ORIGINAL ARTICLES

Expression of MUC5AC and MUC5B mucins in normal and cystic fibrosis lung

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Abstract Hypersecretion of airway mucus is a characteristic feature of chronic airway diseases like cystic fibrosis (CF) and leads via impairment of the muco-ciliary clearance and bacterial superinfection to respiratory failure. The major components of the mucus matrix forming family of mucins in the airways are MUC5AC and MUC5B. To investigate the expression of these glycoproteins in CF, immunohistochemistry was carried out on trachea, bronchi and peripheral lung obtained from CF patients and compared to normal lung tissues. MUC5AC immunohistochemistry demonstrated signals in goblet cells of the epithelial lining. Also, goblet cells inside glandular secretory ducts revealed MUC5AC-positive staining. In comparison to those from normal subjects, CF sections were characterized by inflammatory changes and goblet cell hyperplasia, resulting in increased numbers of MUC5AC-positive cells. Immunohistochemical staining for MUC5B showed abundant staining of submucosal glands and epithelial goblet cells. Inside the glands, the immunoreactivity was restricted to glandular mucous cells. MUC5AC and MUC5B are expressed in the same histological pattern in CF compared to normal tissues with an increase of MUC5AC-positive cells due to goblet cell hyper- and metaplasia.

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INTRODUCTION

Cystic fibrosis (CF), a common inherited, autosomal recessive disorder, is caused by a dysfunction of the epithelial chloride channel CF transmembrane regulator (CFTR) (1). So far, more than 500 mutations of the CFTR gene are known that are associated with CF (2). Apart from gastrointestinal manifestations such as pancreatic insufficiency, the major cause of morbidity results from airway disease (1). The hypersecretory-induced airway changes in CF are characterized by submucosal gland and goblet cell hyper- and metaplasia, leading to mucus over-production and distortion of the muco-ciliary clearance. As a result, airway plugging by mucus leads to chronic inflammatory changes and bacterial colonization (3).

The polymer matrix of airway mucus is made up of large, oligomeric, gel-forming glycoproteins qmucins with molar masses ranging between 10 and 40 million Da (4–7). Seven different mucin apo-proteins which are products of different genes, have been identified in the respiratory tract so far (5). The mucins are produced primarily by two different airway cell types: goblet cells and glandular cells. While MUC2 and MUC5AC expression has been localized to cells of the surface epithelium (9,10), MUC5B and MUC7 mucins are expressed in glandular cells (11,12). Out of the seven mucins, MUC5AC and MUC5B have been identi-

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fied as major gel-forming macromolecules, whereas MUC2 contributes only to a lesser extend to the matrix (13,14).

In view of the high levels of CFTR expression in glandular cells which play an important role in the composition of mucus hydration and their attributed pathophysiological importance in the progression of CF (15), numerous studies have focused on the identification of differences between CF and normal airway secretions (16–19). Alterations such as increased sulphation and fucosylation and decreased sialylation of secreted mucins have been demonstrated. Biochemical studies also indicated a higher heterogeneity of CF mucins in mucus as compared to the normal mucus composition (20).

Pseudomonas aeruginosa infections play a central role in the progression of respiratory failure in CF (21). Because *P. aeruginosa* exoproducts may up-regulate the expression of mucin genes (22–24), the present study was carried out to determine whether there are changes in the pattern and extent of distrubution of the two major airway mucins, MUC5AC and MUC5B, in airway tissues from CF patients as compared to tissues from normal subjects.

MATERIALS AND METHODS

Tissue preparation

CF tissues from explanted lungs were obtained from cystic fibrosis patients (n=6) who had lung transplantation due to progressive respiratory failure. Tracheal (n=3), bronchial (n=4) and peripheral lung (n=3) sections were subjected to immunohistochemistry and compared to sections of normal human lung tissues from patients (n=3) who died without pulmonary involvement. The tissues were either paraffin-embedded or frozen in liquid nitrogen-cooled isopentane.

Antibodies

One polyclonal antibody against MUC5AC, and one polyclonal and one monoclonal antibody against MUC5B were used. The rabbit polyclonal anti-MUC5AC serum (LUM5-1) was raised against a keyhole limpet haemocyanin-conjugated synthetic peptide (sequence RNQDQQGPFKMC) that is present in the carboxy-terminal region and two stretches flanking a tandem repeat region of MUC5AC (9). A rabbit polyclonal anti-MUC5B (LUM5B-2) serum was raised against the sequence RNREQVGKFKMC which is present in the central region of the MUC5B apoprotein (12) and compared to a mouse monoclonal antibody (11C1) against MUC5B (25). Both polyclonal antibodies were characterized recently: preabsorption studies with increasing concentrations of the mucins confirmed the specifity of the antibodies (9,12).

Immunohistochemistry

For immunohistochemistry, 6–8 μm sections of frozen tissues were cut on a cryostat, mounted on gelatin-coated glass slides and air dried for 1 h. Alternatively, 6 μm paraffin-embedded sections were cut on a microtome, deparaffinized through 100% xylene and rehydrated through graded alcohol series. After rinsing in phosphate-buffered saline (PBS), the sections were immersion-fixed in 50% acetone 50% methanol for 20 min and washed in PBS twice for 5 min. Endogenous peroxidase activity was quenched by incubation with 0.3% H_2O_2 for 30 min followed by rinses in PBS. Then, non-specific labelling was blocked by coating with preincubation serum (0.1 M phosphate buffer containing 1% bovine serum albumin and 10% normal swine serum) for 1 h at room temperature. After washing in PBS, the tissues were incubated with either the polyclonal rabbit anti-MUC5AC-serum (9) diluted 1:1000 in the preincubation solution, the polyclonal rabbit anti-MUC5B-serum (12) diluted 1:200 or the monoclonal mouse-anti-MUC5B serum (25) diluted 1:2000 for 2 h at room temperature. After incubation and repeated washing steps with PBS, the sections incubated with the polyclonal rabbit primary antisera were subsequently treated with biotinylated goat anti-rabbit IgG (diluted 1:75 in preincubation serum; Vectastain Elite ABC, Vector Laboratories, Burlingame, CA, U.S.A) and the sections incubated with the monoclonal mouse antibody were treated with biotinylated horse anti-mouse IgG (diluted 1:75 in preincubation serum, Vectastain ABC, Vector Laboratories, for 1 h at room temperature. After repeated washing steps, secondary antibodies were detected with the Vectastatin ABC reagent in combination with DAB substrate (Sigma, Poole, Dorset, U.K.). Slides were counterstained with haematoxylin, mounted in carbonate-buffered glycerol (pH 8.6) and viewed using a Zeiss microscope.

RESULTS

Localization of MUC5ACA immunoreactivity in CF and normal tissues

MUC5AC immunohistochemistry resulted in abundant staining of tracheal and bronchial sections. Peripheral lung tissue was negative. MUC5AC-immunoreactivity in trachea and bronchi was restricted to the epithelial lining. In normal tissues, the positive cells were a minority of the epithelial cells which could be identified as goblet cells by their characteristic morphology [Fig. 1 (b)]. Comparison with periodic Schiff acid (PAS) staining demonstrated that all goblet cells within the surface epithelium displayed MUC5AC reactivity. The immunoreactivity was intense and of a non-granular type, and was not associated to the cellular membrane or to any specific intracellular organelle pattern. Goblet cells inside glandular

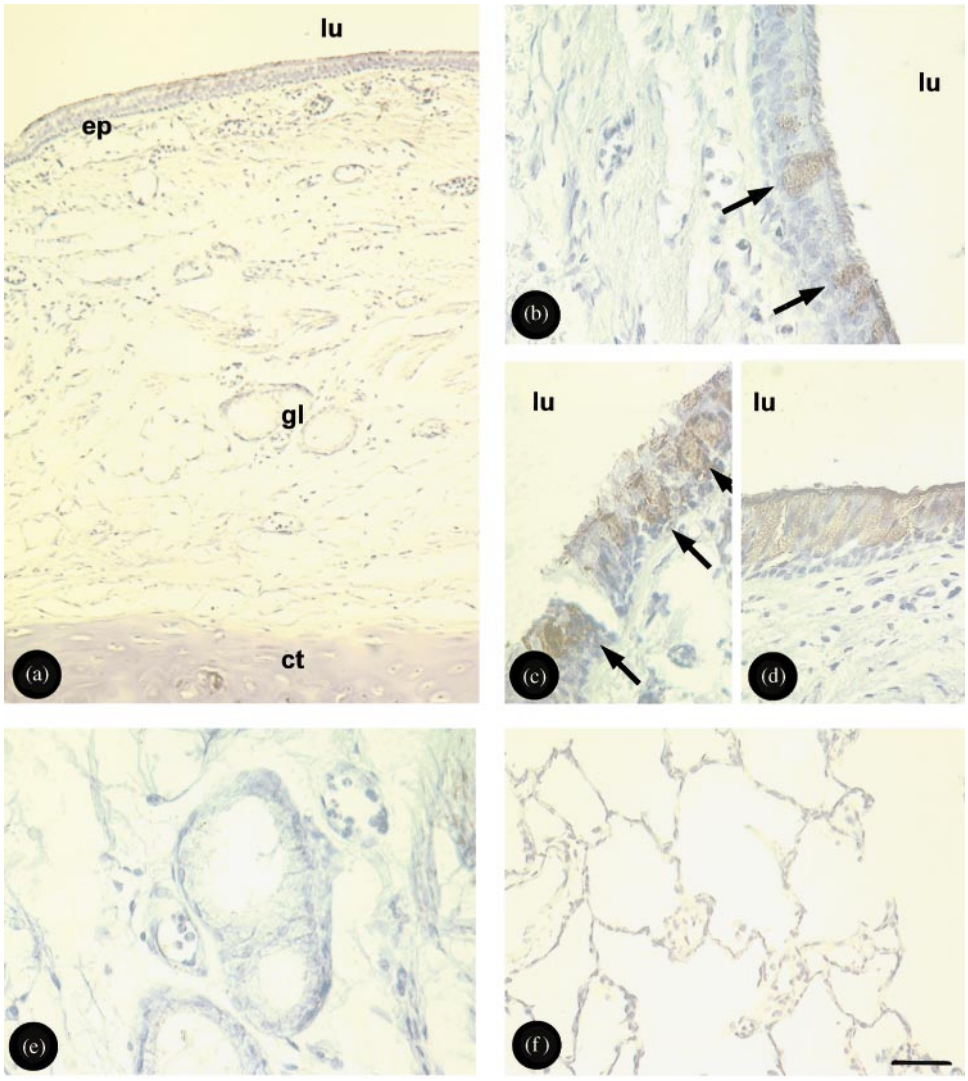

Fig. 1. Cellular localization of MUC5AC immunoreactivity in CF and normal human lung sections. MUC5AC immunoreactivity is localized to goblet cells of CF trachea (a, c), and CF (d) and normal bronchi (b). There are increased numbers of goblet cells, positive for MUC5AC in CF (c, d) as compared to normal tissue (b). But there was no detectable reactivity in submucosal glands (e) and peripheral lung (f). lu: airway lumen, ep: epithelial lining, gl: glands, ct: cartilage. Bar= 105 μm (a), 45 μm (b, c, d), 35 μm(e) and 60 μm (f).

secretory ducts also revealed MUC5AC positive staining. Comparison between samples from normal subjects [Fig. 1(b)] and those from patients with cystic fibrosis [Fig. 1 (a,c,d)] revealed that there was no change in the cellular distribution of MUC5AC immunoreactivity although, parallel to the increased number of goblet cells due to hyperplasia, MUC5AC immunoreactivity was increased compared to normal conditions [Fig. 1 (b)].

Localization of MUC5B immunoreactivity in CF and normal tissues

Immunohistochemistry for MUC5B resulted in abundant staining of submucosal glands. The immunoreactivity was restricted to glandular mucous cells and epithelial goblet cells [Fig. 2 (a–e)]. The morphology of the MUC5B-positive cells was characteristic for mucus cells with basal nuclei in comparison to serous cells [Fig. 2 (b)]. There was MUC5B-positive mucus present in the lumen of secretory ducts [Fig. 3(b)]. There was no difference in the distribution of glandular MUC5B immunoreactivity between CF [Fig. 2 (a, c)] and non-CF tissues [Fig. 2(b)]. The mono-and polyclonal antibodies against MUC5B did not differ in the staining pattern of MUC5B-reactive cells.

DISCUSSION

The primary causes of morbidity and mortality in CF are pulmonary obstruction and bronchiectasis, leading to persistent bacterial infection and respiratory failure.

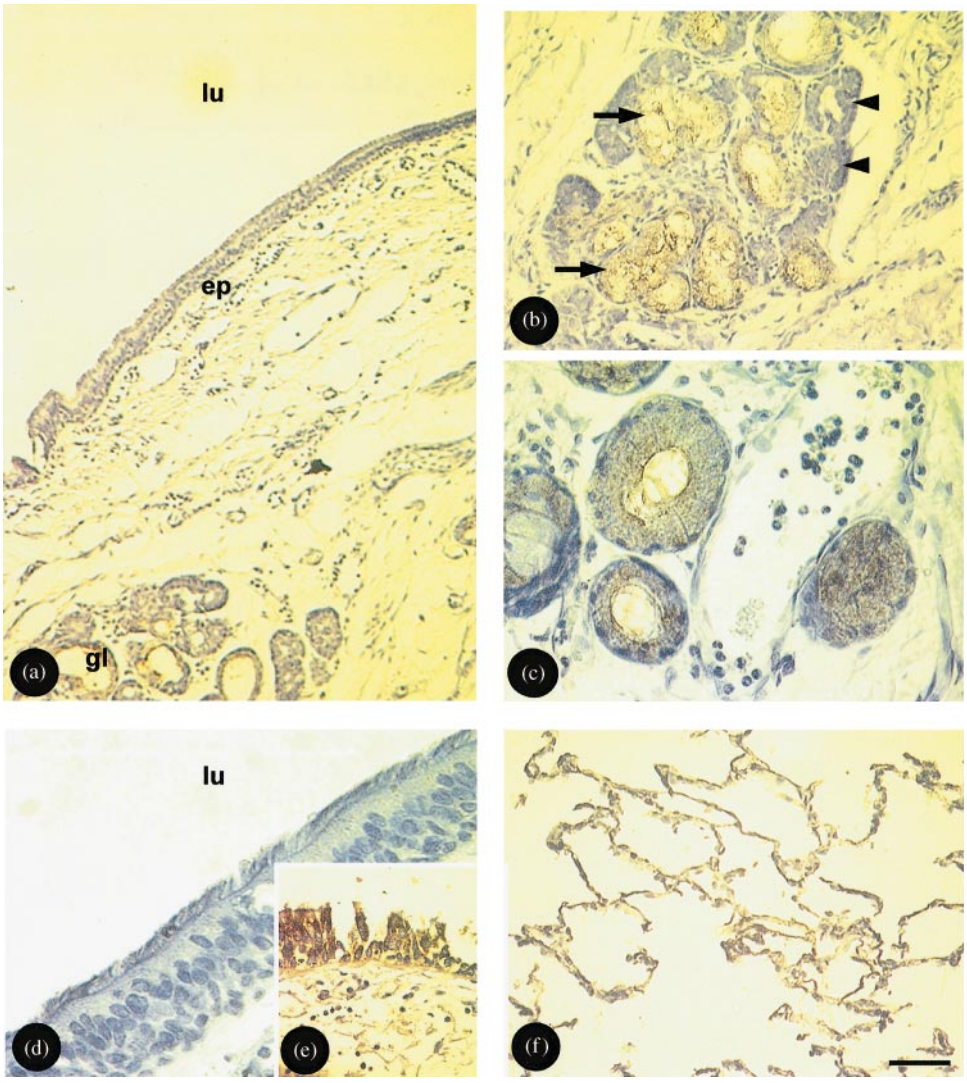

Fig. 2. Localization of MUC5B immunoreactivity in CF and normal human lung. Sections 8 μm of CF (a, c, e, f) and normal (b, d) lung tissues were incubated with MUC5B antisera. Specific MUC5B immunoreactivity is present in mucous cells (arrows in b) but not serous cells (arrowheads in b) of submucosal glands in CF (a, c), normal tissues (b) and in epithelial cells (e). There was no difference in expression between CF (a, c) and normal glands (b). Negative CF peripheral lung (F). lu: airway lumen, ep: epithelial lining, gl: glands. Bar = 105 μm (a), 60 μm (b, e, f), 35 μm (c, d).

The obstructive component of the disease is caused by a distortion of the muco-ciliary clearance and abnormality of the properties of the mucus leading to persistent airway blockage. So far, it is not known whether the hypersecretion and alteration of mucus consistency is paralleled by an up-regulated expression of mucin-encoding genes.

Previously, several mucin gene products were identified in the respiratory system under normal and pathological conditions. MUC2 was localized to basal epithelial and glandular duct cells but not to goblet cells or secretory acini (10,26,27). A significantly greater proportion of the surface epithelium was positive for MUC2 gene expression in CF tissues compared to normal lungs (10). Whereas MUC4, MUC5B, MUC5C and MUC7 gene expression was localized to gland acinar cells (27), MUC1 gene expression was reported to be present in peripheral lung sections (28). For MUC5B and MUC7 protein expression, studies on normal and CF bronchi revealed an expression of MUC5B restricted to glandular mucous cells and of MUC7 restricted to a subpopulation of serous cells (11).

Following the characterization of MUC5AC and MUC5B as major components of tracheobronchial secretions (14,29), we investigated the expression of these mucins in CF and normal tracheal, bronchial and peripheral lung tissues by immunohistochemistry. We found that MUC5AC is expressed in goblet cells of the surface epithelial lining and also in goblet cells in ducts of glands. This observation for normal bronchial and tracheal tis-

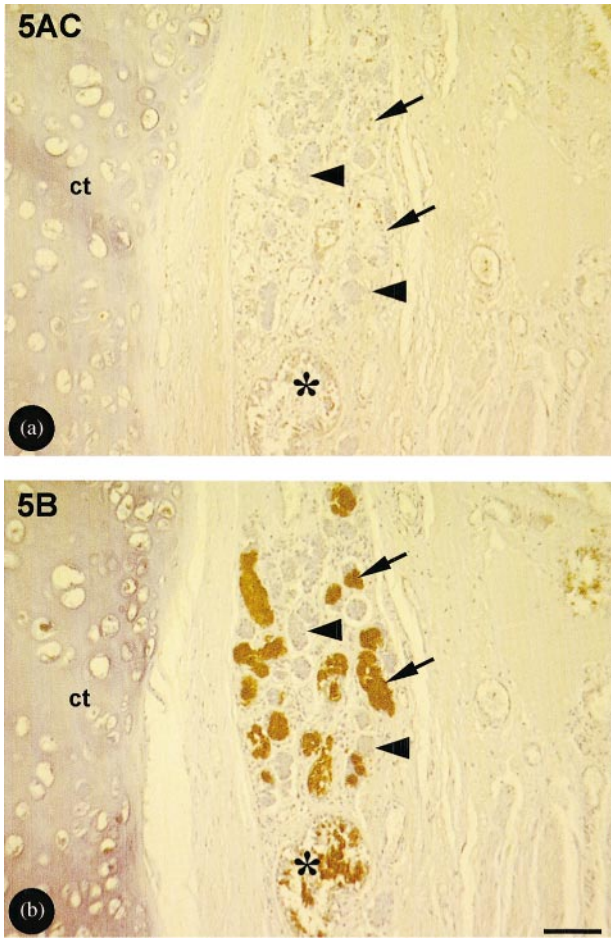

Fig. 3 Comparison of MUC5AC and MUC5B expression in parallel sections of trachea. Alternate incubation with MUC5AC and MUC5B antiserum of parallel sections demonstrated mutual exclusive expression of the two mucin genes. Positive mucous gland cells for MUC5B (arrows in b) in comparison to negative glands for MUC5AC (a) in CF tissues. In the lumen of a secretory duct, MUC5B-but not MUC5AC-positive mucus is present (*). Serous cells do not display MUC5B immunoreactivity (arrowheads). Ct: cartilage. Bar= 105 μm.

sues is confirmed by a previous localization of MUC5AC to goblet cells of tracheal tissue from a single individual (9). In contrast, MUC5B was expressed mainly in mucous cells of submucosal glands and to a lesser extent in epithelial goblet cells. This distribution is consistent with a report on the localization of MUC5B to mucous cells of submandibular, sublingual and labial glands (29). Our results indicate that MUC5AC and MUC5B are expressed by different mucus-producing airway cell types. Together with the finding that MUC7 is produced in serous glandular cells (27), the differential cellular partitioning of mucin gene expression underlines the diversity of airway mucus production. Recently, MUC5B and MUC5AC but not MUC2 were identified to be the main matrix forming components of the gel phase of CF sputum. MUC5AC was enriched in the sol indicating that it may be more susceptible to proteolytic degradation than MUC5B (14). The mucin pattern in the sputum did not alter significantly in acute inflammatory exacerbation or following antibiotic treatment (14).

Although numerous studies have characterized changes in the composition of secreted airway mucins in health and disease, there have been no detailed studies investigating whether these changes reflect changes and plasticity of the cellular phenotypes of mucin-secreting cells or changes in the numbers of these cells. Our results indicate no significant qualitative differences in the pattern of expression of MUC5AC and MUC5B between CF and normal lung. However, due to the well-defined goblet cell hyper- and metaplasia in CF, the MUC5AC expressing cellular compartment appeared to be more abundant. In conclusion, MUC5AC and MUC5B are differentially expressed in goblet cells and submucosal glandular mucous cells. In comparison to normal conditions, CF tissues which are characterized by inflammatory changes displayed an increased number of MUC5AC-positive staining due to goblet cell hyper- and metaplasia. Also, there was increased MUC5B positive mucus present in the airway epithelial lining, possibly representing attached mucus. Qualitatively, MUC5AC and MUC5B are expressed in a similar histological pattern in CF and normal tissues.

Acknowledgements

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