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Factors Relevant to Ciliary Activity of the Airway Mucosa

Ken-ichi HISAMATSU

Mucociliary transport is one of the essential defensive functions of the airway mucosa. Dysfunction of this system facilitates recurrent and chronic mucosal inflammation and allergy of the airway. In this paper, factors relevant to mucociliary function are reviewed with 110 references. The bioactive substances that are reviewed in this paper are as follows : immune complexes, viruses, bacteria, allergen, MBP, elastase, sulfide peptide leukotrienes, platelet activating factor, prostaglandins, histamine, bradykinin, neuropeptides, autonomic neurotransmitters, lysozyme, etc.

key words : ciliary activity, inflammatory mediators, neurotransmitter, neuropeptides, bioactive substances

1. Introduction

The airway mucosal ciliated epithelium is covered with a mucus layer, the so-called mucus blanket, that traps inhaled substances to clean up inspired air, which is further conditioned by warming and humidifying during passing through the airway until reaching the alveoli. Mucociliary transport is one of the essential defensive functions of the airway mucosa. Ciliary movement transports the mucus blanket to the pharynx from both the upper and the lower airway, where it is then swallowed and digested.

Dysfunction of this system allows inhaled substances invade the mucosa and facilitates recurrent and persistent mucosal inflammation and allergy of the airway. In this paper, factors relevant to mucociliary function are reviewed and discussed focusing on methodology for observation of ciliated cell function, and ciliary activity along with recent advances in this field.

2. Ultrastructure of the nasal epithelium

The respiratory epithelium consists of four dominant cell types, ciliated columnar cells, non-ciliated columnar cells, goblet cells and basal cells. Ciliated epithelium covers the posterior 2/3 of the nasal cavity as well as rhinopharynx, larynx, trachea, cartilaginous sections of the bronchial tree, and also the eustachian tube and the protympanum. All ciliated and non-ciliated columnar epithelial cells are covered with about 300 microvilli which retain moisture. Goblet cells are distributed approximately 500 cells per mm^2 in the nasal mucosa.

A ciliated cell of human nasal mucosa has approximately one hundred cilia over the luminal surface of the cell. The cilia are 0.3 μm in diameter and 4-6 μm long. A cross-section of the cilia shows a ring of nine doublet microtubles surrounding a pair of single central microtubles. The energy for ciliary movement is supplied by the conversion of adenosine triphosphate (ATP) by an ATP-ase named dynein. This molecule forms a series of projections called dynein arms that protrude from one side of each of the nine outer doublets. Energy production in the dynein arms causes peripheral microtubles to slide past one another, and the cilium to bend (Satir, 1974)[1].

Cilia beat very actively with a frequency of approximately 1,000 strokes per minute. The beat of a single cilium resembles the arm movement of a swimmer swimming the crawl, consisting a rapid forward beat (effective stroke) and a slow return beat (recovery stroke). Cilia beat synchronously in parallel ranks one after another forming metachronical waves. The mechanism of co-ordination of this activity still remains unknown. Cilia can only act in a fluid medium. Therefore, the surface layer of fluid in the respiratory tract is crucial to ciliary function and in addition to integrate its role in mucociliary transport system.

Lucas and Douglas suggested that the mucus blanket has to be a double fluid layer on the airway surfaces; a superficial viscid sheet of mucus (gel layer) and an underlying layer of serous fluid (sol layer)(Lucas et al., 1934)[2]. The gel layer acts as a conveyor belt moving along on the top of the cilia, and this layer traps various inhaled substances. Although this is still a hypothesis, it is difficult to explain mucociliary clearance without their existence.

Department of Otorhinolaryngology, Yamanashi Medical University

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The cilia are thought to touch the gel layer only with their tips during forward movement. They are always surrounded by the sol layer supplied both from the secretory cells, goblet cells and serous cells of mucosal glands and from tissue fluid transferred through the surface epithelium. The thickness of the sol layer is regulated by water absorption from the microvilli of the epithelial cells, approximating the length of the cilia 4-6 mm in depth.

The mucociliary transport system has the capacity to transport even large particles of up to 0.5 mm in diameter. When the system is intact, bacteria have little chance to settle and invade the epithelium since they are moved over 20 epithelial cells per second. Defense of the mucosa against viral infection is less complete, as viruses may possess a special affinity to receptors on the cilia and cause ciliary dysfunction (Sasaki et al., 1973)[3].

3. Mucociliary function and its disturbance

The activity of the mucociliary transport system in vivo can be assessed by mucociliary transport of markers which can be soluble, for example, radioisotope labeled albumin (Proctor et al, 1965)[4] and saccharin particles (Andersen et al., 1972, 1974)[5,6], or insoluble, e. g. resin, Teflon, charcoal particles or paper disks stained with dye or labeled with radioisotopes. Radioisotope-labeled resin particles are used to measure mean transport distance (mucociliary particle transport rate, PTR). When resin particles, 0.5 mm in diameter, were used, PTR was 5.3 mm/min (0.5-23.6 mm/min) in 181 normal subjects with a mean age of 24 years old (Quinlan et al., 1969)[7]. Sakakura reported that mean PTR was 5.8 ± 3.3 mm/min (mean$\pm$SD) in normal Japanese subjects with a mean age of 26.7 years old (Sakakura et al., 1983)[8].

Andersen et al. used saccharin particles for measurement of mucociliary transport activity. Saccharin particles placed on the inferior turbinate are dissolved in the mucus blanket during transportation to the pharynx, and subjects report a sweet when it reaches the pharynx. Thus, the time required to recognize the sweet taste is easily measured following each 30 sec swallowing (saccharin time: ST). The saccharin method is suitable for clinical use since it is safe, simple and reliable in the same subjects (Vinter, 1978; Ginzel, 1981)[9,10]. ST in normal subjects is 13.7 ± 8.9 min, and it was reduced in chronic sinusitis patients (Sakakura et al., 1983)[8]. The disadvantages of the measurement of mucociliary transport time are as follows: 1) It is inaccurate because of the different anatomical distances of the transport pathway in each subject; 2) unable to repeat during remaining previous taste; and 3) this test cannot be used in subjects with taste disorders, etc.

Eliezer et al. (1970) proposed that there are two transport inhibitory factors; namely, rheological changes in the mucus blanket and dyscoordination between cilia and mucus blanket, in chronic sinusitis patients. They investigated mucus transport time using a frog pharyngeal explant eliminated mucus blanket, and found that the transport time of the mucus obtained from chronic sinusitis patients was significantly decreased compared with that from normal subjects. They presumed that this finding was due to rheological changes of the mucus due to chronic mucosal inflammation. However, in two groups with respective saccharin times of less and 30 min longer than mucus transport time using the frog pharyngeal explant showed no significant differences suggesting the importance of other inhibitory factors, i. e. coordination of mucus and cilia[11].

To date, various changes in components of the mucociliary transport system have been reported in respiratory diseases. These are classified as follows (Hisamatsu, 1987)[12]:

〔1〕 Quality of the mucus layer: 1) Rheological changes; there is an optimum range for integration of the transport function. If viscoelasticity of the mucus is out with the optimum range, mucus transport by ciliary movement cannot function effectively (King et al., 1974; Chen et al., 1978)[13,14]. 2) Changes in components of the mucus layer; inhaled substances, lysozyme, immune complexes, mediators of allergy and inflammation (histamine, bradykinin, PAF, LTD_4, etc.) and other bioactive molecules. These substances might affect mucociliary transport function directly or indirectly. Hisamatsu et al. have studied the effects of inflammatory mediators and bioactive molecules on ciliary activity.

〔2〕 Structural changes in the mucociliary transport system: There have been reports that structural changes inhibit the mucociliary function although the quality of the mucus may change along with subsequent structural alterations. 1) Increase in thickness of the outer mucus layer (Dalhamn, 1956)[15]: SO_2 exposure induces an increase in amount of the outer mucus layer resulting in prolongation of the transport time. 2) Decrease in depth of the inner mucus layer (Litt, 1978)[16]: This was observed in chronic obstructive lung diseases. In this situation the tips of the cilia must make contact with the gel layer in both effective and recovery phases. 3) Increase in depth of the inner mucus layer (Proctor et al., 1978)[17]: Increased amounts of periciliary fluid were reported

in methacholine-administrated dogs. This resulted in detachment of the tips of cilia from the outer mucus layer during the effective stroke. 4) Tethering phenomenon: The outer mucus layer is tethered by high viscoelastic secretion from goblet cells and/or mucosal glands. Both the outer mucus layer and cilia are hardly able to move. This has been reported in chicken following dehydration (Bang et al., 1963)[18], SO_2 exposure (Majima et al., 1981)[19], DNV infection (Sakakura, 1983)[20] and in chronic sinusitis patients (Majima et al., 1986)[21].

〔3〕 Ciliary movement: Ciliary movement is essential to integrate the mucociliary transport function. 1) Inhibited ciliary activity was reported in chronic sinusitis (Hisamatsu et al, 1981)[22]. 2) Dyscoordination of the mucus layer and cilia was reported in a subtype in immotile cilia syndrome (Pedersen et al., 1980)[23] and in the common cold (Pedersen et al., 1983)[24]. 3) Decrease in ciliated area of the epithelium due to chronic inflammation (Itoh et al., 1984)[25].

4. Ciliary movement

Various methods for observation of ciliary movement have been described. Several cilioinhibitory factors are possibly involved in persistent mucosal inflammation in upper and lower respiratory diseases including otitis media with effusion (OME). In OME induced experimentally by PAF, LTD_4 and PGE_2, the author and coworkers observed inhibited clearance of the tubotympanic cavity by otoscopy and tympanometry (Ganbo et al., 1995)[26]. However, there have been no methods reported capable of measuring direct ciliary beat frequency (ciliary activity) in vivo for clinical use.

I. Measurement of ciliary movement in vivo.

Dalhamn (1956)[15] measured ciliary activity of rat tracheal mucosa using analysis of ciliary movement recorded on a chine-film. Alternative method to observe mucociliary activity in vivo has been described. Light reflection due to metachronal wave can be measured photoelectrically using an operating microscope equipped with a photo-cell and a recorder. Hybbinette et al. established this method for studies of the effects of intra-arterial administration with bioactive substances on rabbit maxillary sinus mucosa with a feeding vessel (Hybbinette, 1982)[27]. They described the potentiation of methacholine-induced ciliostimulation by vasoactive intestinal peptide (VIP)[28], ciliary inhibition by the alpha-2-adrenoceptor agonist xylometazoline without blood flow reduction[29], weak ciliostimulation by PGE_1, PGE_2 and $PGF_{2\alpha}$[30], cholinergic and C-fiber mediated mechanisms in ciliostimulation by PGE_1 and $PGF_{2\alpha}$[31], no influence of LTC_4, LTD_4 or PAF on ciliary activity[32], ciliostimulation by histamine[33], calcium-dependent neuropeptide Y (NPY)-induced ciliary inhibition[34], ciliary inhibition by sympathetic nerve stimulation[35], inhibitory modulation of cholinergic ciliostimulation by NPY[36], high dose noradrenaline-induced ciliostimulation via the cyclo-oxygenase pathway[37] and cyclic adenosine monophosphate-induced stimulation of mucociliary activity[38]. The animal model with a feeding vessel has contributed to in vivo studies of mucociliary activity influenced by administration of various substances through the vessel. However, administration into the vessel possibly induces second messengers derived from cell components of the vessel involved in mucociliary activity. Thus, a simplified administration route is required in vitro for clarification of the effects of substances on ciliated epithelial cells.

II. Measurement of ciliary movement in vitro.

1. Analysis of rotation of the ciliated cells (rotating ciliated explant method; Ballenger,1960)[39]. Separated ciliated cells form a rotating explant in cell culture, and the rotation is countable. Corssen et al.(1958)[40], and Nakamura et al.(1968)[41] studied the effects of medicines on ciliated cells. However, accurate counting of rotation of the ciliated explant is difficult because of the three-dimensional movement recorded on a cinefilm.

2. Stroboscopy

This method allows direct measurement of ciliary activity despite difficulties in vewing the lateral aspect of ciliated epithelium and in visualization of each ciliated cell due to residual impression with wide experimental error (Shimizu, 1955)[42]. Saito et al. described a microstroboscopy method using a microscope combined with a stroboscope apparatus that allowed stroboscopy at a magnification of 200x (Saito et al., 1982)[43]. However, this method did not allow long-term observation of the same portion of the mucosa in tissue culture. Therefore, measured ciliary activities were derived from different portion of the ciliated epithelium.

3. High speed video

Rossman et al. studied ciliary activity and ciliary wave form of patients with and without primary ciliary dyskinesia (PCD) using a videomotion analyzer along with ultrastructural study of cilia. In the PCD groups, ciliary activity was significantly reduced, ciliary wave form was grossly abnormal, and both pulmonary and nasal mucociliary transport were

virtually completely absent. The proportion of cilia with ultrastructural abnormalities was significantly greater than those in normal subjects and in patients with non-PCD respiratory disease ($p<0.0001$). These findings demonstrate the relevance of ciliary ultrastructural abnormalities to altered ciliary function (Rossman et al., 1984)[44].

4. Photoelectric measurement of ciliary activity

There are various factors which affect ciliary movement. Therefore, it is essential to consider these factors to accurately measure ciliary activity. 1) Temperature: Ciliary activity increases linearly with environmental temperature up to 40°C. At over 40°C, ciliary cessation occurs. 2) pH: In acidic medium, ciliary activity is inhibited, whereas it is minimally affected in alkaline medium.

The ciliary activity can be measured using photoelectrical methods, which was reported originally by Rylander in 1966[45]. In the past two decades, this technique has been used commonly in studies of ciliary movement. Changes in intensity of light passed through beating cilia are transferred into electrical signals and can be recorded on paper in the form of waves. Thus, ciliary activity can be measured by counting numbers of the waves recorded within a set period. However, conventional techniques without video-recording (VTR) permit only single measurement of a given area of active ciliated epithelium. Recently, a VTR method for measurement of ciliated cells was developed using a CO_2 incubator, thermoregulator, video camera, photo sensor and recorder (Hisamatsu et al., 1987)[12]. This method allows repeatable and simultaneous measurement of ciliary activities of multiple ciliated cells of the mucosa, and also permits investigation of the mucosal epithelial profile at a high magnification (approximately 2,500x) on a video monitor during long observation periods over 24 hours in tissue culture with a regulated temperature and in a humidified atomosphere of 95 % air, 5 % CO_2. This method enabled analytical studies of the effects of bioactive substances on ciliary activity and also cytotoxicity in vitro.

5. Effects of immune complexes on ciliary activity.

Nasal mucosa is the first line of defense, and the mucus blanket contains various immunogloblins, cytokines, etc. The initial response against inhaled substances may occur in the mucus blanket. Therefore, antigens might react with specific antibodies resulting in formation of immune complexes in the nasal secretions. It is very interesting how ciliated cells respond to the antigens in sensitized animals, and this response seems crucial to host defense.

1) Viruses. Chevance et al.(1975)[46] first reported that inactivated influenza virus specifically induced ciliostasis in the tracheal mucosa of sensitized rabbits. However, Aoyagi performed a careful follow-up study and showed a conflicting phenomenon; namely, specific ciliostimulation provoked by inactivated influenza virus in the tracheal mucosa of sensitized rabbits (1980). He found that ciliostasis occurred as a result of the use of hormalin to inactivate influenza virus. Inactivated influenza A/England/47/72 showed a tendency to inhibit ciliary activity of nonsensitized rabbits, whereas it showed significant ciliostimulation in vitro although influenza B virus showed minimal effects on the ciliary activity. A greater ciliostimulatory effect of influenza A/England/47/72 was observed in sensitized animals with higher serum antibody titer. Furthermore, the reaction mixture of inactivated influenza A/England/47/72 and its specific antiserum with or without heating showed significant ciliostimulation suggesting no requirement of complement in this phenomenon (Aoyagi, 1980)[47].

2) Bacteria. There were few bacterioimmunological studies of ciliary activity until Sakuma's report appeared in 1980. He found significant prolongation of ciliated cell survival with ciliostimulation against vital Bordetella bronchiseptica (B. b.) in sensitized rabbits although minimal differences were observed between ciliary activities in the sensitized and nonsensitized animals 20 minutes after exposure to B. b., and ciliostasis was observed in almost ciliated cells by 60 minutes after challenge. Mixtures of vital B. b. and anti-B. b. serum showed ciliostimulation in nonsensitized animals, whereas the mixture of vital B. b. and anti-B. b. serum with heating showed cilioinhibitory and ciliostatic effects (Sakuma, 1980)[48].

3) Allergens. It is not known how ciliated cells respond to inhaled allergens in allergic patients. The author first reported that reaction mixtures of allergen extract and specific IgE antibody-rich serum or of anti-IgE serum and IgE antibody-rich serum showed ciliostimulation in vitro although allergen, anti-IgE serum and IgE antibody-rich serum alone showed minimal effects on ciliary activity (Hisamatsu et al., 1981)[49]. This ciliary response of the mucosa might be mediated by IgE because of the minimal response induced by heated IgE-rich serum. In allergic nasal mucosa, the IgE-mediated ciliostimulation may occur during the early phase of allergen challenge since

mast cell-derived lipid mediators act as cilioinhibitory factors and eosinophil accumulation and activation must lead to damage of ciliated epithelial cells. Further analytical studies remain to be performed.

6. Effects of inflammatory mediators on ciliary activity

Mucociliary dysfunction and morphological changes of cilia have been reported in chronic inflammatory airway diseases, and some cilioinhibitory factors have been reported in sputum from patients with chronic respiratory diseases such as cystic fibrosis and bronchial asthma. However, the pathogenesis of ciliary dysfunction in chronic inflammatory airway diseases has not been clarified. Thus, it is crucial to study the effects of inflammatory mediators on ciliated cells to understand the pathogenesis of these diseases.

There have been several in vivo and in vitro studies of leukotrienes with regard to ciliary activity ; however, the results are unreliable due to the technical difficulty of measurement of ciliary activity. Since 1986, the author and co-workers have been studying the effects of inflammatory mediators on ciliated epithelium using the newly developed VTR method.

1. Eosinophil granule major basic protein (MBP). Four granule proteins of eosinophils have been identified ; MBP, eosinophil cationic protein (ECP)[50], eosinophil peroxidase (EPO)[51] and eosinophil-derived neurotoxin (EDN or EPX)[52,53]. These cationic proteins are cytotoxic (Gleich et al., 1976 ; McLaren et al., 1981)[54,55]. However, systemic analysis of these proteins and their effects on ciliary activity in humans remain to be performed. Frigas et al. demonstrated morphologically that human MBP damages airway mucosal epithelium. The concentration of MBP in the sputum of bronchial asthma is approximately 100 μg/mL or less (1981)[56].

The author and co-workers first reported that human MBP showed cilioinhibitory effects on intact human paranasal sinus mucosa obtained from patients with facial trauma in vitro in a concentration- and time-dependent manner. Irrigation with culture medium after MBP exposure prolonged the time required for ciliary inhibition. The lowest concentration of MBP required to induce ciliary inhibition of intact human paranasal sinus mucosa is 10 μg/mL, although as little as 1 μg/mL is sufficient in allergic inflamed mucosa. This suggests that the inflamed mucosa is already affected by other mediators, possibly lipid mediators, before MBP exposure (Hisamatsu et al., 1990)[57]. However, prior to the studies of the authors group there had been few detailed reports regarding the effects of inflammatory mediators on ciliated cells.

2. Serine protease. This protease was found in sputum obtained from patients with bronchiectasis (Smallman et al., 1984)[58]. This proteolytic enzyme is possibly neutrophil-derived elastase (Tegner et al., 1979)[59]. The protease might be an important cilioinhibitory factor in purulent airway secretions in inflammatory respiratory diseases such as paranasal sinusitis, otitis media and bronchitis.

3. Leukotrienes. SRS-A has been reported to cause dysfunction of the mucociliary transport system in allergic patients[60,61,62]. It has been now demonstrated that SRS-A is a mixture of leukotriene C_4 (LTC_4), LTD_4 and LTE_4. LTC_4 is converted enzymatically to LTD_4 and further to LTE_4. These sulfide peptide leukotrienes are thought to be important lipid mediators causing increases in amounts of secretions and airway resistance due to mucosal edema and contraction of bronchial trees.

1) LTC_4. There have been several reports regarding the effects of leukotrienes on ciliated cells during 20-30-minute observation periods in vitro. LTC_4 was reported to induce ciliostimulation at a concentration of 10^{-8}M in a study using single cells brushed from sheep trachea (Wanner et al., 1983)[63]. On the other hand, 3×10^{-9}M LTC_4 induced ciliary inhibition in a study using human scraped separated cells and this phenomenon was blocked by the SRS-A antagonist, FPL-55712 (Bisgaard et al., 1987)[64]. In a study using chicken tracheal ciliated cells in a form of tissue culture, 10^{-8}M LTC_4 induced ciliary inhibition that was blocked by FPL-55712 (Weisman et al., 1990)[65]. Tamaoki and co-workers described LTC_4 and LTD_4-induced ciliostimulation in vitro[66]. These inconsistent results of previous studies may be attributable to the variety of study designs, species and measurement techniques used. For instance, separated single cells showed spontaneous decreases in ciliary activity[64] and three-dimensional movement of cell bodies made measurement of ciliary activity difficult. Tissue culture allows preservation of cell-cell interactions which are an important component of ciliary activity (Weisman et al., 1990)[65], and allows further observation of the mucosal surface profile during long observation periods. Therefore, tissue culture seems more appropriate than cell culture for measurement of ciliary activity.

Alternatively, the inconsistencies in results in

tissue culture might be due to neuropeptides contained in nerve fibers of the mucosa. Various stimuli might provoke release of preformed SP from the C-fibers in mucosal specimens. Mucus secretion from the mucosal specimens induced by mechanical and/or chemical stimulation was frequently observed during culture within a few days. However, minimal secretion were observed after 7 days in culture. SP-induced ciliostimulation has been reported in dog sinus mucosa in vivo (Lindberg et al., 1986)[67], and in rat in vitro (Khan et al., 1986)[68], but not in humans in vitro (Petersson et al., 1989)[69]. Recently, SP-induced ciliostimulation was demonstrated in an in vitro study using human adenoid explant tissue (Staskowski et al., 1992)[70]. The author and co-workers observed SP-induced ciliostimulation along with mucus secretion from goblet cells in rat nasal septal mucosa in vitro using videomicroscopy (Hisamatsu et al., 1995)[71]. Therefore, the effects of SP on ciliary activity should be considered during short-term observation.

Another crucial factor to be considered in studies on sulfide peptide leukotrienes is rapid metabolism of leukotrienes. In our latest study on LTC_4 regarding ciliary activity, with elimination and inhibition of the mucus blanket to avoid its effect, LTC_4 exposure showed minimal effects on ciliated epithelium during the initial 30 minute of exposure, and thereafter ciliary inhibition was observed in a concentration-and time-dependent manner. This ciliary inhibition was completely blocked by the leukotriene receptor antagonists FPL-55712 and Ly-171883 that interact with receptors of LTC_4 and LTD_4. Furthermore, blocking LTC_4 conversion to LTD_4 by L-serine and sodium tetraborate complex[72] showed minimal LTC_4-induced effects on ciliated epithelium. These findings indicate that the major effect of LTC_4 on ciliated epithelium occurs subsequent to its conversion to LTD_4 (Hisamatsu et al., 1995)[73].

2) LTD_4. In studies using ciliated cell clumps brushed from allergic and non-allergic sheep trachea (Wanner et al., 1986)[74], LTD_4 induced ciliostimulation, whereas this leukotriene was reported to induce ciliary inhibition in a study using human nasal scraped cells during a 60-minute observation period (Bisgaard et al., 1987)[64].

Using apparatus which we developed LTD_4 was found to be the most potent sulfide peptide leukotriene. LTD_4-induced ciliary inhibition and mucosal damage were defined (Nakazawa et al., 1994)[75]. LTD_4 inhibited ciliary activity in a time-and dose-dependent manner at concentrations of 10^{-6}M to 10^{-10}M. Ciliary inhibition was induced within 5 minutes of exposure to 10^{-8}M LTD_4 and showed a continuous reduction for 10 hours. Thereafter, over 25 % of observed cells became ciliostatic. At concentrations of 10^{-9}M and 10^{-10}M LTD_4 significantly inhibited ciliary activity 30 minutes and 8 hours after commencement of exposure, respectively. After cessation of the measurement of ciliary activity in each experiment, over 25 % of all observed cells showed progressive ciliary dysfunction, resulting in increases in ciliostatic cell numbers. Moreover, within 5 minutes of exposure, 10^{-8}M LTD_4 induced ciliary inhibition and eventually ciliostasis along with alterations of the mucosal surface profile. The concentration-response curve for LTD_4 was linear until appearance of ciliostasis in human paranasal sinus mucosa ($r=-0.953$, $n=4$). Both FPL-55712 and Ly-171883, specific leukotriene receptor antagonists, blocked LTD_4-induced ciliary inhibition and mucosal damage. Ciliary inhibition was detected during the early phase in a progressive manner after exposure to LTD_4, while mucosal surface alteration was observed on the video monitor during the late phase after 3 hours of exposure. Transmission electron microscopy showed LTD_4-induced ultrastructural alterations in the epithelial cells even after 1 hour of exposure.

LTD_4 concentrations detected in nasal secretions are in the range of 10^{-9}M to 10^{-10}M[76,77], suggesting that LTD_4 is able to induce cytotoxic phenomena in vivo. In fact, the cytotoxic effect of the peptidoleukotrienes were confirmed to cause experimental otitis media with effusion (Ganbo et al., 1995)[78].

3) LTE_4. LTE_4 showed minimal effects on the ciliary activity and mucosal surface profile in vitro in our study (Nakazawa et al., 1994)[75].

4. Platelet activating factor (PAF). PAF is an important lipid mediator in allergy and inflammation as well as sulphidepeptide leukotrienes. Miadonna et al. reported PAF at approximately 10^{-10}M in nasal washings (1989)[79]. The author and co-workers first reported and clarified PAF-induced ciliary inhibition and mucosal epithelial damage in vitro (Hisamatsu et al., 1987, 1988, 1991 ; Ganbo et al., 1990, 1991)[12,80,81,82,83]. Significant PAF-induced ciliary inhibition was noted after 6 hours of exposure to PAF at concentrations of 10^{-8}M and 10^{-10}M ($p<0.05$). After 10 hours of exposure, significant ciliary inhibition ($p<0.01$) was noted at all concentrations. Ciliary inhibition occurred in a time-and dose-dependent manner. The length of the incubation period at which initial ciliostasis occurred and the level of PAF concentration showed an inverse correlation with $r=-0.918$ ($p<10^{-6}$). PAF also induced mucosal damage that resulted in a coarse profile including ciliostasis and exfoliation of epithelial cells. The length of the incubation period at which the

initial coarse profile occurred on the mucosal surface showed an inverse correlation with the concentrations of PAF ranging from 10^{-6}M to 10^{-10}M with $r=-0.712$ ($P<2\times10^{-4}$). Control medium showed minimal effects on ciliary activity or mucosal surface alteration even after 24 hours of exposure (Hisamatsu et al., 1991)[81]. Furthermore, these effects were reliable, and were blocked by a specific PAF receptor antagonist CV-3988 and/or CV-6209 (Ganbo et al.,1991)[84]. The mechanism of cytotoxicity of PAF is presumably dependent upon increased membrane permeability of ciliated cells and organelles. On the video monitor screen, the mucosal surface of specimens was seen to develop a coarse profile prior to ciliostasis, and then the mucosal epithelium finally detached from the lamina propria as a consequence of PAF exposure[82]. It is known that PAF is converted to lyso-PAF by acetylhydrase (Blank et al., 1981)[85] and lyso-PAF is synthesized to PAF by acetyltransferase (Wykle et al., 1980)[86]. In our experimental system lyso-PAF could be converted to PAF (Inoue et al., 1993 ; Ganbo et al., 1994)[87,88], which affected ciliary activity (Ganbo et al., 1994)[89]. Moreover, PAF delayed middle ear clearance in chinchillas and PGE_2 augmented the PAF-induced inflammatory disorder[26].

5. Prostaglandins (PG_s). $PGF_{2\alpha}$ induced ciliary inhibition in human paranasal sinus mucosa (Hisamatsu et al., 1987)[12]. PGE_2-induced ciliostimulation was reported in sheep tracheal scraped ciliated cells (Wanner et al., 1986)[63,74]. In a rabbit maxillary sinus with a feeding vessel, through which administered PGE_1 and $PGF_{2\alpha}$ but PGE_2 showed a slight accelaration of mucociliary wave frequency (Dolata et al., 1989)[30]. In the author's group using our self-designed apparatus, PGE_2 showed significant but temporary ciliostimulation in chinchilla tubotympanic mucosa in vitro. Interestingly, PGE_2 facilitated LTC_4-and LTD_4-induced experimental OME (Ganbo et al., 1994)[90]. Detailed studies of PGs remain to be performed regarding their effects on ciliary activity.

6. Histamine. This diamine, an important chemical mediator in the early phase of inflammation, was reported to promote ciliary movement of single brushed cells from sheep trachea only at high concentrations above 10^{-5}M (Wanner et al., 1983)[63].

7. Bradykinin. In a study using cultured rabbit tracheal epithelium, bradykinin showed ciliostimulation which was inhibited by a B2-bradykinin receptor antagonist but not by a B1-receptor antagonis (Tamaoki et al., 1989)[91].

7. Neural effects on ciliary activity

1. Neuropeptides. Sensory peptidergic nerves are non-myelinated C-fibers (Uddman et al., 1983)[92]. Bare terminal endings of sensory nerves are present in the epithelium of the nasal mucosa. These nerves branch extensively and extended to vessels, glands and also other epithelial cells. These nerve fibers store such tachykinins as substance P (SP) and neurokinin A (NKA), and calcitonin gene-related polypeptide (CGRP) (Baraniuk et al., 1990)[93]. These neuropeptides take part in regulating secretion from goblet cells (Kamijo et al., 1993)[94] and the mucosal glands (Coles et al., 1980)[95]. Recent studies have demonstrated that neuropeptides play important roles in amplifying topical inflammatory responses.

These tachykinins and CGRP can be released from C-fibers in response to various stimuli. SP was shown to promote secretion and ciliostimulation in vivo (Lindberg et al., 1986)[67], and in vitro (Hisamatsu et al., 1995)[71]. SP and NKA were reported to show ciliostimulation in rabbit and rat nasal mucosa in vivo. NKA (Kahn et al., 1986 ; Lindberg et al., 1986)[68,69], and tachykinin-induced ciliostimulation are mediated by NK1 receptors (Lindberg et al., 1993)[97]. NKA-induced ciliostimulation in vitro was also reported (Kondo et al., 1990)[98]. As described above, the temporary effects of preformed SP and NKA released from C-fibers should be considered in short-term observations of ciliary activity in tissue culture less than 30 minutes after experimental stimuli with tested materials. The author and co-workers reported the parallel effects of neuropeptides on secretions and ciliary activity (Hisamatsu et al., 1995)[71]. CGRP was also reported to promote ciliary activity in vitro (Tamaoki et al., 1989)[99].

Postganglionic parasympathetic nerves contain VIP along with acetylcholine and polypeptides with histidine at the N-terminal and methionine at the C-terminal (PHM)(Lundberget al., 1984 ; Uddman et al., 1979)[100,101]. In an in vitro study, VIP was reported to induce ciliostimulation in rabbits, and using a neutral endopeptidase inhibitor, phosphoramidon, ciliostimulation was shown to be due to the increase in cyclic AMP level in epithelial cells (Sasaki et al., 1991)[102]. Cyclic AMP was reported to cause ciliostimulation in vitro (Tamaoki et al., 1983)[103,104].

2. Autonomic neurotransmitters. Merke and co-workers reported that the parasympathomimetic agonist methacholine and sympathomimetic beta 2-adrenoceptor agonist salbutamol showed ciliostimula-

tion in vivo, and alpha 1- and alpha 2-adrenoceptor agonists, phenylephrine and oxymetazoline retard ciliary activity (1982)[105,106,107].

8. Effects of medicines on ciliary activity

Several drugs have been reported to affect ciliary activity.

Lysozyme. The author and co-workers reported that egg-derived lysozyme induced ciliostimulation in vitro using mucus blanket-eliminated mucosal explants (Hisamatsu et al., 1986)[108].

Ibdilast. A new macrolide antibiotic agent, Ibdilast, showed ciliostimulatory effects in vitro with possible elevation of intracellular cyclic AMP level (Hisamatsu et al., 1995)[109].

Xanthine derivatives. Theophiline and enprophyline induced acceleration of mucociliary wave frequency in vivo (Cervin et al., 1995)[38].

Indomethacin. This nonsteroidal anti-inflammatory drug may enhance ciliary beat frequency in vitro, and the ciliostimulation may be mediated by leukotrienes produced by activation of the lipoxygenase pathway of arachidonic acid metabolism (Tamaoki et al., 1988)[110]. However, this explanation for the indomethacin-induced ciliostimulation seems inappropriate because the author and co-workers elucidated LTD_4-induced ciliary inhibition.

Summary

The factors relevant to mucociliary transport function of the airway mucosa were reviewed focusing on ciliary activity. Recent advances in studies dealing with ciliated cells have been made on the basis of techniques developed for mucosal surface observation and measurement of ciliary activity for long observation periods using a video tape recording system. Conventional techniques are inadequate for observation of ciliated cells in the form of cell or tissue culture for long observation periods over 60 minutes, and previously reported results were inconsistent. Therefore, careful follow-up studies are required.

To understand in vivo phenomena observed in patients with inflammatory and/or allergic respiratory diseases, it is important to study the effects of each inflammatory mediator on the ciliated epithelium under simplified conditions in vitro considering the following factors: 1) specificity of the phenomenon induced by the tested mediator; 2) the lowest effective concentration of mediator; 3) the detectable concentration of the mediator in vivo, i.e., in secretions covering mucosal epithelium; 4) the minimum exposure time required to affect ciliated cells; 5) conversion of the mediators; and 6) involvement of second messengers.

MBP, an eosinophil granule cationic protein, and lipid mediators such as LTC_4, LTD_4 and PAF have been shown to have the capacity to inhibit ciliary activity and damage the airway epithelium. However, the experimental methods used in most reported studies concerning the effects of inflammatory mediators on ciliary activity were insufficiently accurate resulting in inconsistent results. Therefore, careful follow-up studies remain to be performed.

As the next step in these studies, the phenomena also have to be analyzed with regard to the details of their mechanisms; for instance, how ciliary activity is promoted, ciliary activity is inhibited, and mucosal epithelial cells are damaged, etc. Such studies will contribute to our understanding of the clinical pharmacology of respiratory diseases, and the advances in this scientific field will also provide new therapeutic strategies.

References

1) Satir P. (1974) How cilia move. Scientific American, 231: 44-52.
2) Lucas AM, Douglas LC. (1934) Principles underlying ciliary activity in the respiratory tract. II. A comparison of nasal clearance in man, monkey and other mammals. Arch Otolaryngol, 20: 518-541.
3) Sasaki Y, Wagner Y, Hornick RB, Schwartz AR, Proctor DF. (1973) Mucociliary function during experimentally induced rhinovirus infection in man. Ann Otol Rhinol Laryngol, 82: 203-211.
4) Proctor DF, Wagner JHN. (1965) Clearance of perticles from the human nose. Arch Environ Health 11: 366-371.
5) Andersen I, Lundquist GR, Proctor DF. (1972) Human nasal mucosal function under four controlled humidities. Am Rev Respir Dis 106: 438-449.
6) Andersen I, Camner P, Jensen PL, Proctor DF. (1974) Nasal clearance in monozygomatic twins. Am Rev Respir Dis, 110: 301-305.
7) Quinlan MF, Salman SD, Swift DL, Wagner HL Jr, Proctor DF. (1969) Measurement of mucociliary function in man. Am Rev Respir Dis, 99: 13-23.
8) Sakakura Y, Ukai K, Majima Y, Murai S, Harada T, Miyoshi Y. (1983) Nasal mucociliary clearance under various conditions. Acta Otolaryngol

(Stockh), 96 : 167-173.
9) Vinter B, Elbrønd O. (1978) Nasal mucociliary function during penicillin treatment. Acta Otolaryngol (Stockh), 86 : 132-134.
10) Ginzel A, Peter I. (1981) Messung des Schleimtransport in der Nase an Gesunden und im Anshluss an eine Erkältungmittels des Saccharin-sky-blue-Tests. HNO-Praxis, 6 : 31-37.
11) Eliezer N, Sadé J, Silberberg A, Nevo AC. (1970) The role of mucus in transport by cilia. Am Rev Respir Dis, 102 : 48-52.
12) Hisamatsu K (1987) Sino-bronchial syndrom and related factors -A study on mucociliary transport system. J Jpn Bronchoesophagol Soc, 38 : 158-162, 1987.
13) King M, Gilboa A, Meyer FA, Silberberg A. (1974) On the transport of mucus and its rheologic simulants in ciliated system. Am Rev Respir Dis, 110 : 740-745.
14) Chen TM, Dulfano MJ. (1978) Mucus viscoelasticity and mucociliary transport rate. J Lab Clin Med, 91 : 423-431.
15) Dahlamn T. (1956) Mucus flow and ciliary activity in the trachea of healthy rats and rats exposed to respiratory irritant gases. Acta Physiol Scand (Suppl) : 123 : 1-61.
16) Litt M. (1978) Mucus rheology. Relevance to mucociliary clearance. Arch Int Med, 126 : 417-423.
17) Proctor DF, Adams GK, Andersen I, Man SF. (1978) Nasal mucociliary clearance in man. Ciba Found Symp, 54 : 219-234.
18) Bang BG, Bang FB. (1963) Response of upper respiratory mucosa to dehydration and infection. Ann NY Acad Sci, 106 : 625-630.
19) Majima Y, Swift DL, Bang BG, et al. (1981) A study on mechanism of chicken nasal mucociliary dysfunction exposed to SO_2. Pract Otol (Kyoto), 74 : 1191-1203.
20) Sakakura Y. (1983) Changes of mucociliary function during colds. Eur J Respir Dis, 128 (Suppl 1) : 348-354.
21) Majima Y, Sakakura Y, Matsubara T, Miyoshi Y. (1986) Possible mechanisms of reduction of nasal mucociliary clearance in chronic sinusitis. Clin Otolaryngol, 11 : 55-60.
22) Hisamatsu K, Makiyama K, Aoyagi M, Okuno H, Kawamura S, Horikawa Y, Ikeda K. (1981) A study on ciliary function of chronic sinusitis patients using photoelectrical measurement. Oto Rhino Laryngol Tokyo, 27 (Suppl 1) : 243-249.
23) Pedersen M, Mygind N. (1980) Ciliary motility in the 'immotile cilia syndrome'. First results of microphoto-oscillographic studies. Br J Dis Chest, 74 : 239-244.
24) Pedersen M, Sakakura Y, Winther B, et al. (1983) Nasal mucociliary transport, number of ciliated cells, and beating pattern in naturally acquired common colds. Eur J Respir Dis, 64 (Suppl 128) : 355-365.
25) Itoh H, Majima Y, Sakakura Y, Miyoshi Y. (1984) Comparison of the X-ray mucous membrane function test and quantitative surface ultrastructure in chronic sinusitis. Pract Otol (Kyoto), 77 : 1375-1380.
26) Ganbo T, Hisamatsu K, Nakajima M, Inoue H, Murakami Y. (1995) Effect of platelet activating factor (PAF) on mucociliary clearance of the eustachian tube. Ann Otol Rhinol Laryngol, in press.
27) Hybbinette HC. (1982) A method for evaluating the effect of pharmacological substances on mucociliary activity in vivo. Acta Otolaryngologica, 93 : 151-159.
28) Lindberg S, Cervin A, Mercke U, Uddman R. (1988) VIP potentiates cholinergic effects on the mucociliary system in the maxillary sinus. Otolaryngol Head Neck Surg, 99 : 401-407.
29) Cervin A, Bende M, Lindberg S, Mercke U, Olsson P. (1988) Relations between blood flow and mucociliary activity in the rabbit maxillary sinus. Acta Otolaryngologica, 105 : 350-356.
30) Dolata J, Lindberg S, Merke U. (1989) The effect of prostaglandins E_1, E_2 and F_2 alpha on mucociliary activity in the rabbit maxillary sinus. Acta Oto-Laryngologica, 108 : 290-297.
31) Dolata J, Lindberg S, Merke U. (1989) Cholinergic and C-fibre mediated mechanisms in the stimulation of mucociliary activity induced by prostaglandins and histamine. Acta Oto-Laryngologica, 108 : 456-463.
32) Dolata J, Lindberg S, Merke U. (1990) The influence of leukotrienes and platelet activating factor on mucociliary activity in the rabbit maxillary sinus. Acta Oto-Laryngologica, 109 : 149-154.
33) Dolata J, Lindberg S, Merke U. (1990) Histamine stimulation of mucociliary activity in the rabbit maxillary sinus. Ann Oto Rhinol Laryngol, 99 : 666-671.
34) Cervin A, Lindberg S, Merke U. (1991) The effect of neuropeptide Y on mucociliary activity in the rabbit maxillary sinus. Acta Oto-Laryngologica, 111 : 960-966.
35) Cervin A, Lindberg S, Merke U. (1991) Sympathetic nerve stimulation influences mucociliary activity in the rabbit maxillary sinus. Acta

Physiol Scand, 143 : 405-411.
36) Cervin A, Lindberg S, Merke U. Uddman R. (1992) Neuropeptide Y in the rabbit maxillary sinus modulates cholinergic acceleration of mucociliary activity. Acta Oto-Laryngologica, 112 : 872-881.
37) Cervin A, Lindberg S, Merke U. (1993) The effect of noradrenaline on mucociliary activity in the rabbit maxillary sinus. Rhinology, 31 : 17-21.
38) Cervin A, Dolata J, Lindberg S, Merke U. (1995) Cyclic adenosine monophosphate stimulation of mucociliary activity in the upper airways in vivo. Ann Oto Rhinol Laryngol, 104 : 388-393.
39) Ballenger JJ (1960) Experimental effect of cigarette smoke on human respiratory cilia. N Engl J Med, 263 : 832-835.
40) Corssen G, et al. (1958) A comparison of the toxic effects of various local anesthetic drugs on human ciliated epthelium in vtro. Texas Respir Biol Med, 16 : 194-202.
41) Nakamura I. (1968) A study on ciliary activity related to topical therapy for chronic sinusitis. Pract Otol (Kyoto), 61 : 1135-1155.
42) Shimizu H (1955) An experimental study on ciliary movement of the nasal mucosa. Pract Otol (Kyoto), 48 : 635-653.
43) Saito H, Uede K, Terazono T, Miyoshi S, Hoshino A, Takenaka H. (1982) A method for detection of ciliary dysfunction induced by verious drugs in tissue culture - forcusing HPC -. Oto-Rhino-Laryngol Tokyo, (Suppl 4) : 319-329.
44) Rossman CM, Lee RM, Forrest JB, Newhouse MT. (1984) Nasal ciliary ultrastructure and function in patients with primary ciliary dyskinesia compared with that in normal subjects and in subjects with various respiratory diseases. Am Rev Respir Dis, 129 ; 1 : 161-167.
45) Rylander R. (1966) Current techniques to measure alteration in the ciliary activity to intact respiratory epithelium. Am Rev Respir Dis, 93 : 67-85.
46) Chevance LG, Hannoun C. (1975) Influenza virus and ciliary beating of the respiratory epthelium in sensitized animals. Dev Biol Stand, 28 : 181-185.
47) Aoyagi M. (1980) Immunological observation on ciliary movement of tracheal membrane. Otolaryngology Tokyo, 52 : 141-147.
48) Sakuma S. (1980) Bacterio-immunological investigations on the ciliary movement in the respiratory tract. Nihon Univ Med J, 39 : 809-816.
49) Hisamatsu K, Aoyagi M, Sakuma S, Makiyama K, Saito H. (1981) Effect of reaginic antigen-antibody reaction on the ciliary activity of the human nasal mucosa. Rhinology, 19 : 290-294.
50) Olsson I, Venge P. (1972) Cationic proteins of human granulocytes. I. Isolation of the cationic proteins from the granules of leukaemic myeloid cells. Scand J Haematol, 9 : 204-214.
51) Archer RK, Broome J. (1963) Studies on the peroxidase reaction of living eosinophils and other leukocytes. Acta Haematol (Basel), 29 : 147-156.
52) Gleich GJ, Loegering DA, Bell MP, Checkel JL, Ackerman SJ, Mackean DJ. (1986) Biochemical and functional similarities between human eosinophil-derived neurotoxin and eosinophil cationic protein : homology with ribonuclease. Proc Natl Acad Sci USA, 83 : 3146-3150.
53) Gleich GJ, Frigas E, Loegering DA, Wassom DL, Steinmuller D. (1979) Cytotoxic properties of the eosinophil major basic protein. J Immunol, 123 : 2925-2927.
54) Gleich GJ, Loegering DA and Maldonado JE. (1973) Identification of a major basic protein in guinea pig eosinophil granules. J Exp Med, 137 : 1459-1471.
55) McLaren DJ, McKean JR, Olsson I, Venges P, Kay AB. (1981) Morphological studies on the killing of schistosomula of Schistosoma mansoni by human eosinophil and neutrophil cationic proteins in vitro. Parasite Immunol, 3 : 359-373.
56) Frigas E, Loegering DA, Solley GO, Farrow GM, Gleich GJ. (1981) Elevation level of the eosinophil granule major basic protein in the sputum of patients with bronchial asthma. Mayo Clin Proc, 56 : 345-353.
57) Hisamatsu K, Ganbo T, Ogino J, Yamauchi Y, Murakami Y, Koyama H, Makiyama K, Gleich GJ. (1990) Cytotoxicity of human eosinophil granule major basic protein to human nasal sinus mucosa in vitro. J Allergy Clin Immunol, 86 : 52-63.
58) Smallman LA, et al. (1984) Reduction of ciliary beat frequency in vitro by sputum from patients with bronchiectasis : a serine protease effect. Thorax, 39 : 663-667.
59) Tegner H, Ohlsson K, Toremalm NG, von Mecklenburg C. (1979) Effect of human leukocyte enzymes on tracheal mucosa and its mucociliary activity. Rhinology, 17 : 199-206.
60) Santa Cruz R, Landa J, Hirsch J, Sackner MA. (1974) Tracheal mucous velocity in normal man and patients with obstructive lung disease ; effects of terbutaline. Am Rev Respir Dis, 109 : 458-463.
61) Mezey RJ, Cohn MA, Fernandez RJ, Januszkiewicz AJ and Wanner A. (1978) Mucociliary transport in allergic patients with antigen-induced bronchospasm. Am Rev Respir Dis, 118 : 677-684.

62) Ahmed T, Greenblatt DW, Birch S, Marchette B, Wanner A. (1981) Abnormal mucociliary transport in allergic patients with antigen-induced bronchospasm : role of slow reacting substance of anaphylaxis. Am Rev Respir Dis, 124 : 110-114.
63) Wanner A, Maurer D, Abraham WM, Szepfalusi Z, Sielczak M (1983) Effects of chemical mediators on ciliary function. J Allergy Clin Immunol, 72 : 663-667.
64) Bisgaard H, and Andersen M. (1987) SRS-A leukotrienes decrease the activity of human respiratory cilia. Clin Allergy, 17 : 95-103.
65) Weisman Z, Fink A, Alon A, Poliak Z, Tabachnik E, Priscu L, Bentwich Z.(1990) Leukotriene C_4 decreases the activity of respiratory cilia *in vitro*. Clin Exp Allergy 20 : 389-393.
66) Tamaoki J, Sakai N, Kobayashi K, Kanemura T, Takizawa T. (1991) Stimulation of airway ciliary motility by immunologically activated canine pulmonary macrophages: role of leukotrienes. Act Physiol Scand, 141 : 415-420.
67) Lindberg S, Hybbinette JC, Mercke U. (1986) Effects of neuropeptides on mucociliary activity. Ann Otol Rhinol Laryngol 95 : 94-100.
68) Khan AR, Bengtsson B, Lindberg S. (1986) Influence of substance P on ciliary beat frequency in airway isolated preparations. Eur J Pharmacol, 130 : 91-96.
69) Petersson G, McCaffrey TV, Malm L. (1989) Substance P and nasal secretion in dog, rat and man. Ann Allergy, 62 : 410-414.
70) Staskowski PA, McCaffrey TV. (1992) Effect of substance P on ciliary beat frequency in human adenoid explants. Otolaryngol Head Neck Surg, 107 : 553-557.
71) Hisamatsu K, Kamijo A, Terakawa S, Ganbo T, Mizukoshi A, Murakami Y. (1995) Effects of sensory neuro peptides on secretory responses of epithelial goblet cells and ciliary activity. Oto-Rhino-Laryngol Tokyo, 38 : 296-303.
72) Tate SS, Meister A. (1978) Serine-borate complex as a transition-state inhibitor of γ-glutamyl transpeptidase. Proc. Natl. Acad. Sci. USA. 75 : 4806-4809.
73) Hisamatsu K, Ganbo T, Nakajima M, Nakazawa T, Murakami Y. (1995) Effect of leukotriene C_4 exposure on ciliated cells of the nasal mucosa. Prostaglandins (in press).
74) Wanner A., Silczak M, Abraham WM. (1986) Ciliary responsiveness in allergic and nonallergic airways. J Appl Physiol, 60 : 1967-1971.
75) Nakazawa T, Hisamatsu K, Uchida T. (1994) Leukotriene D_4-induced mucosal damage during long observation periods in vitro. Prostaglandins, 47 : 379-392.
76) Creticos PS, Peters SP, Adkinson JNF, Naclerio RM, Hayes EC, Norman PS, Lichitenstein LM. (1984) Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. N Engl J Med, 310 : 1624-1630.
77) Okuda M, Watase T, Mezawa A, Liu CM. (1988) The role of LTD_4 in allergic rhinitis. Ann Allergy, 60 : 537-540.
78) Ganbo T, Hisamatsu K, Shimomura S, Nakajima T, Inoue H, Murakami Y. (1995) Inhibition of mucociliary clearance of eustachian tube by leukotriene C_4 and D_4. Ann Otol Rhinol Laryngol, 104 : 231-236.
79) Miadonna A, Tedeschi A, Arnoux B, Sala A, Zanussi C, Benveniste J. (1989) Am Rev Respir Dis, 140 : 142-147.
80) Hisamatsu K , Ganbo T, Ogino J, Yamauchi Y, Murakami Y, Koyama H, Makiyama K, Gleich GJ. (1988) Cytotoxicity of platelet activating factor (PAF) to the human nasal mucosa. Oto-Rhino-Laryngology Tokyo 31Supp.7 : 919-920.
81) Hisamatsu K, Ganbo T, Nakazawa T, Murakami Y. (1991) Platelet activating factor induced respiratory mucosal damage. Lipids, 26 : 1287-1291.
82) Ganbo T, Hisamatsu K. (1990) Mucosal dysfunction and damage induced by platelet activating factor (PAF). Acta Otolaryngol (Stockh), 110 : 427-438.
83) Ganbo T, Hisamatsu K, Nakazawa T, Kamijo A, Murakami Y. (1991) Platelet activating factor (PAF) effects on ciliary activity of human paranasal sinus mucosa in vitro. Rhinology, 29 : 231-237.
84) Ganbo T, Hisamatsu K, Nakazawa T, Kamijo A, Murakami Y (1991) : Platelet activating factor (PAF) effects on ciliary activity of human paranasal sinus mucosa in vitro. Rhinology, 29 : 231-237.
85) Blank ML, Lee T, Fitzgerrald V, Synder F. (1981) A specific acetylhydrase for 1-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine (a hypotensive and platelet-activating lipids). J Biol Chem, 256 : 175-178.
86) Wykle RL, Malone B, Synder F. (1980) Enzymatic synthesis of 1-alkyl-2-acetyl-sn-glycero-3-phosphorylcholine, a hypotensive and platelet-aggregating lipids. J Biol Chem, 255 : 10256-10260.
87) Ganbo T, Hisamatsu K, Inoue H, Horiguchi S, Shimomura S, Murakami Y : Turn-over of PAF in cultures of human paranasal sinus mucosa. Rhinology, 32 : 127-129, 1994.

88) Inoue H, Ganbo T, Hisamatsu K , Ishida H : Study on the metabolism of lyso-platelet activating factor (lyso-PAF) in human paranasal sinus mucosa. The cultured ciliated epithelium can convert lyso-PAF to PAF. Life Science 52 : 227-232, 1993.
89) Ganbo T, Hisamatsu K, Inoue H, Nakamima T, Kamijo A, Murakami Y : The effect of lyso-PAF on ciliated cells of human paranasal simus mucosa. American Journal of Rhinology, 8 (5) : 320-321, 1994.
90) Ganbo T, Shimomura S, Hisamatsu K, Nakazawa T, Nakajima T, Nozawa I, Murakami Y. (1994) The effect of leukotriene C_4, D_4, and prostaglandin E_2 on mucociliary transport of the eustachian tube. Otol Jpn, 4 : 90-94.
91) Tamaoki J, Kobayashi K, Sakai N, Chiyotani A, Takizawa T. (1989) Effect of bradykinin on airway ciliary motility and its modulation by neutral endopeptidase. Am Rev Respir Dis, 140 : 430-435.
92) Uddman R, Malm L, Sundler F. (1983) Substance-P-containing nerve fibers in the nasal mucosa. Arch Otorhinolaryngol, 238 : 9-16.
93) Baraniuk JN, Lundgren JD, Goff J, et al. (1990) Calcitonin gene-related peptide in human nasal mucosa. Am J Physiol 258 (Lung Cell Mol Physiol 2) : L81-L88.
94) Kamijo A, Terakawa S, Hisamatsu K (1993) Neurotransmitter-induced exocytosis in goblet and acinar cells of rat nasal mucosa studied by video microscopy. American Journal of Physiology, 265 : 1200-1209.
95) Coles SJ, Bhaskar KR, O'Sullivan DD, Neill KH, Reid LM. (1980) Airway mucus : composition and regulation of its secretion by neuropeptides in vitro. In : Mucus and Mucosa, Ciba Foundation Symposium 109, Pitman, London, pp40-60.
96) Lindberg S, Dolata J, Mercke U. (1986) Effects of neurokinin A and calcitonin gene-related peptide on mucociliary activity in rabbit maxillary sinus. Regulatory Peptides, 16 : 15-25.
97) Lindberg S, Dolata J. (1993) NK1 receptors mediate the increase in mucociliary activity produced by tachkinins. Eur J Pharmacol, 231 : 375-380.
98) Kondo M, Tamaoki J, Takizawa T. (1990) Neutral endopeptidase inhibitor potentiates the tachykinin-induced increase in ciliary beat frequency in rabbit trachea. Am Rev Respir Dis, 142 : 403-406.
99) Tamaoki J, Kanemura T, Kobayashi K, et al. (1989) Effects of calcitonin gene-related peptide on airway epithelial functions in dogs. Peptides 10 : 1007-1011.
100) Lundberg JM, Fahrenkrug J, Larson O, Anggard A. (1984) Corelease of vasoactive intestinal polypeptide and peptide histidine isoleucine in relation to atropine-resistant vasodilation in cat submandibular salivary gland. Neurosci Lett 52 : 37-42.
101) Uddman R, Sundler F. (1979) Vasoactive intestinal polypeptide nerves in human upper respiratory tract. ORL 41 : 221-226.
102) Sasaki N, Tamaoki J, Kobayashi K, Kanemura T, Isono K, Takeyama K, Takeuchi S, Takizawa T. (1991) Vasoactive intestinal peptide stimulates ciliary motility in rabbit tracheal epithelium : modulation by neutral endopeptidase. Regulatory Peptides, 34 : 33-41.
103) Tamaoki J, Kondo M, Takizawa T. (1989) Effect of cAMP on ciliary function in rabbit tracheal epithelial cells. J Appl Physiol, 66 : 1035-1039.
104) Tamaoki J, Kondo M, Takizawa T. (1989) Adenosine-mediated cyclic AMP-dependent inhibition of ciliary activity in rabbit tracheal epithelium. Am Rev Respir Dis, 139 : 441-445.
105) Merke U, Hybbinette JC, Lindberg S. (1982) Parasympathetic and sympathetic influences on mucociliary activity in vivo. Rhinology, 20 : 201-204, 1982.
106) Hybbinette JC, Merke U. (1982) Effects of parasympathomimetic drug methacholine and its antagonist atropine on mucociliary activity. Acta Otolaryngol, 93 : 465-473.
107) Hybbinette JC, Merke U. (1982) Effects of sympathomimetic agonists and antagonists on mucociliary activity. Acta Otolaryngol, 94 : 121-130.
108) Hisamatsu K, Yamauchi Y, Uchida M, Murakami Y. (1986) Promotive effect of lysozyme on the ciliary activity of the human nasal mucosa. Acta Otolaryngol (Stockh), 101 : 290-294.
109) Hisamatsu K, Ganbo T, Mizukoshi A, Kamijo A, Kou J, Nakajima T, Murakami Y. (1995) Effect of Ibudilast (KC-404) on ciliary activity of human paranasal sinus mucosa in vitro. Jpn Pharmacol Ther, 23 : 849-853.
110) Tamaoki J, Kondo M, Takizawa T. (1988) Stimulation of ciliary activity by indomethacin in rabbit tracheal epithelium organ culture. Respir, 54 : 127-131.