gene expression. The increase of sialyl-Le^x in colonic cancers may be due to an increase of MUC-1 as well as FTIII overexpression.

S20.19

Mucin Secretion by Clones Derived from a Human Lung Adenocarcinoma Cell Line

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Primary or secondary cultures of tracheal epithelial cells from different animals have been reported to secrete high molecular weight mucins, but the study of these mucins has been limited by the quantity of material available.

Since a few cells from the human lung adenocarcinoma cell line Calul (ATTCC ATB 54) were labelled by an antiserum directed against apomucins [1], a cloning of mucin secreting cells was performed: two clones were obtained by limite dilution (JMP-1) and by exposure to sodium butyrate (JMP-2) respectively.

The two clones expressed MUC 1 and MUC 2 genes [2]. MUC 4 gene [3] transcripts were exclusively found in the clone obtained by limit dilution (JMP-1). Evidences were obtained for the secretion of mucins in the cells culture supernatant: (i) the Sepharose CL-4B elution profile of media from cells incubated with [^{3}H] fucose showed a radiolabelled peak in the void volume; (ii) the protease-resistant fraction obtained after pronase treatment of the cell supernatant migrated in agarose gel electrophoresis like mucin glycopeptides prepared from sputum, and were stained with Schiff/periodate and/or toluidine blue. The MUC genes expression was higher when the cells were plated on collagen matrix than when they were grown on plastic.

[1] Perini *et al.*, 1991, *Eur. J. Biochem.*, **196**, 321 – 328; [2] Gendler *et al.*, 1987, *Proc. Natl. Acad. Sci. USA*, **84**, 6060 – 6066; [3] Porchet *et al.*, 1991, *Biochem. Biophys. Res. Com.*, **175**.

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Bovine Trachea as a Model for Mucin Secretion in the Airways

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Many physiological studies of airway mucus secretion have relied upon the incorporation of radioactive glycoprotein precursors into mucins in various models of respiratory epithelium. This approach may, however, give an incomplete picture if some mucins do not become radiolabelled and/or if certain populations are over-represented due to a very high rate of synthesis and secretion. We are currently identifying and characterising the major mucin species in bovine trachea. The surface epithelium and submucosal tissues are studied separately in order to obtain samples enriched in mucins from the goblet cells and submucosal glands respectively. The release of mucins from bovine trachea in organ culture is then studied with chemical methods rather than with radioactive precursors.

Mucin glycopeptides, corresponding to the highly glycosylated regions of the macromolecules, were prepared from the surface epithelium and submucosal tissue after reduction/alkylation followed by trypsin and nuclease digestion. Chromatography on a Superose 6 column revealed the presence of three major species of large mucin glycopeptides in the surface epithelium. In contrast, the predominant mucin glycopeptides from the submucosa were much smaller, although some larger species (similar to those in the surface epithelium) were also present. At least four populations of high-M_r glycopeptides were thus identified, the relative proportions of which differed between the surface epithelium and submucosa. Whole mucins were prepared from the surface and submucosal tissue by isopycnic density-gradient centrifugation first in 4 M- and then in 0.5 M-guanidinium chloride/caesium chloride after extraction in 6 M-guanidinium chloride buffer, pH 6.5 containing proteinase inhibitors. This showed the presence of three populations of whole mucins in the surface epithelium, two of which had buoyant densities typical for mucins whereas one was found at the top of the gradient. The submucosa contained one major population of mucins with a buoyant density slightly lower than that of the 'typical' mucins from the surface epithelium.

Bovine trachea, maintained in organ culture, has been shown to secrete a mucus gel containing at least two mucin populations, as identified with density-gradient centrifugation. The more dense mucin gave rise to two populations of large glycopeptides (similar to those in the surface epithelium) whereas the less dense one afforded much smaller glycopeptides.