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REVIEW

Airway goblet cells: responsive and adaptable front-line defenders

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Airway goblet cells: responsive and adaptable front-line defenders. D.F. Rogers. ©ERS Journals Ltd 1994.

ABSTRACT: Goblet cells are situated in the epithelium of the conducting airways, often with their apical surfaces protruding into the lumen, a location which fits them for a rapid response to inhaled airway insults. Together with the submucosal glands, goblet cells secrete high molecular weight mucus glycoproteins (mucins), which confer upon the airway surface fluid the requisite biochemical and biophysical properties which determine the efficiency of entrapment and transportation of inhaled irritants, particles and micro-organisms. The diversity of glycosylation of airway mucins may be important in facilitating adherence of micro-organisms to mucus prior to mucociliary clearance. Other secretory products, including lipids and "small" glycoproteins, may also be produced by goblet cells.

It is possible that goblet cells have the potential to produce markedly more mucus than do the glands. Mucins are tightly packed in the intracellular granules of the goblet cell. The morphology of these granules varies with fixation technique, and release of mucins may be *via* a combination of merocrine and apocrine secretion. Discharge of mucus is accomplished remarkably rapidly (tens of milliseconds) and vast quantities of mucus are released (size expansions from the granule of many hundredfold).

Depending upon species and preparation, goblet cells discharge mucus in response to a wide variety of stimuli, including proteinases, irritant gases, inflammatory mediators, reactive oxygen species, nerve activation and changes in the biophysical environment. Under normal conditions, goblet cell proliferation and differentiation, particularly to ciliated cells, contributes to maintenance of the airway epithelial cell population. In addition to participating in acute airway defence, goblet cells increase in number in response to chronic airway insult, with a resultant increase in output of mucus. The increase in number of cells is *via* hyperplastic and metaplastic mechanisms. Early triggers for the development of a hypersecretory epithelium include excessive discharge of mucus and increased expression of airway mucin messenger ribonucleic acid (mRNA). Cessation of chronic airway stress rapidly reverses the increased number of goblet cells. Irritant-induced increases in number of goblet cells can be inhibited by a variety of drugs with anti-inflammatory and mucoregulatory properties, and the reversal to normal numbers after cessation of the irritation is speeded by these drugs.

The ability of goblet cells to be progenitors of ciliated cells, to rapidly produce vast quatities of mucus in response to acute airway insult, and to change in number according to variations in chronic insult indicates that these cells are vitally important responsive and adaptable front-line defenders of the airways.

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Goblet cells are one of at least 22 principal cell types identified in the respiratory tract and their distribution varies with species, airway level and disease status [1]. They are situated in the epithelium of the conducting airways, which indicates that their phenotype is appropriate for contributing to first-line defence of the mucosa. A major part of this first-line defence is responding to inhalation of airborne irritants, particles and micro-organisms with the production of a fluid barrier to overlie and protect the airway epithelial surface. Once produced, the fluid has to have appropriate biochemical and biophysical properties to enable it to trap these particles before many more of them impinge upon the epithelial surface and cause damage. Once trapped, the particles have to be removed from the airways. Removal is accomplished by the ciliated cells, which transport the mucus on the tips of beating cilia towards the throat, a process termed mucociliary clearance. The properties of the airway surface fluid which are important in determining its ability to interact successfully with the cilia are its viscosity

and elasticity. The combination of the two properties, termed viscoelasticity, affects the efficiency of mucociliary transport. High elastic recoil with intermediate viscosity is considered the best rheological combination for maximal transport velocity [2]. The principal components of the fluid which confer viscoelasticty are the high molecular weight glycoconjugates, the mucus glycoproteins or mucins [3].

Mucins are produced and secreted by specialized cells in the epithelium, including the serous cell, the goblet cell and, possibly, the ciliated cell, and by seromucous glands in the submucosa. Serous cells are in low numbers in airways of animals which are not specific pathogen-free (SPF), and although containing intracellular secretory granules mucin production may be subsidiary to other protective functions of this cell type. Ciliated cells, although not containing secretory granules, may be involved in the production of "surface mucosubstance". However, the precise role of the ciliated cell in contributing to the mucin content of airway surface fluid remains ill-defined and speculative. In contrast, goblet cells have as their most distinctive feature numerous intracellular granules which contain mucins [4, 5]. In the present review, the term granule rather than vesicle will be used, because the latter may imply immature granules without any membrane, as frequently identified in airway cell culture. It is often considered that, because of their greater prominence in histological section, submucosal glands rather than goblet cells contribute the greater quantity of mucus to airway surface fluid. However, detailed morphometric analysis of the amount of stainable stored secretory product in the tracheobronchial airways of the Rhesus monkey reveals that there is at least twice as much mucus in the surface epithelium as in the submucosal glands [6] (fig. 1). In the lower airways of the Rhesus monkey, as in the majority of other animal species studied [1], glands are absent and goblet cells are the sole source of mucus. Admittedly, measurements merely of amount of intracellular mucus do not take into account possible differences in the rate of secretion between gland and goblet cell or of differences in ability to sustain secretion. It is also of particular interest to determine, using these morphometric techniques, the ratio of goblet cell to gland mucus content in human airways. Nevertheless, the histological measurements above do indicate that, at least in the Rhesus monkey, goblet cells can represent a much greater potential for secretion than do the submucosal glands. Thus, it can be argued that the goblet cell represents the principal front-line defender of the airway.

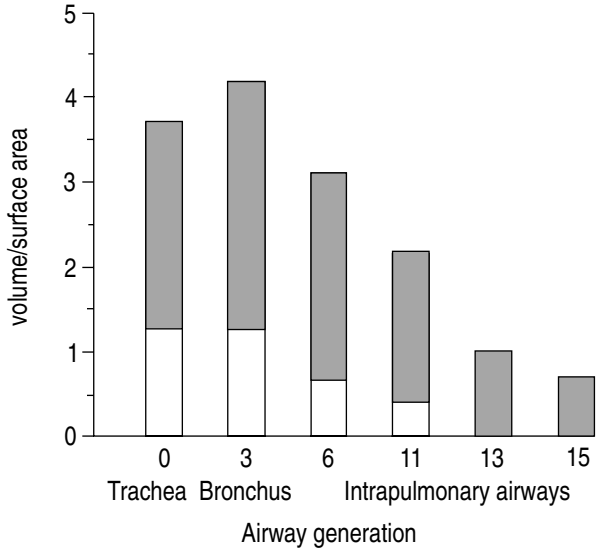

Fig. 1. – Morphometric comparison of the volume of stored stainable secretory product ($mm^3 \times 10^{-3}$) per unit surface area basal lamina (mm^2) in tracheobronchial airways of the rhesus monkey. Airway sections were stained with Alcian blue and periodic-acid-Schiff in sequence, and the amount of stained intracellular product was quantified using an image analyser. ▭ : surface epithelium; □ : submucosal glands. More than two-thirds of the total secretory product is contained in the surface epithelium in all airway generations (No. 0=trachea; No. 3=lobar bronchus of right middle lobe; Nos 6–15= intrapulmonary airways with No. 15 being pulmonary artery side of the respiratory bronchiole). (Recalculated and redrawn after PLOPPER *et al*. [6], 1989).

New information demonstrates that goblet cells can discharge vast quantities of mucus in fractions of a second, a property integral to airway defence against acute insult. Goblet cells can also increase in number in response to continued airway insult, a property integral to defence against chronic stress, but which may also precipitate airway diseases associated with hypersecretion of mucus, including chronic bronchitis [7], asthma [8, 9] and cystic fibrosis [10]. New research is shedding light on the cellular and molecular mechanisms underlying changes in airway goblet cell number, and should prove invaluable in the further understanding of the pathogenesis of hypersecretory airway disease. The present review will focus on the two above-mentioned principal characteristics of these important cells, namely their ability to react rapidly to acute insult and their ability to change in number in response to chronic stress.

Morphology: is "goblet" an appropriate descriptive term?

Goblet cells were identified by early histologists as those cells, principally in the gut lining, which had a cup-shaped apical aspect filled with secretory granules, called the "theca", atop a tapering base, akin to the stem of a goblet, which extended to the basal lamina of the epithelium (see [11]). In the conducting airways, surface epithelial cells distended by secretory granules can be readily identified both by light and electron microscopy; and, although these cells do not possess an obvious theca, the term goblet cell is frequently applied to them. However, the morphology of the goblet cell varies with the preparative technique used, so much so that, under certain conditions, the characteristic "goblet" shape is lost altogether.

After standard chemical fixation in gluteraldehyde, transmission electron microscopy reveals goblet-like epithelial cells which bulge with electron-lucent granules, many of which coalesce (fig. 2a and b). The nuclei are irregular and contain condensed chromatin. In contrast, after quick-freezing and cryosubstitution, these same cells now contain highly electron-dense granules which do not

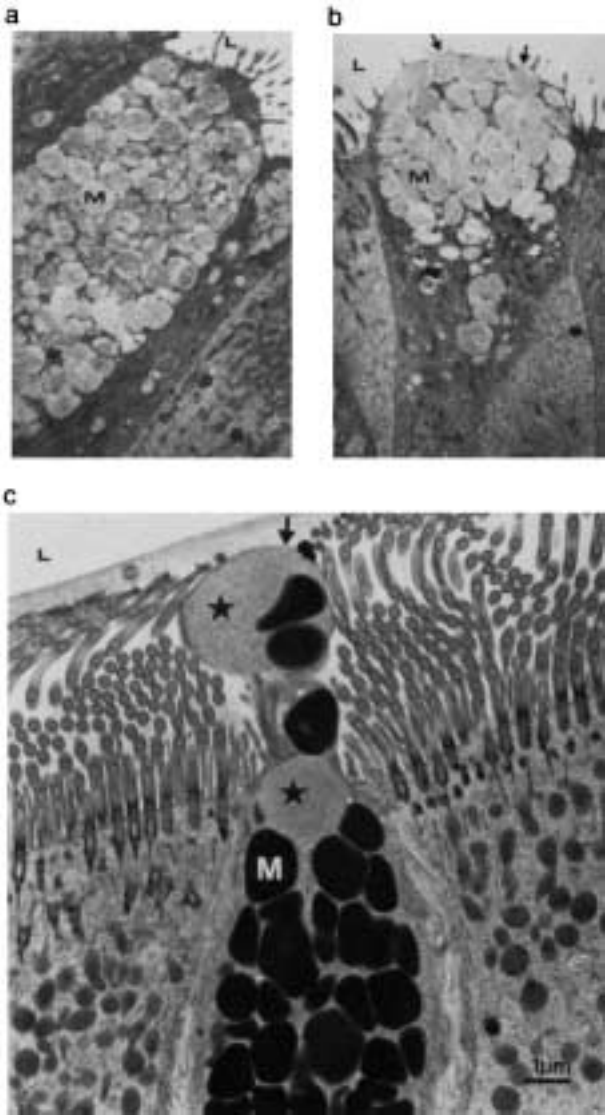

Fig. 2. – "Goblet" cells in respiratory epithelium: fixation-dependent differences in morphology and mode of mucus discharge (transmission electron microscopy). a) and b) Goblet cells in human bronchus after gluteraldehyde fixation and post-fixation in osmium tetroxide, demonstrating classically-described intracellular mucus granules (M) of varying electron-lucency, many of which coalesce: a) undischarged cell, packed and distended by granules; b) partially discharged cell, with remaining granules converged at apex (which protrudes into the airway lumen (L)) demonstrating merocrine secretion of mucin from individual granules (arrows). (Magnification ×5,200). c) Epithelial mucous secretory cell flanked by ciliated cells in cryofixed and cryosubstituted frog palate (a mucociliary respiratory epithelium) demonstrating electron-dense mucus granules (M) of varying size and which do not coalesce [14]. Partially lucent circular areas (★) at cell apex and within the cilia boundary correspond to cytoplasm containing mucus granules and represent apocrine secretion. A fragment of the mucous cell apical membrane is visible at the apex of the upper cytoplasmic extrusion (arrow). (Magnification ×6,000). (Electron micrograph (c) kindly supplied by E. Puchelle, INSERM 314, Reims, France).

coalesce or distend the cell [12–14] (fig. 2c). The nucleus is ovoid and contains dispersed chromatin. Quick-freezing is considered to be superior to chemical fixation for the preservation of the fine structure of tissues. The highly hydrophilic nature of intragranular mucus is, in particular, responsible for many of the problems associated with chemical fixation of mucous cells and may contribute to swelling *in situ* of the intracellular granules. It is possible, therefore, that the term "goblet" cell is descriptive of a fixation artefact.

It should be noted, however, that neither of the morpholgies described above is likely to represent the appearance of the living cell, and in the present article both the bulging and nonbulging cells demonstrated by different fixation techniques for electron microscopy will be termed goblet cells. In this review, the term goblet cell will also be used to define any surface epithelial cell in the conducting airways which by light microscopy contains acidic mucin, for example staining blue or heliotrope with sequential Alcian blue and periodic acid-Schiff (PAS) [15]. These cells are usually distended by secretion and their apices protrude above the upper aspect of the epithelium. Other cells, for example cells in culture, which are derived from the epithelium and can be demonstrated to secrete mucus macromolecules may be considered to be goblet cells. However, to acknowledge that their precise phenotype is ill-defined, this category of cells will be referred to in this review as epithelial secretory cells.

Mucin structure

Immunocytochemical studies demonstrate that goblet cells contain mucins [4, 5] which, at masses of up to 7,000 kDa and lengths of up to 6 µm [16], are among the largest molecules in nature. Much of the information concerning the structure of airway mucins has been surmised from samples of airway fluid obtained from patients with airway diseases associated with mucus hypersecretion: subjects without airway disease produce insufficient airway fluid from which to extract and purify mucins, although the newly-utilized technique of inducing sputum with inhaled hypertonic saline should allow sampling of airway fluid from normal subjects. It is possible, therefore, that some of the structural information currently available is not applicable to "normal" human mucins. In particular, it should be noted that it is those molecules, isolated by gel filtration, with a high relative molecular mass (Mr) which are selected for detailed study. Selection of particular molecules may limit investigation of other smaller but, nevertheless, important secretory moieties. Further identification of the genes which encode for mucin and the nature of their product is required before a complete understanding of mucin structure will be achieved. Nevertheless, a number of general principles concerning mucin structure can currently be identified. These principles apply to mucins in general and are not specific for the secretory product of any particular secretory cell type, including goblet cells: there is little evidence that goblet cell mucin is the same or different from submucosal gland mucin.

The most defensible proposed structure for airway mucins is of numerous glycoprotein subunits joined end-to-end by disulphide bonds to form a long thread-like polymer [13, 16, 17]. The subunits consist of a peptide

core, comprising approximately 20% of the polymer, bristling with short carbohydrate side-chains (comprising the remaining 80%). Seventy to eighty percent of the core is highly glycosylated. The remaining 20–30% comprises unglycosylated regions (or "naked" regions) containing cysteine residues, which represent cleavage sites. The oligosaccharide side-chains are attached to the peptide core *via* O-glycosidic bonds, namely linkages between N-acetylgalactosamine on the side-chain and serine and threonine on the core. The side-chains are remarkably diverse, with several hundred different types expressed in one person [18]. The side-chains can be linear or branched and vary considerably in length (2–20 monosaccharides long) and in monosaccharide composition. They are invariably sulphated and terminate in sialic acid, both of which factors confer polyanionic properties on the mucin molecule. The biological significance of diversity in glycosylation of airway mucins is ill-defined, but one function may be interaction with micro-organisms and facilitation of their removal by mucociliary clearance [18]. Many carbohydrates expressed on the surface of host cells are recognized by adhesins and haemagglutinins of many species of fungi, bacteria, mycoplasmas and viruses, a number of which are respiratory pathogens. Similar carbohydrate moieties are detected in airway mucins and may represent recognition sites for micro-organisms, allowing mucus entrapment and clearance, and contributing to protection of the underlying mucosa. The final mucus polymer is produced by the long mucin molecules becoming entangled to produce a loosely-woven random network, which is held together by the tangles as well as, perhaps, by low energy inter-chain bonds [13]. A tangled polymer network explains a number of properties of mucus, including its non-Newtonian behaviour (viscosity changing with flow rate) and the ability of different mucous gels to anneal.

It should be noted, however, that in addition to mucus, hamster tracheal epithelial secretory cells in culture produce secretions where the mucus-like glycoproteins are associated with lipids and "small" molecular weight glycoproteins [19]. The physiological significance of these other components of secretion remains to be clarified, but the presence of lipids and small glycoproteins demonstrates that goblet cell secretions may be even more complex than hitherto defined.

Mucin packaging and discharge

Mucus-like glycoproteins from hamster tracheal epithelial secretory cells appear to be released into the airway lumen *via* three mechanisms; namely, a constitutive and a regulated pathway, both of which involve mucin secretion from intracellular granules, and by cell surface release by the action of proteinases [19]. The latter mechanism is characteristic of release of mucins from the "surface mucosubstance" [20]. The constitutive pathway may be represented by small intracellular granules ("vesicles") which continuously secrete a small amount of mucus for minimal but maintained airway protection. The regulated pathway is represented by granules from which mucins are discharged in response to stimuli, either environmental or pathophysiological.

The enormous mucin molecules described above have to be packaged within the intracellular granules of the goblet cells, ready for discharge. Packaging involves keeping the mucus molecules as small as possible during synthesis for retention within the secretory granules, a process termed condensation. Discharge involves the mucus molecules in a rapid expansion to enormous size, a process termed decondensation.

Condensation and decondensation

The polyanionic nature of the mucus polymer means that the individual strands of the network are separated by electrostatic repulsion. Mucus condensation is achieved by the granules containing high concentrations of Ca^{2+}, which acts as a "shielding" cation to nullify the repulsive forces within the molecule [21]. Mucus discharge, if similar to exocytotic processes in other systems [22], probably involves fusion of the outer surface of the intracellular granule with the inner surface of the apical aspect of the goblet cell, and the development of a pore which is comparatively unique in that it spans two membranes, those of the granule and the cell. Opening of the pore between intragranular space and extracellular space permits water to enter the vesicle and Ca^{2+} to exit. Loss of charge-shielding allows electrostatic repulsion to rapidly expand the mucin polymer network, which facilitates hydration with the incoming water, and the resultant vastly expanded network erupts from the cell in the fashion of a "jack-in-the-box" [13, 21].

The above model explains many of the principal events in discharge of mucus from goblet cells. However, a number of factors pertaining to secretion remain to be explained. For example, where do the cell-surface attached mucins come from? Are they perhaps secretory mucins which did not disengage from the inner surface of the granule, and which have now become extracellular on the outer surface of the cell membrane of which the vesicle has become a part? In addition to mucins, a number of important nonmucin proteins are found in goblet cell granules, including endoperoxidases and protease inhibitors [23, 24]. How these molecules are packaged with mucins in the secretory granules, and how they may interact with mucins in the discharge process, are questions which are presently unexplored.

Rapidity of mucus discharge

The process of mucin discharge obeys first order kinetics, and size expansions of many hundredfold are achieved in only tens of milliseconds. In isolated epithelial secretory cells of the guinea-pig trachea, the eventual size of the discharged secretory product (before dispersion into the medium is evident) is comparable to that of the secretory cell itself (fig. 3). The rapidity of exocytosis

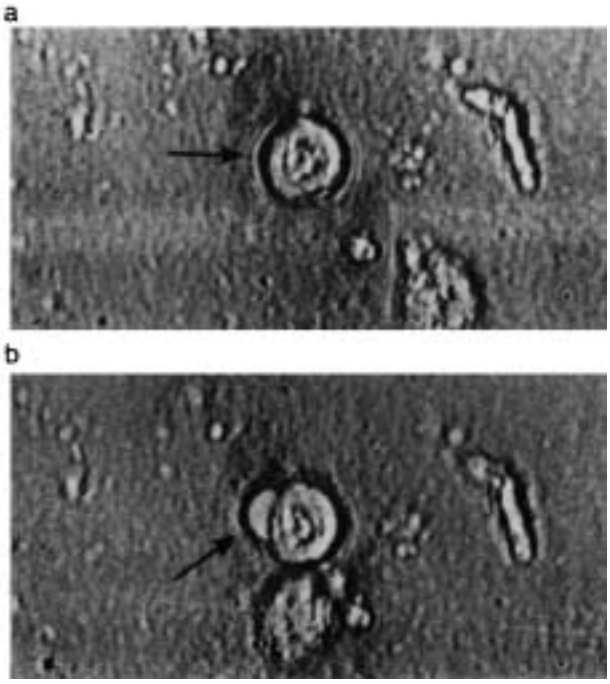

Fig. 3. – Spontaneous mucus discharge *in vitro* by an isolated cultured guinea-pig tracheal epithelial secretory cell. a) Quiescent cell. b) Mucus secretion is seen as a rapidly-developing volumetric expansion from the surface of the cell, which later disperses in the bathing medium. The size of the exocytosed mucus globule is equivalent to that of the secretory cell. (Videorecordings at ×600 magnification).

by goblet cells in explanted canine tracheal epithelial sheets can be visualized by differential interference video microscopy as the sudden disappearance from the cell surface of a single secretory granule (termed a "degranulation event"), and the approximate speed of the degranulation event can be assessed [25]: by 67 ms after time 0 (considered to be the beginning of the exocytotic process) the vesicle was already only partially visualized (presumed to be undergoing exocytosis), and by 134 ms the vesicle had gone (exocytosis was presumed to be complete) (fig. 4). Using similar video microscopic technology, goblet cell exocytosis has been found to be equally rapid in explants from rat nasal mucosa [27], and human nasal and tracheobronchial mucosa [26] (figs 5 and 6). In each of the three studies above, there was very little baseline secretion. Interestingly, despite minimal spontaneous degranulation, secretory granules in some goblet cells could be seen to be moving slowly about within the cell [25, 27]. With stimulation, granule movement within the cells was increased, often vigorously. Degranulation events were discrete and separate; goblet cells did not appear to release groups of vesicles together.

The rapidity of degranulation by isolated airway goblet cells *in vitro* is not repeated *in vivo*. Significant reduction in the amount of stainable intracellular mucin in guinea-pig airways (presumed to represent secretion of mucus from goblet cells) is only apparent a few minutes (3–15 min depending upon the stimulus used) after administration of the stimulus to secrete [28–30]. In *in vivo* experiments, accessibilty of drug and the involvement of indirect mechanisms undoubtedly slows determination of the secretory response. Nevertheless, even *in vivo*, goblet cell discharge can be comparatively rapid with maximal discharge achieved within a few minutes.

Merocrine versus *apocrine secretion*

The observations described above using video microscopy of live airway mucosal explants indicate that goblet cells secrete mucus *via* a purely merocrine process (*i.e.*

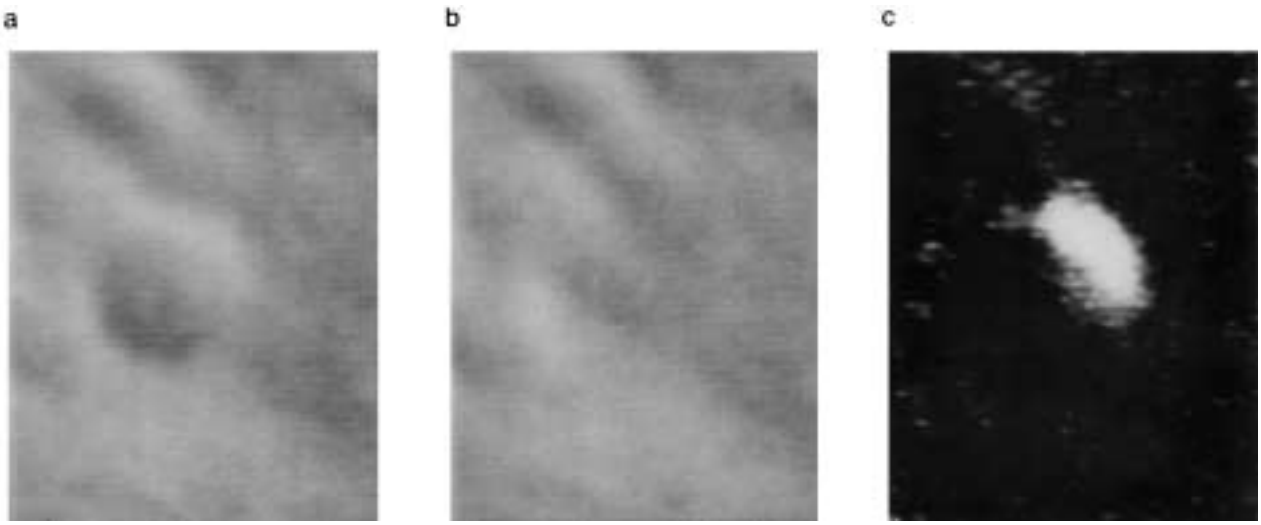

Fig. 4. – Detection of mucin granule exocytosis *in vitro* by digital image subtraction (for details of methods, see [25, 26]). a) A 2 µm granule in a canine tracheal epithelial cell just before exocytosis. b) After exocytosis 134 ms later (granule has ceased to be visualized). Images acquired by imaging system from videotape. c) Result of subtracting b) and a). Because of the "shadow-cast" nature of images acquired by Normaski microscopy, only one half of the granule is visualized (the bright white object) in the subtracted image; the other half of the granule is black. (Video images kindly provided by C.W. Davis and M.I. Lethem, University of North Carolina, Chapel Hill, USA. Current address for M.I. Lethem, Dept of Pharmacy, University of Brighton, Brighton BN2 4GJ, UK).

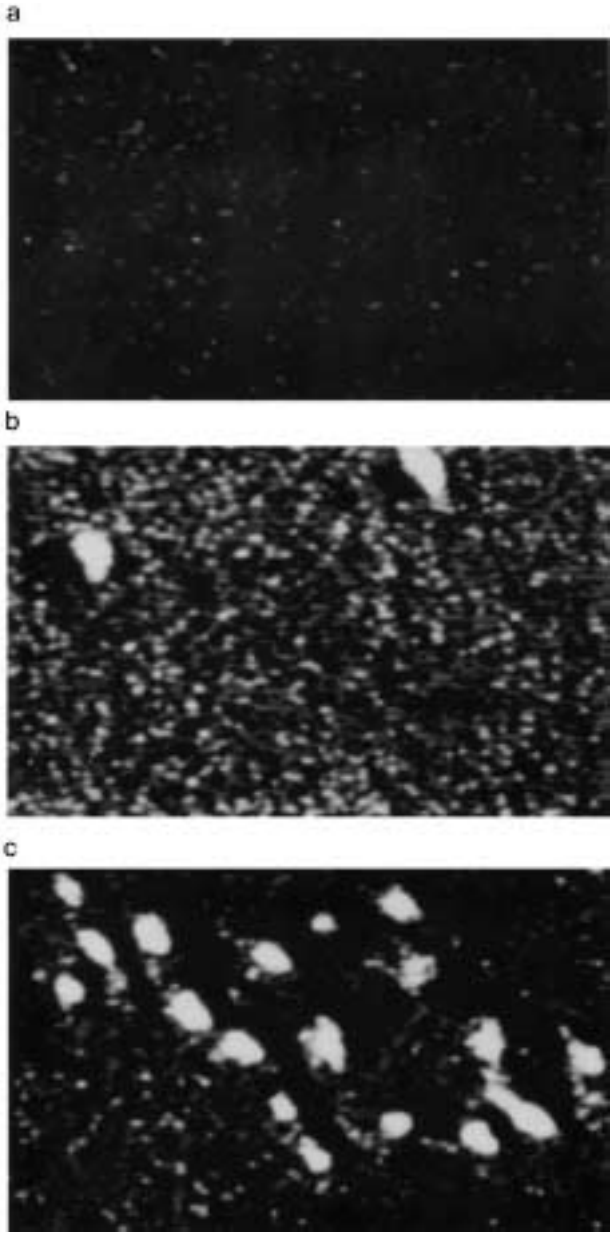

Fig. 5. – The first two seconds of secretion by a human airway epithelial secretory cell *in vitro*, as visualized by image subtraction (acquired from an S-VHS video tape recorder) (for details of methods, see [25, 26]). Images are the results of subtracting an image (two-frame averages at 1 s intervals) obtained 1 s prior to the commencement of secretion a), and at 1 s b) and 2 s c) after commencement of secretion, in response to luminally-applied adensone triphosphate (ATP; 0.1 mM). Individual degranulations are visualized as bright white objects (granules nominally 1 μm across). (Video imaging kindly provided by C.W. Davis and M.I. Lethem (see legend to fig. 4)).

discharge of secretory product alone from the intact cell). However, histological observation of mouse and rat trachea [31, 32], human nasal mucosa [33], and cryofixed frog palate (a model respiratory epithelium) [14] has demonstrated secretory extrusions suggestive of apocrine secretion (fig. 2c). The extent to which apocrine secretion contributes to discharge of mucus by goblet cells is not apparent from the latter studies. In living cells of rat nasal mucosal explants, evidence of apocrine secretion was frequently found in preparations which were "exhausted" but was

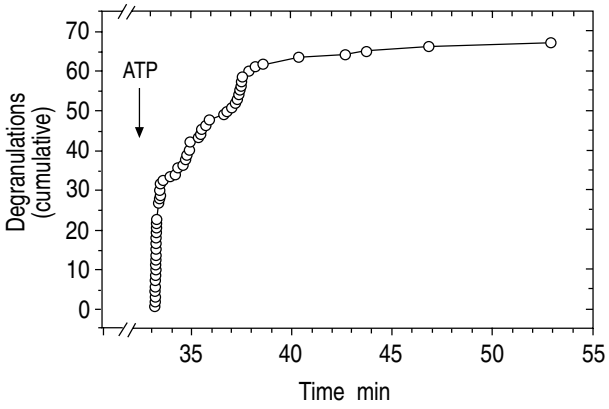

Fig. 6. – Time-course of mucus exocytosis by a single human airway epithelial secretory cell *in vitro*, in response to luminally-applied adenosine triphosphate (ATP; 0.1 mM applied at arrow) (for details of methods, see [25, 26]). Data are cumulative degranulations or exocytoses. Initial rapid burst of degranulations is accomplished within seconds and reaches a maximum within minutes. (Figure kindly provided by C.W. Davies and M.I. Lethem (see legend to fig. 4)).

not found in fresh preparations [27]. It is tempting to speculate from the latter observations that merocrine secretion is the principal mechanism of discharge of mucus from goblet cells and that apocrine secretion may represent an end-stage response by exhausted cells. Conversely, it may be that apocrine secretion is an adjunct to merocrine secretion under conditions of stimulation following mechanical or chemical injury. Further studies are required to clarify the relative contributions of apocrine and merocrine mechanisms of secretion to discharge of mucus from airway goblet cells.

Induction of goblet cell secretion

Irritant gases, proteinases, a variety of inflammatory mediators and neural agents, and changes in the biophysical environment will induce release of mucus by airway goblet cells (table 1). Significantly, and perhaps not unexpectedly, the effect of many of these agents varies with the type of preparation used. In particular, there is a discrepancy between the secretory responses by *in vivo* or *in vitro* explant preparations compared with the responses by primary cultures of confluent hamster tracheal surface epithelial cells, which are enriched with secretory cells [19]. The latter preparations produce glycoconjugates, which have been termed "mucin-like glycoproteins" to acknowledge incomplete characterization. Nevertheless, these cultures produce glycoproteins with the size, charge and carbohydrate composition characteristic of airway mucins. One concern would be that the cultures also produce proteoglycans, which indicates that the secretory cells are expressing a phenotype in culture that is not normally expressed *in vivo*. The "culture shock" of isolation from their accustomed surroundings and finding themselves at a density greater than normal may contribute to the difference in response by these secretory cells to a number of humoral agents, compared to goblet cells in uncultured and unenriched preparations (see below). The specific reasons for the differences in secretory response between the enriched

Table 1. – Inducers of airway goblet cell secretion

Inducer		*In vivo*/explant	Enriched culture
Proteinases		Potent [32, 34, 35]	Yes [19]
Irritant gases:	cigarette smoke	Yes [29, 36]	NR
	sulphur dioxide	Yes [37]	NR
	ammonia vapour	Yes [38]	NR
Prostaglandins		Yes [39]	No [19]
Leukotrienes		Yes [40]	No [19]
Platelet-activating factor		Small/no effect [41–43]	NR
Histamine		Variable [27, 40, 51]	No [19]
Cyclic nucleotides		NR	Yes [25, 26, 44, 45]
Oxygen metabolites (oxidants)		Yes [39]	NR
pH (acidic or basic)		NR	Yes [19]
Mechanical strain:	hypo-osmolality	NR	Increased [19]
	hyperosmolality	NR	Decreased [19]
	gel contraction	NR	Increased [19]
Calcium ionophore (A 23187)		NR	No [19]
cAMP analogues (or IBMX)		NR	No [19]
Nerve stimulation:	cholinergic	Yes [30, 46]	NR
	adrenergic	?No [30, 46]	NR
	NANC	Yes [30, 46, 47]	NR
Cholinoceptor agonists		Variable [27, 40]	No [19]
Adrenoceptor agonists		No [46]	No [19]
Capsaicin		Yes [28]	No [48]*
Substance P (and neurokinin A)		Yes [28]	Yes [27, 48]*
Calcitonin gene-related peptide		Weak [28]	NR

Enriched culture: hamster tracheal epithelial cells at confluence where the proportion of secretory cells is increased (secretory cell metaplasia) [19]. cAMP: cyclic adenosine monophosphate; IBMX: isobutyl-1-methylxanthine; NANC: nonadrenergic noncholinergic (neural mechanisms); Variable: reported effects at variance; *: in isolated cells, not enriched culture; NR: not reported; [ ]: references.

cultures and other *in vitro* and *in vivo* systems are unclear, but may be related to differences in receptor expression (perhaps certain receptors are internalized or rendered unresponsive under conditions of enriched culture), or to differences in intracellular signal transduction mechanisms (perhaps receptors and signalling mechanisms become uncoupled or otherwise disrupted). The latter suggestion is, however, not applicable to all stimuli, because activation of signal transduction mechanisms can be demonstrated in epithelial secretory cells from the enriched culture in response to exogenously-administered adenosine triphosphate (ATP) [44]. Further direct comparative studies are required to determine the mechanisms of differences between preparations and their overall relevance to goblet cell secretion *in vivo*.

Measurement of goblet cell secretion

Apart from experimental discrepancies between different *in vitro* culture techniques and *in vivo* preparations, measurement of secretion of mucins by airway goblet cells is invariably made indirectly. Unlike human intestinal goblet cells, where a specific trefoil factor has been identified as a marker for secretion [49], there is currently no direct selective method to detect and quantify secretion of airway goblet cell mucus. No technique is without its advantages and disadvantages, the relative importance of which will be determined by the context in which the technique is being used.

Radiolabelled precursors of mucin are often used to quantify secretion *in vitro* and *in vivo*. Similarly, lectin staining, antibodies to mucus, staining with "mucus" stains (in particular Alcian blue and PAS), or utilization of endogenous markers for mucus (for example fucose) may each be used to quantify the magnitude of airway goblet cell secretion. The reliability of each technique is dependent upon the selectivity for goblet cell mucus of the radiolabel, stain, antibody or other marker. Selectivity requires characterization in the particular experimental situation in which the mucus marker is to be used. In preparations where there may be more than one source of mucus, selectivity of these markers will be reduced. Use of preparations in which the source of mucus is restricted, for example the goose trachea, which has no submucosal glands, or goblet cell cultures, may heighten selectivity but may also give inconclusive data on mechanisms of secretion, because of different emphasis on control mechanisms compared with preparations where the source of secretion is not restricted. Thus, measurement of goblet cell secretion is imprecise and the nature of the secretory product invariably ill-defined. In the following discussion, the technique and marker used to measure mucus will be identified, in order to allow assessment of the validity of the measurements, of what is being measured, and from what source the secretion is derived.

Effect of proteinases, irritants, inflammatory mediators, superoxide, nucleotides and biophysical stress on goblet cell secretion

Proteinases are highly potent *in vitro* in inducing output of radiolabelled glycoconjugates (presumed to be predominantly mucins) by epithelial cells, both in rabbit

and guinea-pig tracheal explants and in enriched hamster tracheal cell culture [19, 32, 34, 35]. The secretory response by goblet cells to these enzymes would be consistent with airway defence against bacterial invasion and in response to airway inflammation and the influx of neutrophils. Merocrine secretion appears not to be the primary mechanism of mucus output from the secretory cells. Instead, the mechanisms underlying the secretory response appear to be either cleavage of cell-surface-attached mucins followed by their subsequent proteolytic degradation, or proteolytic damage of the cell membrane, which causes apocrine leakage of secretory vesicles and cytoplasm.

Irritant gases, including cigarette smoke, sulphur dioxide and ammonia vapour are notable in inducing secretion of mucus by goblet cells, measured either as radiolabel output or as loss of Alcian blue-PAS-stainable material from the secretory cells [29, 36–38]. Antigen challenge, by trimellitic anhydride, of sensitized guinea-pigs *in vivo* also induces loss of stainable intracellular mucin from tracheal goblet cells [50]. In a similar manner to the response by goblet cells to proteinases, a rapid secretory response to inhaled irritants or antigen would facilitate airway defence by providing a protective fluid layer against further damage. The mechanisms of action of sulphur dioxide or ammonia in inducing goblet cells to secrete are ill-defined. However, changes in pH of the airway surface fluid induced by these soluble gases may cause mucus output, because alterations in pH to either acidic or basic increases radiolabel output by cultured hamster tracheal epithelial cells, a response mediated *via* damage to the cell membrane [19]. The secretory effect of cigarette smoke and trimellitic anhydride challenge on goblet cell discharge in guinea-pig airways *in vivo* is mediated, at least in part, *via* activation of nerves [29, 50].

A number of inflammatory mediators influence goblet cell secretion, although to varying degrees depending upon the preparation used. Products of arachidonic acid metabolism, prostaglandins (PG) E_2 and $F_{2\alpha}$ and leukotrienes (LT) C_4 and D_4, induce secretion of mucus in organotypic culture of guinea-pig tracheal epithelial cells [39], and in guinea-pig airways *in vivo* [40], but not in enriched hamster tracheal epithelial secretory cell culture [19]. Generation of $PGF_{2\alpha}$ is the principal mechanism underlying release of high molecular weight glycoconjugates by cultured guinea-pig and rabbit tracheal epithelium in response to oxygen metabolites (oxidants) [39]. Platelet-activating factor (PAF), when given at high concentrations (10 mM), induces small increases in secretion of mucus in tracheal explants from rat, rabbit and guinea-pig [41], species where the primary source of secretion is the goblet cells. PAF also stimulates secretion *in vitro* by cultured guinea-pig and canine tracheal epithelial secretory cells [41, 42], but has no significant effect on secretion of fucose by guinea-pig trachea *in vivo* [43]. The differences in response to PAF between the *in vitro* and *in vivo* systems may be due to other effects of PAF in the intact animal masking the secretory response, for example effects on plasma exudation and bronchoconstriction. The secretory response to histamine is similarly variable: histamine may [51] or may not [19, 27, 40] induce goblet cell secretion. Cyclic nucleotides, in particular ATP, increase degranulation rate *in vitro* by isolated canine and human goblet cells [25, 26] (fig. 6) and induce secretion by cultured hamster tracheal epithelial cells [44, 45], the latter *via* interaction with P_2 purinoceptors and subsequent activation of a signal transduction mechanism involving pertussis-toxin-sensitive guanine nucleotide binding (G)-protein(s) coupled to phospholipase C.

Hypo-osmolality or contraction of the gel support of the culture system induces secretion from cultured hamster tracheal epithelial secretory cells [19]. Both of these physical factors would tend to compact the cells, and might cause discharge of intracellular vesicles as a compensatory mechanism to decrease cell volume, either directly or indirectly *via* release of mediators. Interestingly, hyperosmolality, which would tend to increase cell volume, decreased secretion by these cultured cells. The inhibitory mechanism underlying the decrease in mucus output is unknown, but would be worth exploring because it would appear to be one of the very few endogenous mechanisms which inhibit goblet cell secretion rather than increasing secretion.

Neural control of goblet cell secretion

Demonstration of neural control of goblet cell secretion is dependent upon species (table 1). Electrical stimulation of the oesophageal nerves in the anaesthetized goose induces secretion of radiolabelled macromolecules into an isolated tracheal segment, a preparation where the source of secretion is presumed to be the goblet cells because of the absence of submucosal glands [46]. Similarly, in rats [47] and guinea-pigs [30, 52], species with sparse airway submucosal glands, electrical stimulation of the vagus nerves induces loss of stainable intracellular mucus granules from the goblet cells. In contrast, in the cat, a species with abundant airway glands, nerve stimulation does not induce goblet cell secretion [53, 54]. Taken together, these limited observations indicate that where the proximal lower airways (*i.e.* trachea) do not contain submucosal glands, the goblet cells secrete in response to neural stimulation (presumably these cells are "innervated"). In contrast, where glands are abundant, the goblet cells are not innervated. It would be interesting to determine whether goblet cells are innervated in the lower airways, from which glands are normally absent, in species with abudant glands in the upper airways.

In the goose and guinea-pig *in vivo*, the principal neural pathway mediating tracheal goblet cell secretion is parasympathetic (cholinergic), with very little evidence of sympathetic (adrenergic) innervation [30, 46]. Consistent with these observations is the finding that acetylcholine increases degranulation event rate by goblet cells in explants of rat nasal mucosa [27]. In contrast, in guinea-pig lower airways *in vivo*, a 1% aerosol of acetylcholine does not induce goblet cell discharge [40]. We have found that the secretory response to acetylcholine *in vivo* by guinea-pig goblet cells is only apparent at $pM \cdot kg^{-1}$ doses, a response blocked by atropine, but was

not apparent at higher doses (H-P. Kuo, unpublished observations). The reasons for the secretory stimulation by parasympathetic nerve stimulation and the variable effect of acetylcholine are unclear, but may relate to the sensitivity of goblet cells and the accessibilty of drug. Further direct comparative studies are required to clarify these discrepancies.

In addition to parasympathetic neural mechanisms inducing goblet cell discharge, in goose, guinea-pig and rat tracheae, a small but significant degree of goblet cell secretion is evident after cholinoceptor inhibition by atropine [30, 46, 47], which indicates that nonadrenergic, noncholinergic (NANC) mechanisms contribute to neural control of goblet cell secretion in these species. At the simplest level, the NANC neural pathways mediating airway mucus secretion are twofold: an orthodromic (efferent) neural system and a "sensory-efferent" neural system [55]. The orthodromic system comprises cholinergic nerves and adrenergic nerves, in which neuropeptides are co-localized and co-released with the "classical" neurotransmitters. The neuropeptides fulfil a number of criteria which identify them as neurotransmitters, and include vasoactive intestinal peptide (VIP) and peptide histidine isoleucine (PHI: PH-methionine in humans), which are co-localized with acetylcholine in cholinergic nerves, and neuropeptide Y (NPY), which is co-localized with noradrenaline in adrenergic nerves. The effect on goblet cell secretion of VIP, PHI or NPY has not, at present, been reported, and investigation of the relevance of these neuropeptides to endogenous neural control of goblet cell secretion awaits development of more selective receptor antagonist drugs than are currently available [56].

The second NANC neural system comprises a population of C-fibre afferents (sensory nerves) which are sensitive to stimulation by capsaicin, the pungent ingredient of hot *Capsicum* peppers [57]. These capsaicin-sensitive sensory nerves also serve a motor (or efferent) function and have, therefore, been termed "sensory-efferent" [58]. Activation of sensory-efferents induces mucus discharge from goblet cells in guinea-pig trachea *in vivo* [28–30]. The neurotransmitters of these sensory-efferent nerves are peptides which, in mammalian species, include calcitonin gene-related peptide (CGRP) and the tachykinns substance P (SP), neurokinin A (NKA) and NKB which are collectively termed "sensory neuropeptides". Substance P increases the rate of degranulation *in vitro* by guinea-pig tracheal secretory cells [48] (fig. 7) and rat nasal mucosal goblet cells [27], and *in vivo* is markedly more potent in inducing mucus discharge than NKA, NKB or CGRP [28].

Currently, three classes of tachykinin receptor are recognized, termed NK_1, NK_2 or NK_3 according to their preferential affinity for each of the natural mammalian tachykinins [59]. The order of potency of SP>NKA >NKB in inducing goblet cell discharge *in vivo* [28] and *in vitro* [48], and the observation that a putative tachykinin NK_1 receptor antagonist blocks SP-induced secretion *in vitro* [48], indicates that the tachykinin receptor mediating goblet cell secretion, at least in guinea-pig trachea, is of the NK_1 type. Studies in other species and using selective synthetic tachykinin receptor agonists and antagonists are required to clarify the extent and nature of the involvement of sensory-efferent nerves and tachykinins in neural control of goblet cell secretion in the airways.

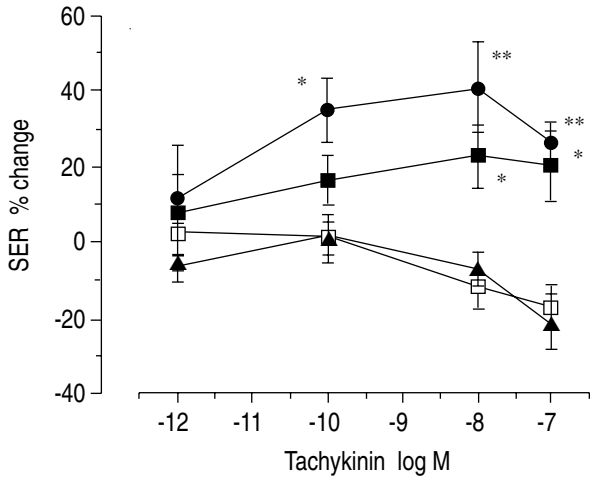

Fig. 7. – Effect of tachykinins on mucus secretion *in vitro* in cultured isolated guinea-pig tracheal epithelial secretory cells. Data are mean percentage change in secretory event rate (SER), a measure of mucus granule exocytosis, compared to prestimulus values (vertical bars are one SEM; n=5–7). —□— : saline vehicle; —●— : substance P; —■— : neurokinin A; —▲— : neurokinin B. *: $p<0.05$; **: $p<0.01$ compared with time-matched saline controls.

Goblet cell hyperplasia

The foregoing has shown that airway goblet cells can respond rapidly to a variety of acutely-administered stimuli and stress factors. In this section of the review, the response by goblet cells to chronic insult will be considered.

Increased numbers of airway goblet cells is a prominent feature of a number of bronchial diseases in humans which are associated with mucus hypersecretion, including chronic bronchitis [7], asthma [8, 9] and cystic fibrosis [10]. In attempts to understand pathophysiology, disease models have been developed in which airway goblet cell number is increased experimentally in laboratory animals, principally rodents. These studies demonstrate that goblet cells increase in number in response to a wide variety of drugs and irritants (table 2).

Experimental "chronic bronchitis"

Subacute exposure (*i.e.* over periods of a few days to weeks) to cigarette or cigar tobacco smoke [36, 60–62] or marijuana smoke [65], or to environmental pollutants, including sulphur dioxide [66], nitrogen dioxide [67], or chlorine [82], or repeated daily injections of adrenoceptor or cholinoceptor agonist drugs [68–71] or synthetic analogues of oestrogen [72], markedly increases goblet cell number in the more proximal airways (trachea, main bronchi and larger intrapulmonary bronchi), and induces the appearance of goblet cells in the more distal airways (smaller intrapulmonary bronchi and bronchioli). A single tracheal instillation of endotoxin

Table 2. – Inducers of airway goblet cell hyperplasia/metaplasia (and associated hypersecretion)

Hyperplasia/metaplasia	Hypersecretion
Tobacco smoke: cigarettes [36, 60–63]	Yes [88]
cigars [60]	NR
Nicotine: "high dose *i.p.* (but not "low" dose *s.c.* or aerosol) [64]	NR
Marijuana smoke [65]	NR
Sulphur dioxide [66]	NR
Nitrogen dioxide [67]	NR
Adrenoceptor agonists (isoprenaline, salbutamol) [68–70]	NR
Cholinoceptor agonists (pilocarpine, methacholine) [68, 71]	NR
Oestrogen analogues [72]	NR
Endotoxin [73]	Yes [73]
Respiratory tract infection [74, 75]	Yes [75]
Ozone [76]	Yes [81]
Elastase [77–79]	NR
Neutrophil lysates [80]	NR

i.p.: intraperitoneal injection; *s.c.*: subcutaneous injection; NR: not reported; [ ]: references.

(lipopolysaccharide) also induces goblet cell increase in rat airways [73]. The proliferative response to cigarette smoke would not appear to be due to an action of nicotine, because nicotine itself, administered at doses producing daily plasma concentrations comparable to those found in cigarette smokers, does not increase airway goblet cell number [64]. In addition to the increase in goblet cell number induced by irritants and drugs, goblet cells are also increased in size and contain an increased amount of intracellular Alcian blue-staining (acidic) mucin. Other histological changes include an increased height of the epithelium and enlargement of the submucosal glands. These latter changes and the increased numbers of goblet cells are characteristic of a "bronchitic" phenotype. The changes are produced comparatively rapidly; up to two weeks exposure is invariably sufficient. Of particular interest is that for a number of these agents, after development of a "bronchitic" mucosa, cessation of administration of the agent halts the course of the changes, and is followed by a slow reversal (over a number of weeks) to a normal morphology [70, 83, 84].

Experimental chronic bronchitis with emphysema

Ozone and elastase have also been used experimentally to model chronic lung disease [76, 77] and, although affecting goblet cell number, these agents are not specific for the induction of a purely "bronchitic" pathophysiology: ozone damages the airway epithelium, focusing on the respiratory bronchioles, and induces an inflammatory cell influx, whereas the principal effect of elastase is the induction of panacinar emphysema. Another departure from the induction of a "bronchitic" phenotype through goblet cell proliferation is that the response to elastase may not involve cell division [78]; goblet cells increase in number in the absence of mitosis. The "apparent" increase in goblet cell number is attributed to an increase in intracellular secretory granules, which reveals previously hidden secretory cells. The proliferative response to marijuana smoke may also be due, in part, to apparent increases in goblet cell number [65]. Whether increased amounts of intracellular secretion are due to increased mucin synthesis or decreased secretion, or a combination of the two, awaits clarification.

Interestingly, in contrast to the potent action of purified human neutrophil elastase in increasing goblet cell number and producing experimental emphysema in hamsters, unpurified neutrophil extract induces an increase in goblet cell number in large intrapulmonary airways but without producing emphysematous changes [80]. The unpurified extract was derived from white blood cell suspensions obtained by leucopheresis and, although presumably containing a high percentage of neutrophils, would contain products from other white cells. In addition, it is possible that the failure of the extract to induce emphysema was because its active agents did not reach the lower airways. The proliferative effect of the unpurified extract was not related to its elastolytic activity, which indicates that, in addition to elastase, neutrophils (or other white cells) contain products which can influence airway goblet cell number. The precise identity of these products awaits clarification, but from the neutrophil alone would include neutral proteases, acid hydrolases, lysozyme, myeloperoxidase, lactoferrin and alkaline phosphatase. Intriguingly, the lack of ability of the unpurified extracts to induce emphysematous changes indicates that these extracts contain products with anti-elastase activity. Again, the precise identity of these products are unknown but would include α_1-antitrypsin and tissue inhibitor of metalloproteinases (TIMP) from monocytes. Whatever the products involved, it is interesting that, unlike purified neutrophil elastase, unpurified neutrophil (?white cell) extracts produce "bronchitic" rather than emphysematous changes. Exposure of the airways to the extracts rather than the purified enzyme is presumably closer to the situation in real life, and investigation of the interations between white cell products may lead to greater understanding of the pathogenesis of chronic bronchitis and emphysema.

Hyperplasia versus *metaplasia*

Hyperplasia (involving cell division) and metaplasia (involving cell differentiation) maintain the normal cell complement in the tracheobronchial epithelium: goblet cells and indeterminate cells comprise the proliferating fraction with basal cells differentiating to both goblet and ciliated cells [63]. Goblet cells can also differentiate into ciliated cells, especially during ciliated cell regeneration. Thus, under normal conditions, the ability of goblet cells to undergo division and differentiation is an important component of the maintenance of the epithelial cell population. Under abnormal conditions, both of these "abilities" of goblet cells appear to contribute to their increased number induced experimentally.

The relative contribution of hyperplasia and metaplasia may vary depending upon airway level. For example, the number of dividing epithelial cells as a percentage of the total population (the "mitotic index") is increased in the airway epithelium of SPF rats exposed either to cigarette smoke [36] or to 500 ppm sulphur dioxide for 4 h·day^{-1}, 5 days a week for up to 3 weeks [66]. The increased mitotic index indicates that hyperplasia was responsible for the increased numbers of goblet cells. However, because the identity of the dividing cells was not reported, the extent to which mitosis accounts for the increase in goblet cells is unclear. In the lower airways of cigarette smoke-exposed or sulphur dioxide-exposed rats, the increased number of goblet cells did not coincide with significantly increased mitosis [36, 66], which indicates that the appearance of goblet cells in these small airways is primarily a metaplastic change (*i.e.* differentiation of pre-existing epithelial cells into goblet cells).

More definitive evidence for the contribution of cell division and cell differentiation to the increased numbers of goblet cells can be obtained by tracing the course of radiolabelled thymidine through the changing epithelial cell population of irritated airways. Using this technique, it was found that in SPF rats exposed to the smoke of 25 cigarettes·day^{-1}, daily for 2 weeks, the increased number of goblet cells arises as a result of two processes, namely differentiation from serous cells already present in the epithelium, and division of new (or pre-existing?) goblet cells [63]. The latter dual profile represents a combined hyperplastic and metaplastic response to airway irritation. Differentiation was inferred on the basis of two observations, namely coincident decreases in numbers of serous cells with increased numbers of goblet cells, and the appearance of cells intermediate in morphology between the two cell types [85, 86]. In these latter cells, both electron-dense and electron-lucent intracellular granules are distinguished. With increasing time of exposure to smoke, the number of epithelial cells with a predominance of electron-dense granules declines, to be replaced by a predominance of cells containing electron-lucent granules. "Intermediate" cells have also been reported in elastase-exposed hamsters [79], and in rats with respiratory tract infections [74]. Thus, it would appear that, in addition to cell division, differentiation from a serous to a mucous (goblet) phenotype is an important mechanism by which the secretory cells in the epithelium contribute to defence against a variety of airway insults.

Hyperplasia and hypersecretion

Although goblet cell number may be increased in response to inhaled gases and irritants or to systemic administration of drugs, it is of interest functionally to determine whether the increase in number of these cells is associated with an increase in mucus secretion into the airways. From autoradiographic analysis of incorporation of radiolabelled mucus-precursors by human bronchial explants, it can be inferred that rates of amino acid uptake and glycoprotein biosynthesis are increased in tissue from patients with chronic bronchitis compared with normal tissue [87]. The latter authors inferred from data for the serous and mucous cells of the submucosal glands that over the time period required for epithelial modification, increased mucin biosynthesis equates with increased mucus secretion. This inference is consistent with the finding that secretion of the mucus marker fucose is increased in an *in situ* laryngotracheal segment in SPF rats previously exposed subacutely to cigarette smoke, compared with air controls [88]. Increased numbers of goblet cells may, therefore, equate with hypersecretion. However, in the latter study, it is not possible to exclude the contribution of secretions from the submucosal glands in the larynx and upper trachea which, although sparse in SPF rats, are increased in size in response to cigarette smoke [89, 90]. The involvement of goblet cells in airway hypersecretion can be implicated from a subsequent study using perfused lungs *ex vivo*, where the trachea was cannulated sufficiently caudal to exclude the submucosal glands, which confirmed that fucose secretion was increased in smoke-exposed rats (fig. 8). Similarly, rates of secretion of radiolabelled glycoproteins are increased in tracheal explants from rats previously

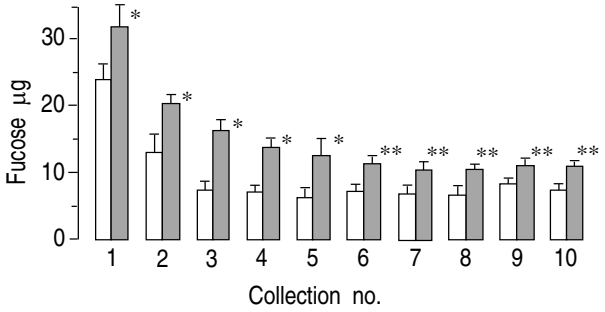

Fig. 8. – Effect of subacute exposure to cigarette smoke on basal secretion of fucose in rat lung *ex vivo*. Rats were exposed either to the smoke of 25 cigarettes·day^{-1} for 14 consecutive days (▭: n=6), or were given a sham exposure to air (□: n=5). The trachea, main bronchi and lungs were removed *en bloc* and were suspended in a moisture chamber at 37°C. The trachea was cannulated for perfusion of the airways with physiological saline. Effluent, containing secretions, passed out through cuts made in the lung parenchyma. Effluent was collected every 15 min for 2.5 h (a total of 10 collections) and each sample analysed for the endogenous mucus marker fucose (for details, see [88]). Data are mean fucose concentration. Vertical bars represent SEM. At each collection there was significantly more fucose in the cigarette smoke-exposed group (*: $p<0.05$; **: $p<0.01$) than in the sham-exposed group.

exposed to ozone (0.8 ppm for 14–90 days), compared to air controls [81]. The latter authors concluded, based on indirect evidence, that the hypersecretion was, in part at least, a result of functional changes in mucin biochemistry rather than merely due to secretory cell hyperplasia. Their conclusion further supports the concept that differentiation, principally involving a change in intracellular mucin chemistry, represents an important response by the airway secretory cells to epithelial insult.

Cellular and molecular mechanisms underlying goblet cell hypersecretion

The mechanisms underlying the development of a hypersecretory airway epithelium are comparatively unknown, but are beginning to receive attention. Presumably the development of the hypersecretory state involves altered regulation of one or more stages of the production of mucus. Alterations may be at any number of levels, including transcription, post-transcriptional processing, the stability of the ribonucleic acid (RNA) transcripts, translation, post-translational modification, or the stability of the protein itself. An early event in the sequence leading to mucus hypersecretion may be discharge of mucus, as an acute response to airway insult, which may in turn initiate goblet cell division. In hamster tracheal epithelial secretory cells in culture, ultrastructural analysis revealed that cells which in profile contained more than five secretory vesicles did not incorporate [^{3}H]-thymidine, whereas cells containing less than five granules did incorporate the label [91]. The latter observations indicate that loss of intracellular mucus to a critical level is a stimulus for goblet cells to enter the cell cycle.

It is interesting that a number of studies *in vivo* have noted that acute stimulation of goblet cell discharge, however potent the stimulus, fails to reduce the number of intra-cellular secretory granules to much below 70% of the number in unstimulated animals [28, 30, 40]. These observations indicate that there are physiological mechanisms which inhibit goblet cells discharging a large proportion of their mucus granules in response to acute stimulation. The observations further indicate that if discharge of mucus granules is minimized, replenishment of a comparatively small proportion of granules does not stimulate mitosis. The latter suggestion is compatible with the combined observations that with exposure to sulphur dioxide incorporation of [^{3}H]-thymidine by PAS-positive cells in rat airways (presumably predominantly goblet cells) [92] does not coincide with the period of induction of mucin mRNA expression [75]. In contrast, repeated discharge associated with chronic airway insult may successively deplete the number of mucus granules to the level where mitosis is stimulated.

The hypothesis above is purely speculative, but each aspect of the argument could be tested experimentally. For example, in the first instance, it is of interest to determine whether repeated administration of secretory agents will completely deplete goblet cells of intracellular secretion and, if so, will these depleted cells subsequently incorporate radiolabelled thymidine.

Another early stage in the development of a hypersecretory epithelium appears to be increased production of mucin mRNA. Infection of SPF rats with parainfluenza I virus, alone or in conjunction with subacute exposure to sulphur dioxide [75], or intratracheal instillation of endotoxin [73], induces goblet cell hyperplasia and associated mucus hypersecretion, and increases steady-state levels of mucin mRNA. Interestingly, mRNA was constitutively expressed in the intestine of these rats, which is consistent with the gut lining's requirement for continuous lubrication. In contrast, mucin mRNA appears to be minimally expressed in the airways under normal circumstances, but is increased in response to airway insult. This suggestion is consistent with the concept that very little mucus is produced by healthy airways but is increased under stress conditions [93]. It should be noted, however, that rat tracheal organ cultures *in vitro* demonstrate a strong basal level of mucin gene expression when incubated in medium containing retinoic acid [94]. Mucin mRNA is barely detectable in the absence of retinoic acid. The reasons underlying the differences in basal mucin gene expression between the *in vivo* study [75] and the *in vitro* study [94] are unclear, but may relate to the dietary status of the rats *in vivo* (*i.e.* the retinoic acid content of their food), or to the differing methodologies employed, in particular the use of molecular probes with marked sequence differences.

Significant increases in mucus secretion and mucin mRNA are detected by Northern blotting two days after endotoxin instillation [73] which, although not proving causality, indicates that the two events are linked. Interestingly, in the latter study, increased mucin mRNA was not detected at 6, 12 or 24 h post endotoxin. This comparatively slow time-course does not appear to be consistent with mucin mRNA induction being the initiating event in the development of a hypersecretory epithelium. However, Northern blotting may not have been sensitive enough to detect small increases in expression. Use of more sensitive molecular biology techniques, including quantitative polymerase chain reaction (PCR) assay, solution hybridization or the ribonuclease (RNAse) protection assay, should give further information on the time-course of mucin mRNA induction, and so clarify the role of mucin mRNA expression in the early events leading to the development of epithelial hypersecretion. Intriguingly, over the same time period of 2 days as induction of mucin mRNA by endotoxin [73], incubation of rat tracheal explants with a number of pharmacological agents including autonomic receptor agonists and histamine did not alter mucin mRNA levels [94]; although, whether these agents induced mucus secretion by the explants was not determined. Nevertheless, the latter observation of no increase in mucin mRNA with agonists compared with the increased expression in response to endotoxin [73] raises the question of whether merely increasing mucus secretion is sufficient to induce mucin mRNA or whether an injurious component, either alone or in addition to induction of mucus secretion, is required before mucin mRNA is increased. It would be of interest to determine just how much of an insult to the airway is required to "trigger" mucin mRNA. Although research

Table 3. – Inhibition and reversal of irritant-induced airway goblet cell hyperplasia/metaplasia

Drug		Inhibition of hyperplasia	Inhibition of hypersecretion	Reversal of hyperplasia
NSAIDs:	indomethacin	Yes [95, 97]	NR	Yes [102]
	flurbiprofen	NR	NR	Yes [102]
	PMO	Yes [36, 61, 96]	NR	NR
Corticosteroids:	dexamethasone	Yes [97, 100]	NR	NR
	prednisolone	Yes [97]	NR	NR
	hydrocortisone	Yes [97]	NR	NR
	budesonide	Yes [9]*	NR	Yes [9]*
"Mucoregulators":	N-acetylcysteine	Yes [98]	Yes [101]	Yes [103]
	S-carboxymethylcysteine	NR	Yes [101]	NR
Antimitotic:	vinblastine	Yes [99]	NR	NR
Flavenoid:	Zy 15850A	Yes [88]	NR	NR

NSAID: nonsteroidal anti-inflammatory drug; PMO: phenylmethyloxadiazole; Zy 15850A: sodium (4H-4-phenyl-thieno-[3,2-c-]-[1]-benzopyran)-2-carboxylate; NR: not reported; *: budesonide given to patients with newly-diagnosed asthma, making effect of drug a combination of inhibition of development of further asthmatic characteristics and reversal of existing characteristics; [ ]: references.

into the cellular and molecular mechanisms underlying airway goblet cell hyperplasia and metaplasia are at an early stage, it would appear that stimulation of mucin gene transcription represents an early event in the development of a hypersecretory epithelium.

Inhibition of goblet cell hyperplasia

The development of animal models, whereby airway goblet cell number can be increased experimentally, has allowed the investigation of whether the proliferative response can be inhibited. Accordingly, a variety of classes of drugs have been found to inhibit the increase in goblet cell number induced by inhaled irritants, in particular cigarette smoke (table 3). Thus, increases in airway goblet cell number in response to subacute exposure to irritant are markedly inhibited by concurrent administration of steroidal or nonsteroidal anti-inflammatory agents [36, 61, 95–97], mucolytic, or more correctly "mucoregulatory", agents [98], antimitotics [99], or flavenoids [88]. Corticosteroid treatment also inhibits the increase in goblet cell number induced in rat trachea by human neutrophil lysates or by elastase [100]. Significantly, in adult patients with asthma, three months of inhaled corticosteroid therapy reduces the number of goblet cells per unit length of epithelium [9]. In the case of two mucoregulatory agents, inhibition by N-acetylcysteine or S-carboxymethylcysteine of cigarette smoke-induced goblet cell hyperplasia in rat airways is associated with inhibition of mucus hypersecretion [101]. More relevant, perhaps, to the situation in human smokers is the observation that if indomethacin, flurbiprofen or N-acetylcysteine are administered to rats after cessation of smoke exposure (*i.e.* after the development of goblet cell hyperplasia and metaplasia), the time taken for the goblet cell population to return to "normal" is markedly reduced, compared with vehicle-treated animals [102, 103].

The precise mechanisms whereby the agents listed above inhibit irritant-induced goblet cell increase are unclear. For example, the effects of N-acetylcysteine or S-carboxymethylcysteine are unlikely to be due solely to their "mucolytic" properties, because, although both inhibit cigarette smoke-induced goblet cell hyperplasia and mucus hypersecretion [98, 101], only N-acetylcysteine has a "free" sulphydryl moiety for breaking of S-S bonds in the mucus macromolecule. These "mucolytics" may instead protect against cigarette smoke-induced injury by maintenance of homeostatic levels of endogenous lung antioxidants, in particular glutathione (in the reduced form). Cigarette smoke may raise the concentration of reactive oxygen species in the airways, which in turn would decrease the levels of reduced glutathione. The mucolytics could make cysteine residues available for maintenance of glutathione levels, which in turn would be available to limit oxidant damage. The inhibitory effect of nonsteroidal anti-inflammatory drugs may be linked to interference with mucin synthesis *via* inhibition of mucin-precursor uptake [104]. The mechanism of the inhibition of precursor uptake is unknown. The inhibitory effect of corticosteroids on cigarette smoke-induced goblet cell hyperplasia [9, 97, 100] will relate to their ability to dampen inflammatory reactions, for example inhibition of release of inflammatory mediators. Part of this dampening may be due to direct effects on mucin synthesis, because prednisolone, albeit at a high dose of 100 μM, inhibits mucin gene expression in rat tracheal organ culture [94]. The latter observation lends further support to the hypothesis that increased mucin gene expression is an early event in the induction of increased mucus synthesis and associated hypersecretion, which may contribute to the initiation of airway goblet cell hyperplasia and metaplasia.

Conclusions

The difficulties associated with isolation of airway goblet cells and in separation of their secretion from those of other sources has limited investigation of the complexities of goblet cell function to but a very few determined researchers. The development over the last few years of

new technologies has enabled a greater number of investigators to enter the field of goblet cell research, and there is currently a renewed interest in these cells as major participants in normal airway function and in the pathophysiology of bronchial diseases associated with mucus hypersecretion, including chronic bronchitis, asthma and cystic fibrosis.

Advances in culture techniques, mucin biochemistry, microvideorecording, and cellular and molecular biological methods have already increased our knowledge of the rapidity with which these cells can discharge mucus in response to stimuli, of some of the intracellular signal transduction mechanisms mediating secretion, and of the possible cellular and molecular triggers for the production of more goblet cells. Development of cellular and molecular probes with greater selectivity than currently available, including antibodies and complementary deoxyribonucleic acids (cDNAs), will allow greater understanding of the nature of the mucins produced by goblet cells, and whether they differ from mucins from other sources, and of the fundamental triggers for goblet cell proliferation. The next few years should witness a number of important developments in the application of cellular and molecular biological techniques to the study of epithelial secretory and proliferative function. These developments will have relevance to understanding the normal functioning of goblet cells, their function in disease, and of therapeutic possibilities for airway hypersecretion.

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