysematous lungs: contradictory results have been obtained [167, 168] and it is not clear whether or not neutrophil elastase is associated to elastic tissue in emphysematous lung. Moreover, when the lungs are free of inflammatory reaction, neutrophils do not seem to contribute significantly to the normal turnover of pulmonary elastin in normal subjects [169].

Cathepsin G accompanies elastase in the azurophil granules of neutrophils. It is also a serine protease of interest in bronchial pathology because it possesses an *in vitro* elastolytic activity [170–173]. It is also able to stimulate bovine airway gland secretion [174]. Free cathepsin G was identified in cystic fibrosis sputum [117], in which it can enhance the activity of leucocyte elastase towards elastin, and is able to cleave fibronectin [175], thus favouring *Pseudomonas aeruginosa* colonization of the upper respiratory tract.

Very little information is available about neutrophil collagenase in bronchial secretions. Presence of neutrophil collagenase, a metallo-protease which is a component of the specific granules [176], was noticed in purulent sputum [177]. It has been considered as an important destructive factor in lung pathologies in which a high turnover of collagen was involved [178].

Cathepsin B, which is a thiol-protease, can also degrade *in vitro* native collagen [179]. Its presence has been reported in bronchial secretions and bronchoalveolar lavage fluids obtained from patients with chronic obstructive lung diseases [180], bronchiectasis [165] or lung damage associated with cigarette smoking [181]. The enzyme purified from purulent sputum [182] is slightly different from that of liver. It could originate from bronchial epithelium and serous cells [183], or alveolar macrophages.

Although it does not share proteolytic properties, myeloperoxidase can be classified among the destructive lung enzymes: it is now well known that protein and

tissue damage in the lung may occur by an oxidative attack caused by the very effective system composed of myeloperoxidase, halide and hydrogen peroxide [184]. Myeloperoxidase is synthesized by promyelocytes and stored in azurophil granules of the neutrophils (localization of the gene at locus 17q11). Its presence in bronchoalveolar lavage fluids was considered as being a marker of local neutrophil activity [185] and an additional factor in the development of emphysema in smokers [105, 186]. In sputum samples from patients with cystic fibrosis, high myeloperoxidase amounts were correlated with high amounts of neutrophil elastase [117].

In addition to neutrophil proteases, bacterial proteases could also be involved in proteolytic lung injury. It is well known that *Pseudomonas aeruginosa* is the major pathogen (with *Staphylococcus aureus*) associated with the bronchial infections of cystic fibrosis patients. Therefore, in their bronchial secretions, *Pseudomonas aeruginosa* elastase has been considered as the main deleterious enzyme. *Pseudomonas aeruginosa* elastase (PsE) was first prepared and studied by MORIHARA [187] in 1965. It has a typical metallo-enzyme inhibition profile with one Zn atom per molecule. The gene has now been studied [188, 189]. *In vitro* PsE is able to degrade lung elastin [190], basement membranes [191], complement C<sub>3</sub> [192], and some components of bronchial secretions: immunoglobulins [193] and lysozyme [194]. Some controversy still exists about the degradation of leucocyte elastase inhibitors:  $\alpha_1$ -proteinase inhibitor ( $\alpha_1$ PI) is effectively cleaved at a single peptide bond located just before the active site [195], but recent studies have demonstrated that this phenomenon does not occur in the presence of leucocyte elastase [196], which is largely represented in cystic fibrosis sputum samples. Nor is the bronchial mucous inhibitor inactivated in the presence of leucocyte elastase [197], in contrast to some previous findings [198]. Moreover, PsE would represent only 2% of sputum elastolytic activity [197]. However, the occurrence of this enzyme in cystic fibrosis bronchial secretions is not controversial [199]; in fact PsE induces production of antibodies present in bronchial secretions, responsible for a neutralization of the enzyme. In bronchoalveolar fluids, an excess of antigenic PsE compared to elastolytic activity was demonstrated [200].

A thiol-protease from *Staphylococcus aureus* was shown to be elastolytic *in vitro* [201]; its presence has not been reported in bronchial secretions, but this enzyme could be a candidate for lung injury in cystic fibrosis patients infected by *Staphylococcus aureus*. Another very specific protease might be involved in a lowering of lung defences: it is immunoglobulin A<sub>1</sub> protease which cleaves the IgA molecule into Fab and Fc fragments. These IgA proteases originate from several bacterial species: among them *Haemophilus influenzae* and *Streptococcus pneumoniae*, which are often detected in bronchial infections. The exact cleavage site is dependent on the bacterial strain, but always follows one of the proline residues in the hinge region. The gene of the protease from *Haemophilus*

*influenzae* has been cloned [202]. Such enzymes may promote perturbations in mucosal immune defence mechanisms [203].

**Antiproteases.** Inasmuch as neutrophil elastase is the major aggressive enzyme in bronchial secretions, the protective role of neutrophil elastase inhibitors against proteolytic lung injury has been widely explored.

These studies were initiated by the observation of the link between  $\alpha_1$ PI deficiency and emphysema by LAURELL and ERIKSSON [204]. Human airway secretions obtained either by cough (sputum samples) or by lavage (bronchial and bronchoalveolar washings) were analysed to define the presence of inhibitors and their properties [162].

Isolation methods, properties [205] and genetic data [206] concerning  $\alpha_1$ PI have been reviewed. Moreover, important data have recently been obtained concerning the expression of  $\alpha_1$ PI gene in alveolar macrophages [207–209]. Therefore,  $\alpha_1$ PI in bronchial secretions may originate from the blood by passive diffusion and from alveolar macrophages by active secretion (at least in alveolar lining fluid). Its elimination is mediated by the serpin-enzyme complex (SEC) receptor localized on the surface of human hepatoma cells and mononuclear phagocytes [210]. According to kinetic constants,  $\alpha_1$ PI is a very rapid and, therefore, effective inhibitor of neutrophil elastase [211]. The inhibition process of this enzyme leads to the formation of an equimolar complex, of which the size, isoelectric point and antigenicity are different from those of both elastase and  $\alpha_1$ PI. In addition to this complex, which showed the presence of elastase in the lung before the moment that airway secretions had been collected, one can find the so-called  $\alpha_1$ PI\* which represents a proteolysed form of  $\alpha_1$ PI, due to either degradation of the complex or proteolytic cleavage by proteases non-inhibited by  $\alpha_1$ PI or proteolytic cleavage of oxidated  $\alpha_1$ PI (for example by myeloperoxidase) [212, 213].

In bronchial secretions, many studies have focused on the relative importance of these three forms (native, complexed, proteolysed) because neither the complex nor  $\alpha_1$ PI\* are able to inhibit some newly released neutrophil elastase or cathepsin G. This was generally achieved by electrophoretic methods followed by an immunological reaction [214–217], but the results are strictly dependent on the greater or lesser specificity of the antibodies for the different  $\alpha_1$ PI forms [218, 219].

Presence of the complex  $\alpha_1$ PI-elastase in bronchial secretions may constitute evidence of the protective role of  $\alpha_1$ PI. In bronchoalveolar lavage fluids, it appears to be a marker of the severity of emphysema in non- $\alpha_1$ PI-deficient subjects [220]. It has been shown that  $\alpha_1$ PI displays a chemotactic activity [221], and the newly recruited neutrophils contribute to the continuous delivery of elastase in the lungs.

Alpha<sub>1</sub>-proteinase inhibitor is not the only inhibitor involved in the defence of the bronchial tree against leucocyte elastase damage. It is now well known that mucus proteinase inhibitor (MPI) is present in the bronchial tree, its physiological role as a serine

proteinase inhibitor being to protect the larger airways [222]. MPI is the common name given to the low-molecular mass (12 kDa) inhibitors present in several mucous secretions [223], which are encoded by a single gene [224]. This molecule is synthesized in the respiratory tract, mainly in the serous cells of the submucosal glands of the bronchial epithelium, together with lactoferrin [76] and lysozyme [82]. MPI consists of 107 amino acid residues [225, 226], which are organized into two domains. Recently, it was demonstrated that the proteinase inhibitory activities are only located in the C-terminal domain [227–229]. This molecule has inhibitory properties against different serine proteinases including leucocyte elastase and cathepsin G. However, its target enzyme is still unknown. This inhibitory activity is suppressed by oxidants from cigarette smoke or phagocyte-derived oxidants [230]. In bronchial secretions, it is partly bound to mucins [231]. Recent studies have demonstrated that  $\alpha_1$ PI and MPI act differently on elastin-bound elastase [232, 233]: MPI is able to efficiently inhibit *in situ* elastin-bound elastase, while  $\alpha_1$ PI dissociates elastase from elastin for a further inhibition. Methods for the determination of the concentration of immunoreactive MPI in bronchial secretions have been described [234, 235]: they both give results for total MPI (free and complexed with enzymes). It seems that: 1) MPI concentration is higher in bronchial secretions than in bronchoalveolar lavage fluids; and 2) in the upper respiratory tract, the molar ratio MPI/ $\alpha_1$ PI is always in favour of MPI, contrary to the situation observed in the lower respiratory tract of all subjects, except in  $\alpha_1$ PI deficient subjects [236]. A genetic defect in MPI has not so far been described. On the other hand [237], it was demonstrated that  $\alpha_1$ PI gene expression in human alveolar macrophages is directly regulated by the presence of elastase. Therefore, the question is: has all released elastase to be inhibited in the bronchial tree?

Alpha<sub>1</sub>-antichymotrypsin is a member of the serpin superfamily; its sequence has been established comparative as with that of  $\alpha_1$ PI [238]. It is a potent inhibitor of all chymotrypsin-like enzymes, including leucocyte cathepsin G [239]. Its presence in bronchial secretions was reported by different groups [240–242]. It enters the lung by passive diffusion, but can be synthesized and secreted by alveolar macrophages [243]. The role of  $\alpha_1$ -antichymotrypsin in lung secretions should be the inhibition of leucocyte cathepsin G, but  $\alpha_1$ -antichymotrypsin in lung secretions was shown not to be an effective inhibitor of cathepsin G [244]. Recent studies may cast new lights on possible roles of  $\alpha_1$ -antichymotrypsin in lungs: 1)  $\alpha_1$ -antichymotrypsin-cathepsin G complexes have been shown to stimulate the synthesis of interleukin-6 by fibroblasts in culture [245]; 2)  $\alpha_1$ -antichymotrypsin inhibits neutrophil superoxide anion generation [246], this property being independent of the protease inhibitory activity; however, it has no significant effect on degranulation.

In plasma,  $\alpha_2$ -macroglobulin and  $\alpha_1$ -proteinase inhibitor are the major inhibitors of leucocyte elastase. Alpha<sub>2</sub>-macroglobulin is a glycoprotein of 728 kDa and,

therefore, its movements from plasma to lung interstitium are somewhat restricted by its size. However, alveolar macrophages are able to secrete  $\alpha_2$ -macroglobulin [247]. The presence of immunoreactive  $\alpha_2$ -macroglobulin has been noted in sputum samples from chronic bronchitis [241, 248] and cystic fibrosis patients [117], although its contribution to the anti-elastase defence is very low [249]. In adult respiratory distress syndrome,  $\alpha_2$ -macroglobulin in bronchoalveolar fluids is complexed with elastase [250] and may be involved in the inhibition of metalloproteinases such as collagenase or *Pseudomonas aeruginosa* elastase.

A low-molecular weight inhibitor called BSI-E I, isolated from bronchial mucus, was shown to be specific for porcine pancreatic and leucocyte elastases [251]. In bronchial secretions from healthy subjects, it is present in an active form, and mainly as a complex with elastase in sputum from chronic bronchitis patients [252].

Two other elastase inhibitors have been isolated from bronchial secretions [253, 254]. Their origin and functions are as yet unknown. Cystatin C and cystatin S (from saliva) appeared as constituents of bronchial secretions [255, 256] without any knowledge about their effective inhibitory activity. Tissue inhibitor of metalloproteinases (TIMP) has been identified in sputum and bronchoalveolar lavage fluid [257]; it is assumed that it forms the major defence against metalloproteinases, but *in vitro* studies have demonstrated the preferential binding of collagenase to  $\alpha_2$ -macroglobulin in the presence of TIMP [258].

It is clear that, in healthy nonsmoking subjects, the antiprotease screen is largely sufficient to prevent proteolysis of the lung matrix. In contrast, it is very evident that the emphysematous ongoing process in  $\alpha_1$ PI-deficient subjects can be stopped (or slowed down) by locally increasing the anti-elastase screen. In contrast, in patients suffering from bronchiectasis, cystic fibrosis or adult respiratory distress syndrome, active (hence, uncontrolled) proteases are present, without giving rise to the development of emphysematous lesions. Therefore, except in emphysema of  $\alpha_1$ PI-deficient subjects, a pathological role for proteases as destructive enzymes in human lung diseases is so far not proven. Hence, have antiproteases a role to play in the defence of the lung? Are they essential by their other properties, only some of them being known? Are proteases only implicated by their non-proteolytic properties (for example inducing cell metaplasia and gland secretion). To our current knowledge, these questions remain unanswered.

### Conclusion and future directions

Respiratory mucus actively participates in the airway epithelial protection. In physiological situations, the biochemical components taking place in the antibacterial and antioxidant protection as well as in the antiprotease screen appear to be efficient in preventing infection, inflammation, hypersecretion and, in some cases, the proteolysis of lung matrix occurring in human



respiratory diseases. In physiopathological conditions, following epithelium injury, biochemical modifications of the airway secretions may induce a dysregulation in the natural protective function of the airway mucosa. The cellular mechanisms inducing changes in the intrinsic programme of synthesis and regulation of secretory products are not completely elucidated. Future studies are needed to gain better insights into the understanding of the molecular and cellular processes which shift the normal secretion pattern to abnormal airway secretions. In order to study these processes (i.e. synthesis and regulation of secretory products from individual airway cell types, control of airway cell growth and differentiation, intracellular mechanisms regulating airway secretion, specific gene expression, ...), systems for culturing airway epithelial cells from human and animal sources have been developed. *In vitro* cell culture could represent an interesting approach to the understanding of the specific cellular events that control mucus secretion in health and diseases.

#### References

1. Reid L. - Measurement of the bronchial mucous gland layer: a diagnostic yardstick in chronic bronchitis. *Thorax*, 1960; 15: 132-141.
2. Basbaum CB, Finkbeiner WE. - Mucus-producing cells of the airways. In: *Lung Cell Biology*. D. Massaro ed., Dekker, 1989; pp. 37-79.
3. Nathanson I, Nadel JA. - Movement of electrolytes and fluid across airways. *Lung*, 1984; 162: 125-137.
4. Rechkemner GR. - The molecular biology of chloride secretion in epithelia. *Am Rev Respir Dis*, 1988; 138: S7-S9.
5. Hunter JA, Finkbeiner WE, Nadel JA, Goetzi EJ, Holtzman MJ. - Predominant generation of 15-lipoxygenase metabolites of arachidonic acid by epithelial cells from human trachea. *Proc Natl Acad Sci*, 1985; 82: 4633-4637.
6. Churchill L, Chilton FH, Resau JH, Bascom R, Hubbard WC, Proud D. - Cyclooxygenase metabolism of endogenous arachidonic acid by cultured human tracheal epithelial cells. *Am Rev Respir Dis*, 1989; 140: 449-459.
7. Salari H, Chan-Yeung M. - Release of 15-hydroxyeicosatetraenoic acid (15-HETE) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) by cultured human bronchial epithelial cells. *Am J Respir Cell Mol Biol*, 1989; 1: 245-250.
8. Kumlin M, Hamberg M, Granstrom E, Bjorck T, Dahlen B, Matsuda H, Zetterstrom D, Dahlen SE. - 15 (S)-hydroxyeicosatetraenoic acid is the major arachidonic acid metabolite in human bronchi. Association with airway epithelium. *Arch Biochem Biophys*, 1990; 282: 254-262.
9. Adler KB, Holden-Stauffer WJ, Repine JE. - Oxygen metabolites stimulate release of high-molecular-weight glycoconjugates by cell and organ cultures of rodent respiratory epithelium via an arachidonic acid-dependent mechanism. *J Clin Invest*, 1990; 85: 75-85.
10. Marom Z, Shelhamer JH, Kaliner M. - Effects of arachidonic acid, monohydroxyeicosatetraenoic acid and prostaglandins on the release of mucous glycoproteins from human airways *in vitro*. *J Clin Invest*, 1981; 67: 1695-1702.
11. Hahn HI, Purnama I, Lang M, Sannwald U. - Effects of platelet activating factor on tracheal mucus secretion. *Eur J Respir Dis*, 1986; 69 (Suppl. 146): 277-284.
12. Holtzman MJ. - Arachidonic acid metabolism. Implications of biological chemistry for lung function and disease. *Am Rev Respir Dis*, 1991; 143: 188-203.
13. Reid LM. - Pathology of chronic bronchitis. *Lancet*, 1954; 1: 275-278.
14. Robinson NP, Kyle H, Webber SE, Widdicombe JG. - Electrolyte and other chemical concentrations in the tracheal airway and mucus. *J Appl Physiol*, 1989; 66: 2129-2135.
15. Frizzel RA. - Role of absorptive and secretory processes in hydration of the airway surface. *Am Rev Respir Dis*, 1988; 138: S3-S6.
16. Potter JL, Matthews LM, Spector S, Lenn J. - Studies on pulmonary secretions. II. Osmolarity and the ionic environment of pulmonary secretions from patients with cystic fibrosis, bronchiectasis and laryngectomy. *Am Rev Respir Dis*, 1967; 96: 83-87.
17. Boucher RC, Stutts MJ, Knowles MR, Cantley L, Gatzky JT. - Na<sup>+</sup> transport in cystic fibrosis respiratory epithelia. Abnormal basal rate and response to adenylate cyclase activation. *J Clin Invest*, 1986; 78: 1245-1252.
18. Li MJ, McCann JD, Welsh MJ. - Apical membrane Cl<sup>-</sup> channels in airway epithelial: anion selectivity and effect of an inhibitor. *Am J Physiol*, 1990; 259: C295-C301.
19. Li MJ, McCann JD, Liedtke CM, Nairn AC, Greengard P, Welsh MJ. - cAMP-dependent protein kinase opens chloride channels in normal but not cystic fibrosis airway epithelium. *Nature*, 1988; 331: 358-360.
20. Boucher RC, Cheng EHC, Paradiso AM, Stutts JM, Knowles MR, Earp HS. - Chloride secretory response of cystic fibrosis human airway epithelia: preservation of calcium but non-protein kinase C and A dependent mechanisms. *J Clin Invest*, 1989; 84: 1424-1431.
21. Smith JJ, McCann JD, Welsh MJ. - Bradykinin stimulates airway epithelial Cl<sup>-</sup> secretion via two second messenger pathways. *Am J Physiol*, 1990; 258: L369-L377.
22. Mason SJ, Paradiso AM, Brown HA, Harden TK, Boucher RC. - Extracellular adenosine triphosphate (ATP) induces calcium release, inositol phosphate (IP) production and chloride secretion by cystic fibrosis and normal human airway epithelium. *Pediatr Pulmonol*, 1990; (Suppl. 5): (Abstract 125).
23. Anderson MP, Welsh MJ. - Fatty acids inhibit apical membrane chloride channels in airway epithelia. *Proc Natl Acad Sci*, 1990; 87: 7334-7338.
24. Tam PY, Verdugo P. - Control of mucus hydration as a Donnan equilibrium process. *Nature*, 1981; 292: 340-342.
25. Aitken ML, Verdugo P. - Donnan mechanism of mucin release and conditioning in goblet cells: the role of polyions. In: *Mucus and Related Topics*. E. Chantler, N.A. Ratcliffe eds, Company of Biologists, 1989; pp. 1-8.
26. Verdugo P. - Goblet cells secretion and mucogenesis. *Ann Rev Physiol*, 1990, 52, 157-176.
27. Widdicombe JG. - Airway mucus. *Eur Respir J*, 1989; 2: 107-115.
28. Deffebach ME, Islami H, Price A, Webber SE, Widdicombe JG. - Prostaglandins alter methacholine induced secretion in ferret *in vitro* trachea. *Am J Physiol*, 1990; 258: L75-80.
29. Tom-Moy M, Basbaum CB, Nadel JA. - Localization and release of lysozyme from ferret trachea: effects of adrenergic and cholinergic drugs. *Cell Tissue Res*, 1983; 228: 549-562.
30. Lopez-Vidriero MT, Das I, Reid LM. - Airway secretion: source, biochemical and rheological properties. In: *Respiratory Defense Mechanisms*. J.D. Bain, D.F. Practor, L. Reid eds, Dekker, New York, 1977; pp. 289-356.

31. Johnson LG, Cheng PW, Boucher PC. - Albumin absorption by canine bronchial epithelium. *J Appl Physiol*, 1986; 66: 2772-2777.
32. Jacquot J, Goldstein G, Sommerhoff C, Benali R, Puchelle E, Basbaum CB. - Synthesis and secretion of an albumin-like protein by cultured bovine tracheal gland serous cells. *Biochem Biophys Res Commun*, 1988; 155: 857-862.
33. Jacquot J, Dupuit F, Benali R, Spilmont C, Puchelle E. - Modulation of albumin-like protein and lysozyme production by bovine tracheal gland serous cells. *FEBS Lett*, 1990; 274: 131-135.
34. List SJ, Findla BP, Forstner GG, Forstner JF. - Enhancement of the viscosity of mucin by serum albumin. *Biochem J*, 1978; 175: 565-571.
35. Halliwell B. - Albumin: an important extracellular antioxidant? *Biochem Pharmacol*, 1988; 37: 569-571.
36. Slayter HS, Lamblin G, Le Trent A, Galabert C, Houdret N, Degand P, Roussel P. - Complex structure of human bronchial mucus glycoprotein. *Eur J Biochem*, 1984; 142: 209-218.
37. Potter JL, Matthews LW, Lemm J, Spector S. - Human pulmonary secretion in health and disease. *Ann NY Acad Sci*, 1963; 106: 692-697.
38. Warembourg H, Havez R, Sezille, G, Scherperell P, Roussel P, Degand P. - Les lipides de l'expectoration. Isolement et caractérisation du surfactant pulmonaire dans l'expectoration. In: *Hypersécrétion bronchique. Colloque International de Pathologie Thoracique*. Lille Poinot eds Clichy, 1968; pp. 181-192.
39. Lewis RW. - Lipid composition of human bronchial mucus. *Lipids*, 1971; 6: 859-861.
40. Sahu S, Lynn WS. - Lipid composition of airway secretions from patients with asthma and patients with cystic fibrosis. *Am Rev Respir Dis*, 1977; 115: 233-238.
41. Slomiany A, Murty VLN, Aono M, Snyder CE, Herp A, Slomiany BL. - Lipid composition of tracheobronchial secretions from normal individuals and patients with cystic fibrosis. *Biochim Biophys Acta*, 1982; 710: 106-111.
42. Galabert C, Filliat C, Lamblin G. - Lipid analysis of sputum from patients with chronic bronchial diseases. *Bull Eur Physiopathol Respir*, 1981; 17: 197-208.
43. Widdicombe JG. - Role of lipids in airway function. *Eur J Respir Dis*, 1987; 71 (Suppl. 153): 197-204.
44. Lhermitte M, Lamblin G, Degand P, Roussel P, Mazzuca M. - Affinity of bronchial secretion glycoprotein and cells of human bronchial mucosa for *Ricinus communis* lectins. *Biochimie*, 1977; 59: 611-620.
45. Bhaskar KR, Reid LM. - Application of density gradient methods for the study of mucus glycoprotein and other macromolecular components of the sol and gel phases of asthmatic sputa. *J Biol Chem*, 1981; 256: 7583-7589.
46. Woodward H, Morsey B, Bhavamandan VP, Davidson E. - Isolation, purification and properties of respiratory mucus glycoproteins. *Biochemistry*, 1982; 21: 694-701.
47. Slayter HS, Lamblin G, Le Trent A, Galabert C, Houdret N, Degand P, Roussel P. - Complex structure of human bronchial mucus glycoprotein. *Eur J Biochem*, 1984; 142: 209-218.
48. Houdret N, Péroni JM, Galabert C, Scharfman A, Humbert P, Lamblin G, Roussel P. - The high lipid content of respiratory mucins in cystic fibrosis is related to infection. *Biochim Biophys Acta*, 1986; 880: 54-61.
49. Bhaskar KR, O'Sullivan DD, Opaskar-Hincman H, Reid LM. - Lipids in airway secretions. *Eur J Respir Dis*, 1987; 71 (Suppl. 153): 215-221.
50. Clements JA, Oyarzun MJ, Baritussio A. - Secretion and clearance of lung surfactant: a brief review. In: *Progress in Respiratory Research*, Vol. 15. P Von Wichert ed., Karger, Basel, 1981; pp. 20-26.
51. Fisher HK, Hyman MH, Ashcraft SJ. - Alveolar surfactant phospholipids are not cleared via trachea. *Fed Proc*, 1979; 38: 1373.
52. Coles SJ, Bhaskar KR, O'Sullivan DD, Neill KH, Reid LM. - Airway mucus composition and regulation of its secretions by neuropeptides *in vitro*. In: *Mucus and Mucosa*. J. Nugent, M. O'Connor eds, Pitman, London, 1984; pp. 40-60.
53. Jozwiak Z, Snyder CE, Murty VLN, Slomiany A, Slomiany B, Herp A. - Lipid composition of the secretion from human bronchial explant culture. *Biochim Biophys Acta*, 1984; 802: 282-286.
54. Bhaskar KR, O'Sullivan DD, Opaskar-Hincman H, Reid LM, Coles SJ. - Density gradient analysis of secretions produced *in vitro* by human and canine airway mucosa: identification of lipids and proteoglycans in such secretions. *Exp Lung Res*, 1986; 10: 401-422.
55. Bhaskar KR, O'Sullivan DD, Lopez-Vidriero MT, Reid LM. - Characterisation of sol and gel phase of infected and mucoid sputum samples from a chronic bronchitic patient. In: *Mucus in Health and Disease*. II. E.N. Chantler, J.B. Elder, M. Elstein eds, Plenum Press, New York and London, 1982; pp. 361-364.
56. Mautone AJ, Scarpelli EM. - Phospholipid synthesis and secretion by isolated airway epithelium. *Fed Proc*, 1987; 46: 813: 2895 (Abstract).
57. Kim KC, Opaskar-Hincman H, Bhaskar KR. - Secretion from primary hamster tracheal surface epithelial cells in culture: mucin-like glycoproteins, proteoglycans and lipids. *Exp Lung Res*, 1989; 15: 299-314.
58. Kim KC, Singh BN. - Association of lipids with mucins take place prior to secretion: studies with primary hamster epithelial cells in culture. *Biorheology*, 1990; 27: 491-501.
59. Kim KC, Singh BN. - Hydrophobicity of mucin-like glycoproteins secreted by cultured tracheal epithelial cells: association with lipids. *Exp Lung Res*, 1990; 16: 279-292.
60. Girod S, Fuchey C, Galabert C, Lebonvallet S, Bonnet N, Ploton D, Puchelle E. - Identification of phospholipids in secretory granules of human submucosal gland respiratory cells. *J Histochem Cytochem*, 1991; 39: 193-198.
61. Barrow RE. - Chemical structure of phospholipids in the lungs and airways of sheep. *Respir Physiol*, 1990; 79: 1-8.
62. Kao YJ, Lichtenberger LM. - Localization of phospholipid-rich zones in rat gastric mucosa - possible origin of a protective hydrophobic luminal lining. *J Histochem Cytochem*, 1987; 11: 1285-1298.
63. Lichtenberger LM, Graziani LA, Dial EJ, Butter BD, Hillks BA. - Role of surface active phospholipids in gastric cytoprotection. *Science*, 1983; 219: 1327-1329.
64. Sarosiek J, Slomiany A, Takagi A, Slomiany BL. - Hydrogen ion diffusion in dog gastric mucus glycoprotein: effect of associated lipids and covalently bound fatty acids. *Biochem Biophys Res Commun*, 1984; 118: 523-531.
65. Slomiany BL, Piasek A, Sarosiek J, Slomiany A. - The role of surface and intracellular mucus in gastric mucosal protection against hydrogen ions. *Scand J Gastroenterol*, 1985; 20: 1191-1196.
66. Bienenstock J. - Mucosal immunological protection mechanisms in the airways. *Eur J Respir Dis*, 1986; 69 (Suppl. 147): 62-71.
67. Bell DY, Haseman JA, Spock A, McLennan G, Hoo GER. - Plasma proteins of the bronchoalveolar surface of the lung; smokers and nonsmokers. *Am Rev Respir Dis*, 1981; 124: 72-79.

68. Tomasi TB, Tan EM, Solomon A, Prendergast RA. - Characteristics of an immune system commune to certain external secretions. *J Exp Med*, 1965; 121: 101-124.
69. Breitfeld PP, Harris JM, Mostov KE. - Post-endocytotic sorting of the ligand for the polymeric immunoglobulin receptor in Madin-Darby canine kidney cell. *J Cell Biol*, 1989; 109: 475-486.
70. Daniele RP. - Immunoglobulin secretion in the airways. *Ann Rev Physiol*, 1990; 52: 177-195.
71. Kerr A. - The structure and function of human IgA. *Biochem J*, 1990; 271: 285-296.
72. Breitfeld PP, Casanova JE, Simuster NE, Ross SA, McKinnon WC, Mostov KE. - Transepithelial transport of immunoglobulin: a model of protein sorting and transcytosis. *Am Respir Cell Mol Biol*, 1989; 1: 257-262.
73. Goodman MR, Link DW, Brown WR, Nakane PK. - Ultrastructural evidence of transport of secretory IgA across bronchial epithelium. *Am Rev Respir Dis*, 1981; 123: 115-119.
74. Jacquot J, Tournier JM, Carmona TG, Puchelle E, Chazalotte JP, Sadoul P. - Protéines des sécrétions bronchiques dans la mucoviscidose. Rôle de l'infection. *Bull Eur Physiopathol Respir*, 1983; 19: 453-458.
75. Masson PL, Heremans SF, Shonn E. - Lactoferrin, an iron-binding protein in neutrophil leukocytes. *J Exp Med*, 1969; 130: 643-658.
76. Bowes D, Clark AE, Corrin B. - Ultrastructural localization of lactoferrin and glycoprotein in human bronchial glands. *Thorax*, 1981; 36: 108-115.
77. Spik G, Montreuil J. - Rôle de la lactotransferrine dans les mécanismes moléculaires de la défense antibactérienne. *Bull Eur Physiopathol Respir*, 1983; 19: 123-130.
78. Delforge A, Stryckmans P, Prieels JP, Bieva C, Ronge-Collard E, Schlussegger J, Eflra A. - Lactoferrin: its role as a regulator of human granulopoiesis? *Ann NY Acad Sci*, 1985; 485: 85-96.
79. Roiron-Lagroux D, Figarella C. - Evidence for a different mechanism of lactoferrin and transferrin translocation on HT 29-D4 cells. *Biochem Biophys Res Commun*, 1990; 170: 837-842.
80. Fleming A. - On a remarkable bacteriolytic element found in tissues and secretions. *Proc Roy Soc Lond, B Biol Sci*, 1922; 93: 306-317.
81. Hinrasky J, Chevillard M, Puchelle E. - Immunocytochemical demonstration of quantitative differences in the distribution of lysozyme in human airway secretory granule phenotypes. *Biol Cell*, 1990; 68: 239-243.
82. Konstan MW, Chen PW, Sherman JM, Thomassen MJ, Wood RE, Boat TF. - Human lung lysozyme: sources and properties. *Am Rev Respir Dis*, 1981; 123: 120-124.
83. Jacquot J, Benali R, Zahm JM, Puchelle E. - Identification of different molecular forms of human airway lysozyme. *Anal Biochem*, 1987; 160: 227-232.
84. Glick AD, Rautrand AM, Cole RM. - Degradation of group A streptococcal cell walls by egg-white lysozyme and human lysosomal enzymes. *Infect Immun*, 1972; 6: 403-413.
85. Pollock JJ, Shoda J, McNamara TF, Cho MI, Campbell A, Iacono VJ. - *In vitro* and *in vivo* studies of cellular lysis of oral bacteria by a lysozyme - protease - inorganic monovalent anion anti-bacterial system. *Infect Immun*, 1984; 45: 610-617.
86. Jacquot J, Puchelle E, Zahm JM, Beck E, Plotkowski MC. - Effect of human airway lysozyme on the *in vitro* growth of type I *Streptococcus pneumoniae*. *Eur J Respir Dis*, 1987; 71: 295-305.
87. Puchelle E, Jacquot J, Zahm JM. - *In vitro* restructuring effect of human airway immunoglobulins A and lysozyme on airway secretions. *Eur J Respir Dis*, 1987; 71: 117-122.
88. Gordon LI, Douglas SD, Kay NE, Yamada O, Osseman EF, Jacob HS. - Modulation of neutrophil function by lysozyme. Potential negative feedback system of inflammation. *J Clin Invest*, 1979; 64: 226-232.
89. Prior C, Barbee RA, Evans PM, Townsend PJ, Primett ZS, Fylviquist F, Gronhagen-Riska C, Haslam PL. - Lavage versus serum measurements of lysozyme, angiotensin-converting enzyme and other inflammatory markers in pulmonary sarcoidosis. *Eur Respir J*, 1990; 3: 1146-1154.
90. Bhaskar KR, Brown R, O'Sullivan DD, Melia S, Guggan M, Reid L. - Bronchial mucus hypersecretion in acute quadriplegia. *Am Rev Respir Dis*, 1991; 71: 117-122.
91. Coles SJ, Reid L. - Glycoprotein secretion *in vitro* by human airway: normal and chronic bronchitis. *Exp Mol Pathol*, 1978; 29: 326-341.
92. Coles SJ, Said SI, Reid LM. - Inhibition by vasoactive intestinal peptide of glycoconjugate and lysozyme secretion by human airways *in vitro*. *Am Rev Respir Dis*, 1981; 124: 531-539.
93. Barnes PJ. - Airway neuropeptides. In: Asthma: basic mechanisms and clinical management. P.J. Barnes, I.W. Rodger, N.C. Thomson eds, Academic Press, 1988; pp. 395-413.
94. Ollerenshaw S, Jarvis DL, Woolcock AJ, Sullivan CE, Scheibner T. - Absence of immunoreactive vasoactive intestinal polypeptide from the lungs of patients with asthma. *N Engl J Med*, 1989; 320: 1244-1248.
95. Baraniuk JN, Lundgreen JD, Okayama M, Mullen J, Merida M, Shelhamer JH, Kaliner MA. - Vasoactive intestinal peptide in human nasal mucosa. *J Clin Invest*, 1990; 86: 825-831.
96. Krivan HC, Ginsburg V, Roberts DD. - Many pulmonary pathogenic bacteria bind specifically to the carbohydrate sequence GalNAc $\beta$ 1-4Gal found in some glycolipids. *Proc Natl Acad Sci USA*, 1988; 85: 6157-6161.
97. Krivan HC, Ginsburg V, Roberts DD. - *Pseudomonas aeruginosa* and *Pseudomonas cepacia* isolated from cystic fibrosis patients bind specifically to gangliotetraosylceramide (asialo GM1) and gangliotriaosylceramide (asialo GM2). *Arch Biochem Biophys*, 1988; 260: 493-496.
98. Ramphal R, Pyle M. - Further characterization of the tracheal receptor for *Pseudomonas aeruginosa*. *Eur J Clin Microbiol*, 1985; 4: 160-162.
99. Thompson LK, Horowitz PM, Bentley KL, Thomas D, Alderete JF, Klebe RJ. - Localization of the ganglioside-binding site of fibronectin. *J Biol Chem*, 1986; 261: 5209-5214.
100. Coonrod JD. - Role of surfactant free fatty acids in antimicrobial defences. *Eur J Respir Dis*, 1987; 71 (Suppl. 153): 209-214.
101. Odeberg H, Olsson I. - Antibacterial activity of cationic proteins from human granulocytes. *J Clin Invest*, 1975; 56: 1118-1124.
102. Lehrer RI, Ganz TT. - Antimicrobial polypeptides of human neutrophils. *Blood*, 1990; 76: 2169-2181.
103. Spitznagel JK. - Antibiotic proteins of human neutrophils. *J Clin Invest*, 1990; 86: 1381-1386.
104. Bangalore N, Travis J, Onunka VS, Pohl J, Shafer WM. - Identification of the primary antimicrobial domains in human neutrophil cathepsin G. *J Biol Chem*, 1990; 265: 13854-13858.
105. Travis J. - Oxidants and antioxidants in the lung. *Am Rev Respir Dis*, 1987; 135: 773.
106. White CW, Repine JE. - Pulmonary antioxidant defense mechanisms. *Exp Lung Res*, 1985; 8: 81-96.

107. Cantin AM, North SL, Fells GA, Hubbard RC, Crystal RG. - Oxidant-mediated epithelial cell injury in idiopathic pulmonary fibrosis. *J Clin Invest*, 1987; 79: 1665-1673.
108. Cordier JF. - Oxidant-antioxidant balance. *Bull Eur Physiopathol Respir*, 1987; 23: 273-274.
109. Voisin C, Aerts C, Wallaert B. - Prevention of *in vitro* oxidant-mediated alveolar macrophage injury by cellular glutathione and precursors. *Bull Eur Physiopathol Respir*, 1987; 23: 309-313.
110. Schraufstatter IU, Hyslop PA, Jackson J, Revak SD, Cochrane CC. - Oxidant and protease injury of the lung. *Bull Eur Physiopathol Respir*, 1987; 23: 297-302.
111. Peden DB, Hohman R, Brown ME, Mason RT, Berkebile C, Fales HM, Kaliner MA. - Uric acid is a major antioxidant in human nasal airway secretions. *Proc Natl Acad Sci*, 1990; 87: 7638-7642.
112. McCusker K, Hoidal J. - Selective increase of anti-oxidant enzyme activity in the alveolar macrophages from cigarette smokers and smoke-exposed hamsters. *Am Rev Respir Dis*, 1990; 141: 678-682.
113. Simon RH, De Hart PD, Nadeau DM. - Resistance of rat pulmonary alveolar epithelial cells to neutrophil- and oxidant-induced injury. *Am J Respir Cell Mol Biol*, 1989; 1: 221-229.
114. Cantin AM, Fells GA, Hubbard RC, Crystal RG. - Antioxidant macromolecules in the epithelial lining fluid of the normal human lower respiratory tract. *J Clin Invest*, 1990; 86: 962-971.
115. Tan YH, Tischfield J, Ruddle FH. - The linkage of genes for the human interferon-induced antiviral protein and indophenol oxidase-B traits to chromosome G-21. *J Exp Med*, 1973; 137: 317-330.
116. Ackerman AD, Fackler JC, Tuck-Muller CM, Tarpey MM, Freeman BA, Rogers MC. - Partial monosomy 21, diminished activity of superoxide dismutase and pulmonary oxygen toxicity. *N Engl J Med*, 1988; 318: 1666-1669.
117. Goldstein W, Döring G. - Lysosomal enzymes from polymorphonuclear leukocytes and proteinase inhibitors in patients with cystic fibrosis. *Am Rev Respir Dis*, 1986; 134: 49-56.
118. Cantin AM, North SL, Hubbard RC, Crystal RG. - Normal epithelial lining fluid contains high levels of glutathione. *J Appl Physiol*, 1987; 63: 152-157.
119. Cantin AM, Hubbard RC, Crystal RG. - Glutathione deficiency in the epithelial lining fluid of the lower respiratory tract in idiopathic pulmonary fibrosis. *Am Rev Respir Dis*, 1989; 139: 370-372.
120. Linden M, Hakansson L, Ohlsson K, Sjödin K, Tegner H, Tunek A, Venge P. - Glutathione in bronchoalveolar lavage fluid from smokers is related to humoral markers of inflammation cell activity. *Inflammation*, 1989; 13: 651-658.
121. Lundberg JM, Hokfelt T, Martling CR, Saria A, Cuello S. - Substance P - immunoreactive sensory nerves in the lower respiratory tract of various animals including man. *Cell Tissue Res*, 1984; 235: 251-261.
122. Lundberg JM, Saria M. - Capsaicin induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants. *Nature*, 1983; 302: 251-253.
123. McDonald DM. - Respiratory tract infections increase susceptibility to neurogenic inflammation in the rat trachea. *Am Rev Respir Dis*, 1988; 137: 1432-1440.
124. Gashi AA, Borson DB, Finkbeiner WE, Nadel JA, Basbaum CB. - Neuropeptides degranulate serous cells of ferret tracheal glands. *Am J Physiol*, 1986; 251: C223-C229.
125. Dusser DJ, Umeno E, Graf PD, Djokic TD, Borson DB, Nadel JA. - Airway neutral endopeptidase-like enzyme modulates tachykinin-induced bronchoconstriction *in vivo*. *J Appl Physiol*, 1988; 65: 2585-2591.
126. Johnson AR, Ashton J, Schulz WW, Erdos EG. - Neutral metalloendopeptidase in human lung tissue and cultured cells. *Am Rev Respir Dis*, 1985; 132: 564-568.
127. Stimler-Gerard NP. - Neutral endopeptidase-like enzyme controls the contractile activity of substance P in guinea-pig lung. *J Clin Invest*, 1987; 79: 1819-1825.
128. Iwamoto I, Ueki IF, Borson DB, Nadel JA. - Neutral endopeptidase modulates tachykinin-induced increase in vascular permeability in guinea-pig skin. *Int Arch Allergy Appl Immunol*, 1989; 88: 288-293.
129. Kohrogi H, Nadel JA, Malfroy B, Gorman C, Bridenbaugh R, Patton JS, Borson DB. - Recombinant human enkephalinase (neutral endopeptidase) prevents cough induced by tachykinins in awake guinea-pigs. *J Clin Invest*, 1989; 84: 781-786.
130. Munck A, Mendel DB, Smith LI, Orti E. - Glucocorticoids receptors and actions. *Am Rev Respir Dis*, 1990; 142: S2-S10.
131. Sebaldt RJ, Sheller JR, Oates JA, Roberts II LJ, Fitzgerald GA. - Inhibition of eicosanoid biosynthesis by glucocorticoids in humans. *Proc Natl Acad Sci*, 1990; 87: 6974-6978.
132. Koehler L, Hass R, Dewitt DL, Resh K, Goppelt-Struebe M. - Glucocorticoid-induced reduction of prostanoid synthesis in TPA-differentiated U937 cells is mainly due to a reduced cyclooxygenase activity. *Biochem Pharm*, 1990; 40: 1307-1316.
133. Lundgren, JD, Kaliner MA, Shelhamer JH. - Mechanisms by which glucocorticosteroids inhibit secretion of mucus in asthmatic airways. *Am Rev Respir Dis*, 1990; 141: S52-S58.
134. Peers SH, Flower RJ. - The role of lipocortin in corticosteroid actions. *Am Rev Respir Dis*, 1990; 141: S18-S21.
135. Goulding NJ, Godolphin JL, Sharland PR, Peers SH, Sampson M, Maddison PJ, Flower RJ. - Anti-inflammatory lipocortin 1 production by peripheral blood leucocytes in response to hydrocortisone. *Lancet*, 1990; 335: 1416-1418.
136. Smith SF, Tetley TD, Guz A, Flower RJ. - Detection of lipocortin 1 in human lung lavage fluid: the control of inflammatory mediators and inflammation. *Environ Health Persp*, 1990; 85: 135-144.
137. Jacquot J, Dupuit F, Elbtaouri H, Hinnrasky J, Antonicelli F, Haye B, Puchelle E. - Production of lipocortin-like proteins by cultured human tracheal submucosal gland cells. *FEBS Lett*, 1990; 274: 131-135.
138. Ambrose MP, Hunninghake GW. - Corticosteroids increase lipocortin 1 in alveolar epithelial cells. *Am J Respir Cell Mol Biol*, 1990; 3: 349-353.
139. Flower PJ. - Lipocortin and the mechanism of action of the glucocorticosteroids. *Br J Pharmacol*, 1988; 94: 987-1015.
140. Cirino G, Peers SH, Flower RJ, Browning JL, Pepinsky RB. - Human recombinant lipocortin 1 has acute local anti-inflammatory properties in the rat paw oedema test. *Proc Natl Acad Sci*, 1988; 86: 3428-3432.
141. Errasfa M, Russo-Marie F. - A purified lipocortin share the anti-inflammatory effects of glucocorticoids *in vivo* in mice. *Br J Pharmacol*, 1989; 97: 1051-1081.
142. Maridonneau-Parini I, Errasfa M, Russo-Marie F. - Inhibition of O<sub>2</sub>-generation by dexamethasone is mimicked by lipocortin 1 in alveolar macrophages. *J Clin Invest*, 1989; 83: 1936-1940.

143. Wirthmueller U, L de Weck A, Dahinden CA. - Studies on the mechanism of platelet-activating factor production in GM-CSF primed neutrophils: involvement of protein synthesis and phospholipase A2 activation. *Biochem Biophys Res Commun*, 1990; 170: 556-562.
144. Bronnegard M, Andersson O, Edwall D, Lund J, Norstedt G, Carlstedt-Duke J. - Human calpactin II (lipocortin 1) messenger ribonucleic acid is not induced by glucocorticoids. *Mol Endocrinol*, 1988; 2: 732-739.
145. Bienkowski MJ, Petro MA, Robinson LJ. - Inhibition of thromboxane synthesis in U937 cells by glucocorticoids: lack of evidence for lipocortin 1 as the second messenger. *J Biol Chem*, 1989; 264: 6536-6544.
146. Hullin F, Raynal P, Ragab-Thomas JMF, Fauvel J, Chap H. - Effect of dexamethasone on prostaglandin synthesis and on lipocortin status in human endothelial cells. *J Biol Chem*, 1989; 264: 3506-3513.
147. Ernst JD, Haye E, Blackwood RA, Mok TL. - Identification of a domain that mediates vesicle aggregation reveals functional diversity of annexin repeats. *J Biol Chem*, 1991; 266: 6670-6673.
148. Lieberman J. - Involvement of leukocytic proteases in emphysema and antitrypsin deficiency. *Arch Environ Health*, 1973; 27: 196-200.
149. Tournier JM, Basbaum C. - *In vitro* secretion of gelatin-degrading enzyme by bovine tracheal gland serous cell in culture. *Eur Respir J*, 1990; 3 (Suppl. 10): (Abstract 641): 1915.
150. Collier IE, Wilhem SM, Eisen AZ, Marmer BL, Grant GA, Seltzer JL, Kronberger A, He C, Bauer EA, Goldberg GI. - H-ras oncogen-transformed human bronchial epithelial cells (TBE-1) secrete a single metalloprotease capable of degrading basement membrane collagen. *J Biol Chem*, 1988; 263: 6579-6587.
151. Tretz G, Erdel M, Spiess E, Ebert W. - Detection of cathepsin B, plasminogen activators and plasminogen activator inhibitor in human non-small lung cancer cell lines. *Biol Chem Hoppe-Seyler*, 1990; 371: 617-624.
152. MacNee W, Selby C. - Neutrophil kinetics in the lungs. *Clin Sci*, 1990; 79: 97-107.
153. Hubbard R, McElvaney N, Crystal R. - Amount of neutrophil elastase carried by neutrophils may modulate the extent of emphysema in  $\alpha_1$ -antitrypsin deficiency. *Am Rev Respir Dis*, 1990; 141: A682.
154. Takahashi H, Nukiwa T, Yoshimura K, Quick CD, States DJ, Holmes MD, Whang-Peng J, Knutsen T, Crystal RG. - Structure of the human neutrophil elastase gene. *J Biol Chem*, 1988; 263: 14739-14747.
155. Sinha S, Watorek W, Karr S, Giles J, Bode W, Travis J. - Primary structure of human neutrophil elastase. *Proc Natl Acad Sci USA*, 1987; 84: 2228-2232.
156. Mainardi CL, Hasty DL, Seyer JM, Kang AH. - Specific cleavage of human type III collagen by human polymorphonuclear leukocyte elastase. *J Biol Chem*, 1980; 255: 12006-12010.
157. Gadek JE, Fells GA, Wright DG, Crystal RG. - Human neutrophil elastase functions as a type III collagen collagenase. *Biochem Biophys Res Commun*, 1980; 95: 1815-1822.
158. Pipoly DJ, Crouch EC. - Degradation of native type IV procollagen by human neutrophil elastase. Implications for leukocyte-mediated degradation of basement membrane. *Biochemistry*, 1987; 26: 5748-5754.
159. MacDonald JA, Kelley DG. - Degradation of fibronectin by human leukocyte elastase. Release of biologically active fragments. *J Biol Chem*, 1980; 255: 8848-8858.
160. Watanabe H, Mattori S, Katsuda S, Nakanishi I, Nagai Y. - Human neutrophil elastase: degradation of basement membrane components and immunolocalization in the tissue. *J Biochem*, 1990; 108: 753-759.
161. Starkey PM, Barrett AJ. - Human lysosomal elastase: catalytic and immunological properties. *Biochem J*, 1976; 155: 265-271.
162. Janoff A. - Elastases and emphysema: current assessment of the protease-antiprotease hypothesis. *Am Rev Respir Dis*, 1985; 132: 417-433.
163. Stockley RA, Hill SL, Morrison HM, Starkie CM. - Elastolytic activity of sputum and its relation to purulence and to lung function in patients with bronchiectasis. *Thorax*, 1984; 39: 408-413.
164. Suter S, Schaad UB, Tegner H, Ohlsson K, Desgrandchamps D, Waldvogel FA. - Levels of free granulocyte elastase in bronchial secretions from patients with cystic fibrosis: effect of antimicrobial treatment against *Pseudomonas aeruginosa*. *J Infect Dis*, 1986; 153: 902-909.
165. Buttle DJ, Burnett D, Abrahamson M. - Levels of neutrophil elastase and cathepsin B activities, and cystatins in human sputum: relationship to inflammation. *Scand J Clin Lab Invest*, 1990; 50: 509-516.
166. Bruce MC, Poncz L, Klinger JD, Stern RC, Tomashefski JF, Dearborn DG. - Biochemical and pathological evidence for proteolytic destruction of lung connective tissue in cystic fibrosis. *Am Rev Respir Dis*, 1985; 132: 529-535.
167. Damiano VV, Tsang A, Kucich U, Abrams WR, Rosenbloom J, Kimbel P, Fallahnejad M, Weinbaum G. - Immunolocalization of elastase in emphysematous lungs. *J Clin Invest*, 1986; 78: 482-483.
168. Fox B, Bull TB, Guz A, Harris E, Tetley TD. - Is neutrophil elastase associated with elastic tissue in emphysema? *J Clin Pathol*, 1988; 41: 435-440.
169. Janoff A. - Do neutrophils play a major role in elastin turnover of normal tissues? *Am Rev Respir Dis*, 1983; 127: 782-783.
170. Reilly CF, Travis J. - The degradation of human lung elastin by neutrophil proteinases. *Biochem Biophys Acta*, 1980; 621: 647-657.
171. Boudier C, Holle C, Bieth J. - Stimulation of the elastolytic activity of leukocyte elastase by leukocyte cathepsin G. *J Biol Chem*, 1981; 256: 10256-10258.
172. Reilly CF, Fukunaga Y, Powers JC, Travis J. - Effect of neutrophil cathepsin G on elastin degradation by neutrophil elastase. *Hoppe-Seyler's Z Physiol Chem*, 1984; 365: 1131-1135.
173. Smyrlaki M, Davril M, Hayem A. - Column separation using Bio-Gel P100 for the characterization of the products of human lung elastin degradation by leukocyte elastase and cathepsin G. *Biomed Chrom*, 1986; 1: 27-30.
174. Sommerhoff CP, Nadel JA, Basbaum CB, Caughey GH. - Neutrophil elastase and cathepsin G stimulates secretion from cultured bovine airway gland serous cells. *J Clin Invest*, 1990; 85: 682-689.
175. Suter S, Schaad UB, Morgenthaler JJ, Chevallier I, Schnebli HP. - Fibronectin activity in bronchial secretions of patients with cystic fibrosis. *J Infect Dis*, 1988; 158: 89-100.
176. Murphy G, Reynolds JJ, Bretz U, Baggiolini M. - Collagenase is a component of the specific granules of human neutrophil leukocytes. *Biochem J*, 1977; 162: 195-197.
177. Ohlsson K, Tegner H. - Granulocyte collagenase, elastase and plasma protease inhibitors in purulent sputum. *Eur J Clin Invest*, 1975; 5: 221-227.

178. Gadek JE, Kelman JA, Fells G, Weinberger SE, Horwitz AL, Reynolds HY, Fulmer JD, Crystal RG. - Collagenase in the lower respiratory tract of patients with idiopathic pulmonary fibrosis. *N Engl J Med*, 1979; 301: 737-742.
179. Burleigh MC, Barrett AJ, Lazarus GS. - Cathepsin B1: a lysosomal enzyme that degrades native collagen. *Biochem J*, 1974; 137: 387-398.
180. Burnett D, Crocker J, Stockley RA. - Cathepsin B-like cysteine proteinase activity in sputum and immunohistologic identification of cathepsin B in alveolar macrophages. *Am Rev Respir Dis*, 1983; 128: 915-919.
181. Chang JC, Lesser M, Yoo OH, Orlowski M. - Increased cathepsin B-like activity in alveolar macrophages and bronchoalveolar lavage fluid from smokers. *Am Rev Respir Dis*, 1986; 134: 538-541.
182. Buttle DJ, Bonner BC, Burnett D, Barrett AJ. - A catalytically active high-Mr form of human cathepsin B from sputum. *Biochem J*, 1988; 254: 693-699.
183. Stockley RA, Ward C, Burnett D. - Proteases in the human lung. *Eur Respir J*, 1990; 3 (Suppl. 10): (Abstract 1014).
184. Klebanoff SJ. - Phagocytic cells: products of oxygen metabolism. In: Inflammation: basic principles and clinical correlates. J.I. Gallin, I.M. Goldstein, R. Snyderman eds, Raven Press, New York, 1988; pp. 391-444.
185. Schmekel B, Karlsson SE, Linden M, Sundström C, Tegner H, Venge P. - Myeloperoxidase in human lung lavage. I. A marker of local neutrophil activity. *Inflammation*, 1990; 14: 447-454.
186. Frysmark V. - Myeloperoxidase activity in smokers: an additional factor in the development of pulmonary emphysema. *Eur J Respir Dis*, 1985; 67: 81-83.
187. Morihara K. - *Pseudomonas aeruginosa* elastase. Isolation, crystallization and preliminary characterization. *J Biol Chem*, 1965; 240: 3295-3304.
188. Yamamoto S, Fukushima J, Atsumi Y, Takeuchi H, Kawamoto S, Okuda K, Morihara K. - Cloning and characterization of elastase structural gene from *Pseudomonas aeruginosa* IFO 3455. *Biochem Biophys Res Commun*, 1988; 152: 1117-1122.
189. Bever RA, Iglewski BH. - Molecular characterization and nucleotide sequence of the *Pseudomonas aeruginosa* elastase structural gene. *J Bacteriol*, 1988; 170: 4309-4314.
190. Hamdaoui A, Wund-Bisseret F, Bieth JG. - Fast solubilization of human lung elastin by *Pseudomonas aeruginosa* elastase. *Am Rev Respir Dis*, 1987; 135: 860-863.
191. Bejarano PA, Lengeveld JPM, Hudson BG, Moelken ME. - Degradation of basement membranes by *Pseudomonas aeruginosa* elastase. *Infect Immun*, 1989; 57: 3783-3787.
192. Suter S, Schaad VB, Roux L, Nydegger VE, Waldvogel FA. - Granulocyte neutral proteases and *Pseudomonas aeruginosa* elastase as possible causes of airway damage in patients with cystic fibrosis. *J Infect Dis*, 1984; 149: 523-531.
193. Döring G, Obernesser HJ, Botzenhart K. - Extracellular toxins of *Pseudomonas aeruginosa*. II. Effect of two proteases on human immunoglobulins IgG, IgA and secretory IgA. *Zentralbl Bakteriell Mikrobiol Hyg (A)*, 249: 89-98.
194. Jacquot J, Tournier JM, Puchelle E. - *In vitro* evidence that human airway lysozyme is cleaved and inactivated by *Pseudomonas aeruginosa* elastase and not by human leukocyte elastase. *Infect Immun*, 1985; 47: 555-560.
195. Morihara K, Tsuzuki H, Harada M, Iwata J. - Purification of human plasma  $\alpha_1$ -proteinase inhibitor and its inactivation by *Pseudomonas aeruginosa* elastase. *J Biochem*, 1984; 95: 795-804.
196. Padrines M, Bieth JG. - *Pseudomonas aeruginosa* elastase does not inactivate  $\alpha_1$ -proteinase inhibitor in the presence of leukocyte elastase. *Infect Immun*, 1989; 57: 3793-3797.
197. Tournier JM, Jacquot J, Puchelle E, Bieth JG. - Evidence that *Pseudomonas aeruginosa* elastase does not inactivate the bronchial inhibitor in the presence of leukocyte elastase. Studies with cystic fibrosis sputum and with pure proteins. *Am Rev Respir Dis*, 1985; 132: 524-528.
198. Johnson DA, Carter-Ham B, Dralle WM. - Inactivation of human bronchial mucosal proteinase inhibitor by *Pseudomonas aeruginosa* elastase. *Am Rev Respir Dis*, 1982; 126: 1070-1073.
199. Döring G, Obernesser HJ, Botzenhart K, Flehmig B, Hoiby N, Hofmann A. - Proteases of *Pseudomonas aeruginosa* in patients with cystic fibrosis. *J Infect Dis*, 1983; 147: 744-750.
200. Fick RB, Hata JS. - Pathogenetic mechanism in lung diseases caused by *Pseudomonas aeruginosa*. *Chest*, 1989; 95: 2065-2135.
201. Potempa J, Dubin A, Korzus G, Travis J. - Degradation of elastin by a cysteine proteinase from *Staphylococcus aureus*. *J Biol Chem*, 1988; 263: 2664-2667.
202. Poulsen J, Brandt J, Hjorth P, Thogersen HC, Kilian M. - Cloning and sequencing of the immunoglobulin A1 protease gene (IgA) of *Haemophilus influenzae* serotype B. *Infect Immun*, 1989; 57: 3097-3105.
203. Kilian M, Reinholdt J, Mortensen SB, Sorensen CH. - Perturbation of mucosal immune defense mechanisms by bacterial IgA proteases. *Bull Eur Physiopathol Respir*, 1983; 19: 99-104.
204. Laurell CB, Eriksson S. - The electrophoretic alpha<sub>1</sub>-globulin pattern of serum in alpha<sub>1</sub>-antitrypsin deficiency. *Scand J Clin Invest*, 1963; 15: 132-140.
205. Heidtmann H, Travis J. - Human  $\alpha_1$ -proteinase inhibitor. In: Proteinase inhibitors. A.J. Barret, G. Salvesen eds, Elsevier, 1986; pp. 441-456.
206. Crystal RG. -  $\alpha_1$ -antitrypsin deficiency, emphysema and liver disease. *J Clin Invest*, 1990; 85: 1345-1352.
207. Perlmutter DH, Cole FS, Kilbridge P, Rossing TH, Colten HR. - Expression of the  $\alpha_1$ -proteinase inhibitor gene in human monocytes and macrophages. *Proc Natl Acad Sci USA*, 1985; 82: 795-799.
208. Mornex JF, Chytil-Weir A, Martinet Y, Courtney M, Lecocq JP, Crystal RG. - Expression of the alpha<sub>1</sub>-antitrypsin gene in mononuclear phagocytes of normal and alpha<sub>1</sub>-antitrypsin-deficient individuals. *J Clin Invest*, 1986; 77: 1952-1961.
209. Perlino E, Cortese R, Ciliberto G. - The human  $\alpha_1$ -antitrypsin gene is transcribed from two different promoters in macrophages and hepatocytes. *The Embo J*, 1987; 6: 2767-2771.
210. Perlmutter DH, Joslin G, Nelson P, Schastee C, Adams SP, Fallon RJ. - Endocytosis and degradation of  $\alpha_1$ -antitrypsin-protease complexes is mediated by the serpin-enzyme complex (SEC) receptor. *J Biol Chem*, 1990; 265: 16713-16716.
211. Bieth JG. - Pathophysiological interpretation of kinetic constants of protease inhibitors. *Bull Eur Physiopathol Respir*, 1980; (Suppl. 16): 183-195.
212. Matheson NR, Wong PS, Schuyler M, Travis J. - Interaction of human  $\alpha_1$ -proteinase inhibitor with neutrophil myeloperoxidase. *Biochemistry*, 1981; 20: 331-336.
213. Clark RA, Stone PJ, El Hag A, Calore JD, Franzblau C. - Myeloperoxidase-catalysed inactivation of  $\alpha_1$ -protease

- inhibitor by human neutrophils. *J Biol Chem*, 1981; 256: 3348-3353.
214. Burnett D, Stockley RA. - The electrophoretic mobility of  $\alpha_1$ -antitrypsin in sputum and its relationship to protease inhibitory capacity, leucocyte elastase concentrations and acute respiratory infection. *Hoppe-Seyler's Z Physiol Chem*, 1980; 361: 781-789.
215. Stockley RA, Afford SC. - Qualitative studies of lung lavage  $\alpha_1$ -proteinase inhibitor. *Hoppe-Seyler's Z Physiol Chem*, 1984; 365: 503-510.
216. Scharfman A, Hayem A, Davril M, Marko D, Hanothiaux MH, Lafitte JJ. - Special neutrophil elastase inhibitory activity in BAL fluids from patients with silicosis and asbestosis. *Eur Respir J*, 1989; 2: 751-757.
217. Cantin A, Bilodeau G, Begin R. - Granulocyte elastase-mediated proteolysis of  $\alpha_1$ -antitrypsin in cystic fibrosis bronchopulmonary secretions. *Pediatric Pulmonol*, 1989; 7: 12-17.
218. Stockley RA, Afford SC. - The immunological assessment of  $\alpha_1$ -antitrypsin with reference to its function in bronchial secretions. *Clin Sci*, 1983; 65: 373-381.
219. El Yamani J, Hayem A, Lafitte JJ, Gressier B, Mizon J. - Functional and immunoreactive  $\alpha_1$ -proteinase inhibitor in bronchoalveolar lavages: methodological studies. *Bull Eur Physiopathol Respir*, 1986; 22: 359-363.
220. Gast A, Dietemann-Molard A, Pelletier A, Pauli G, Bieth J. - The antielastase screen of the lower respiratory tract of  $\alpha_1$ -proteinase inhibitor - sufficient patients with emphysema or pneumothorax. *Am Rev Respir Dis*, 1990; 141: 880-883.
221. Banda MJ, Rice AG, Griffin GL, Senior RM. -  $\alpha_1$ -proteinase inhibitor is a neutrophil chemoattractant after proteolytic inactivation by macrophage elastase. *J Biol Chem*, 1988; 263: 4481-4484.
222. Kramps JA, Willems LNA, Franken C, Dijkman JH. - Antileukoprotease, its role in the human lung. *Biol Chem Hoppe-Seyler*, 1988; 369 (Suppl.): 83-87.
223. Fritz H. - Human mucus proteinase inhibitor (human MPI). *Biol Chem Hoppe-Seyler*, 1988; 369 (Suppl.): 79-82.
224. Stetler G, Brewer MT, Thompson RC. - Isolation and sequence of a human gene encoding a potent inhibitor of leukocyte proteases. *Nucleic Acids Res*, 1986; 14: 7883-7896.
225. Seemuller V, Arnhold M, Fritz H, Wiedenmann K, Machleidt W, Heinzl R, Appelhans H, Gassen HG, Lottspeich F. - The acid-stable proteinase inhibitor of human mucous secretions (HUSI-I, antileukoprotease). *FEBS Lett*, 1986; 199: 43-48.
226. Thompson RC, Ohlsson K. - Isolation, properties and complete amino acid sequence of human secretory leukocyte protease inhibitor, a potent inhibitor of leukocyte elastase. *Proc Natl Acad Sci USA*, 1986; 83: 6692-6696.
227. Kramps JA, van Twisk C, Appelhans H, Meckelein B, Nikiforov T, Dijkman JH. - Proteinase inhibitory activities of antileukoprotease are represented by its second COOH-terminal domain. *Biochim Biophys Acta*, 1990; 1038: 178-185.
228. Eisenberg SP, Hale KK, Heimdal P, Thompson RC. - Location of the protease-inhibitory region of secretory leukocyte protease inhibitor. *J Biol Chem*, 1990; 265: 7976-7981.
229. Meckelein B, Nikiforov T, Clemen A, Appelhans H. - The location of inhibitory specificities in human mucus proteinase inhibitor (MPI): separate expression of the COOH-terminal domain yields an active inhibitor of three different proteinases. *Protein Engineering*, 1990; 3: 215-220.
230. Carp H, Janoff A. - Inactivation of bronchial mucous proteinase inhibitor by cigarette smoke and phagocyte-derived oxidants. *Exp Lung Res*, 1980; 1: 225-237.
231. Van-Seuningen I, Davril M, Hayem A. - Evidence for the tight binding of human mucus proteinase inhibitor to highly glycosylated macromolecules in sputum. *Biol Chem Hoppe-Seyler*, 1989; 370: 749-755.
232. Bruch M, Bieth JG. - Influence of elastin on the inhibition of leucocyte elastase by  $\alpha_1$ -proteinase inhibitor and bronchial inhibitor. Potent inhibition of elastin bound elastase by bronchial inhibitor. *Biochem J*, 1986; 238: 269-273.
233. Morrison HM, Welgus HG, Stockley RA, Burnett D, Campbell EJ. - Inhibition of leukocyte elastase bound to elastin: relative effectiveness and two mechanisms of inhibitory activity. *Am J Respir Cell Mol Biol*, 1990; 2: 263-269.
234. Tournier JM, Jacquot J, Sadoul P, Bieth G. - Non-competitive enzyme immunoassay for the measurement of bronchial inhibitor in biological fluids. *Anal Biochem*, 1983; 131: 345-350.
235. Kramps JA, Franken C, Dijkman JH. - ELISA for quantitative measurement of low-molecular weight bronchial protease inhibitor in human sputum. *Am Rev Respir Dis*, 1984; 129: 959-963.
236. Stockley RA, Morrison HM. - Elastase inhibitors of the respiratory tract. *Eur Respir J*, 1990; 3 (Suppl. 9): 9s-15s.
237. Perlmutter DH, Travis J, Punsal PL. - Elastase regulates the synthesis of its inhibitor  $\alpha_1$ -proteinase inhibitor, and exaggerates the defect in homozygous PiZZ,  $\alpha_1$ PI deficiency. *J Clin Invest*, 1988; 81: 1174-1180.
238. Chandra T, Stackhouse R, Kidd VJ, Robson JH, Woo SLC. - Sequence homology between human,  $\alpha_1$ -antichymotrypsin,  $\alpha_1$ -antitrypsin and antithrombin III. *Biochemistry*, 1983; 22: 5055-5061.
239. Laine A, Davril M, Hayem A. - Interaction between human serum  $\alpha_1$ -antichymotrypsin and human leukocyte cathepsin G. Complex formation and production of a modified inhibitor. *Biochem Biophys Res Commun*, 1982; 105: 186-193.
240. Ryley HC, Brogan TD. - Quantitative immunoelectrophoretic analysis of the plasma proteins in the sol phase of sputum from patients with chronic bronchitis. *J Clin Pathol*, 1973; 26: 852-856.
241. Laine A, Hayem A. - Identification et caractérisation des constituants protéiques de la sécrétion bronchique humaine. *Clin Chim Acta*, 1976; 67: 159-167.
242. Stockley RA, Burnett D. - Alpha<sub>1</sub>-antichymotrypsin in infected and non-infected sputum. *Am Rev Respir Dis*, 1980; 122: 81-88.
243. Burnett D, McGillivray DH, Stockley RA. - Evidence that alveolar macrophages can synthesize and secrete  $\alpha_1$ -antichymotrypsin. *Am Rev Respir Dis*, 1984; 129: 473-476.
244. Berman G, Afford SC, Burnett D, Stockley RA. -  $\alpha_1$ -antichymotrypsin in lung secretions is not an effective proteinase inhibitor. *J Biol Chem*, 1986; 261: 14095-14099.
245. Kurdowska A, Travis J. - Acute phase protein stimulation by  $\alpha_1$ -antichymotrypsin-cathepsin G complexes. *J Biol Chem*, 1990; 265: 21023-21026.
246. Kilpatrick L, Johnson JL, Nickbarg EB, Wang ZM, Clifford TF, Banach M, Cooperman BS, Douglas SD, Rubin H. - Inhibition of human neutrophil superoxide generation by  $\alpha_1$ -antichymotrypsin. *J Immunol*, 1991; 146: 2388-2393.
247. White RR, Janoff A, Godfrey HP. - Secretion of  $\alpha_2$ -macroglobulin by human alveolar macrophages. *Lung*, 1980; 158: 9-14.

248. Burnett D, Stockley RA. - Serum and sputum  $\alpha_2$ -macroglobulin in patients with chronic obstructive airways disease. *Thorax*, 1981; 36: 512-516.
249. Stockley RA, Morrison HM, Kramps JA, Dijkman JH. - Elastase inhibitors of sputum sol phase: variability, relationships to neutrophil elastase inhibition, and effect of corticosteroid treatment. *Thorax*, 1986; 41: 442-447.
250. Wewers MD, Herzyk DJ, Gadek JE. - Alveolar fluid neutrophil elastase activity in the adult respiratory distress syndrome is complexed to alpha<sub>2</sub>-macroglobulin. *J Clin Invest*, 1988; 82: 1260-1267.
251. Hochstrasser K, Albrecht GJ, Schönberger OL, Rasche B, Lempart K. - An elastase-specific inhibitor from human bronchial mucus. *Hoppe-Seyler's Z Physiol Chem*, 1981; 362: 1369-1375.
252. Hochstrasser K, Naumann R, Wachter E. - Inhibitograms of leukoproteinase inhibitors of human respiratory tract. In: Pulmonary emphysema and proteolysis, 1986. J.C. Taylor, C. Mittman eds, Academic Press, 1987; pp. 331-339.
253. Kramps JA, Klasen EC. - Characterization of a low molecular weight anti-elastase isolated from human bronchial secretions. *Exp Lung Res*, 1985; 9: 151-165.
254. Sallenave JM, Ryle AP. - Purification and characterization of elastase-specific inhibitor. Sequence homology with mucus proteinase inhibitor. *Biol Chem Hoppe-Seyler*, 1991; 372: 13-21.
255. Barrett AJ, Rawlings ND, Davies ME, Machleidt W, Salvesen G, Turk V. - Cysteine proteinase inhibitors of the cystatin superfamily. In: Proteinase inhibitors. A.J. Barrett, G. Salvesen eds, Elsevier, 1986; pp. 515-569.
256. Yamauchi K, Ohtsu I, Ohno I, Andoh Y, Shimura S, Tamura G, Takishima R, Isemura M, Isemura S. - Cystatin S is present in human upper and lower respiratory tract. *Am Rev Respir Dis*, 1989; 139: A199.
257. Burnett D, Reynolds JJ, Ward RV, Afford SC, Stockley RA. - Tissue inhibitor of metalloproteinases and collagenase inhibitory activity in lung secretions from patients with chronic obstructive bronchitis: effect of corticosteroid treatment. *Thorax*, 1986; 41: 740-745.
258. Cawston TE, Mercer E. - Preferential binding of collagenase to  $\alpha_2$ -macroglobulin in the presence of the tissue inhibitor of metalloproteinases. *FEBS Lett*, 1986; 209: 9-12.