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The normal trachea is cleaned by MUC5B mucin bundles from the submucosal glands coated with the MUC5AC mucin

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ABSTRACT

To understand the mucociliary clearance system, mucins were visualized by light, confocal and electron microscopy, and mucus was stained by Alcian blue and tracked by video microscopy on tracheal explants of newborn piglets. We observed long linear mucus bundles that appeared at the submucosal gland openings and were transported cephalically. The mucus bundles were shown by mass spectrometry and immunostaining to have a core made of MUC5B mucin and were coated with MUC5AC mucin produced by surface goblet cells. The transport speed of the bundles was slower than the airway surface liquid flow. We suggest that the goblet cell MUC5AC mucin anchors the mucus bundles and thus controls their transport. Normal clearance of the respiratory tree of pigs and humans, both rich in submucosal glands, is performed by thick and long mucus bundles.

1. Introduction

The respiratory system is kept relatively free from inhaled bacteria and debris by the mucociliary clearance system. The cilia generate the movement by their continuous beating [11]. The major constituents in the mammalian mucus are the MUC5B and the MUC5AC mucins. The MUC5B mucin is normally made by submucosal glands and the MUC5AC mucin by surface goblet cells. These two mucins have similar domain organization, and both form disulfide-bonded dimers by their *C*-termini [14]. MUC5B is arranged as linear molecules, similarly to the related von Willebrand factor (VWF), as it forms disulfide-bonded dimers in its *N*-terminus [9]. The properties of these secreted mucins are highly influenced by the local conditions at release from the mucin-producing cells, as shown for MUC2 in the intestine [4].

The different organization of glands and goblet cells in rodents, pigs and humans suggests differences in how the mucociliary clearance system functions. In contrast to mice, the pig system is similar to the one in humans. Piglets have been used for *in vivo* studies of gross mucociliary clearance using single-particle tantalum microdiscs, showing migration cephalically and from dorsal to ventral side [6]. Strands, suggested to contain mucins, were also observed at the submucosal gland openings, but their relation to the clearance of fluorescent nanospheres (beads) has not been clarified [7]. In a preliminary report we established methods to visualize the mucus bundles and preliminary defined their composition [5]. We now show that submucosal glands secrete MUC5B mucin molecules that form linear polymers and are coated by the MUC5AC mucin. These bundles are transported slower and separately from the airway surface liquid (ASL) mass flow.

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2. Material and methods

2.1. Piglet airway preparation and staining

Piglets were euthanized under Ketamine (Ursotamin®, Serumwerk Bernburg, Germany) and Azaperone (Stresnil®, Elanco Animal Health, Bad Homburg, Germany) anesthesia by intracardial injection of T61® (Intervet, Unterschleissheim, Germany). Airways including the larynx, trachea and lungs were explanted and immersed in chilled Perfadex® solution (XVIVO Perfusion, Gothenburg, Sweden) adjusted to pH 7.2 with 1 M TBS. All connective and pulmonary tissue was removed and the prepared airways transferred to a 50 ml tube with Perfadex® solution before shipping under chilled conditions overnight to Gothenburg. Ethical permissions for the pig experiments were obtained from Regierungen von Oberbauern, Münich, Germany and Jordbruksverket, Jönköping, Sweden.

The distal trachea and proximal part of the primary bronchi were mounted in a Petri dish coated with Sylgard 184 Silicone Elastomer (Dow Corning, Wiesbaden, Germany) using 27G needles. The tissue was covered in oxygenated (95% O_2, 5% CO_2) Krebs-glucose buffer in 116 mM NaCl, 1.3 mM $CaCl_2$, 3.6 mM KCl, 1.4 mM KH_2PO_4, 23 mM $NaHCO_3$, and 1.2 mM $MgSO_4$, 10 mM D-glucose, 5.7 mM pyruvate, 5.1 mM glutamate, pH 7.4, and gradually heated to 37 °C. Tissues were stained with 0.4 mM Alcian blue 8GX, charcoal [4] and/or with 40 nm carboxylate-modified fluorescent (580/605) microspheres (FluoSpheres, Thermo), hereafter called "beads". Tissue was monitored through a stereo microscope with color or monochrome CCD cameras (DS-Fi2 or DS-QiMc, Nikon). The speed of the Alcian blue-stained bundles (mean of five measurements in each time-lapse), and thickness were calculated using NIS elements (Nikon). Bundle movement patterns were calculated

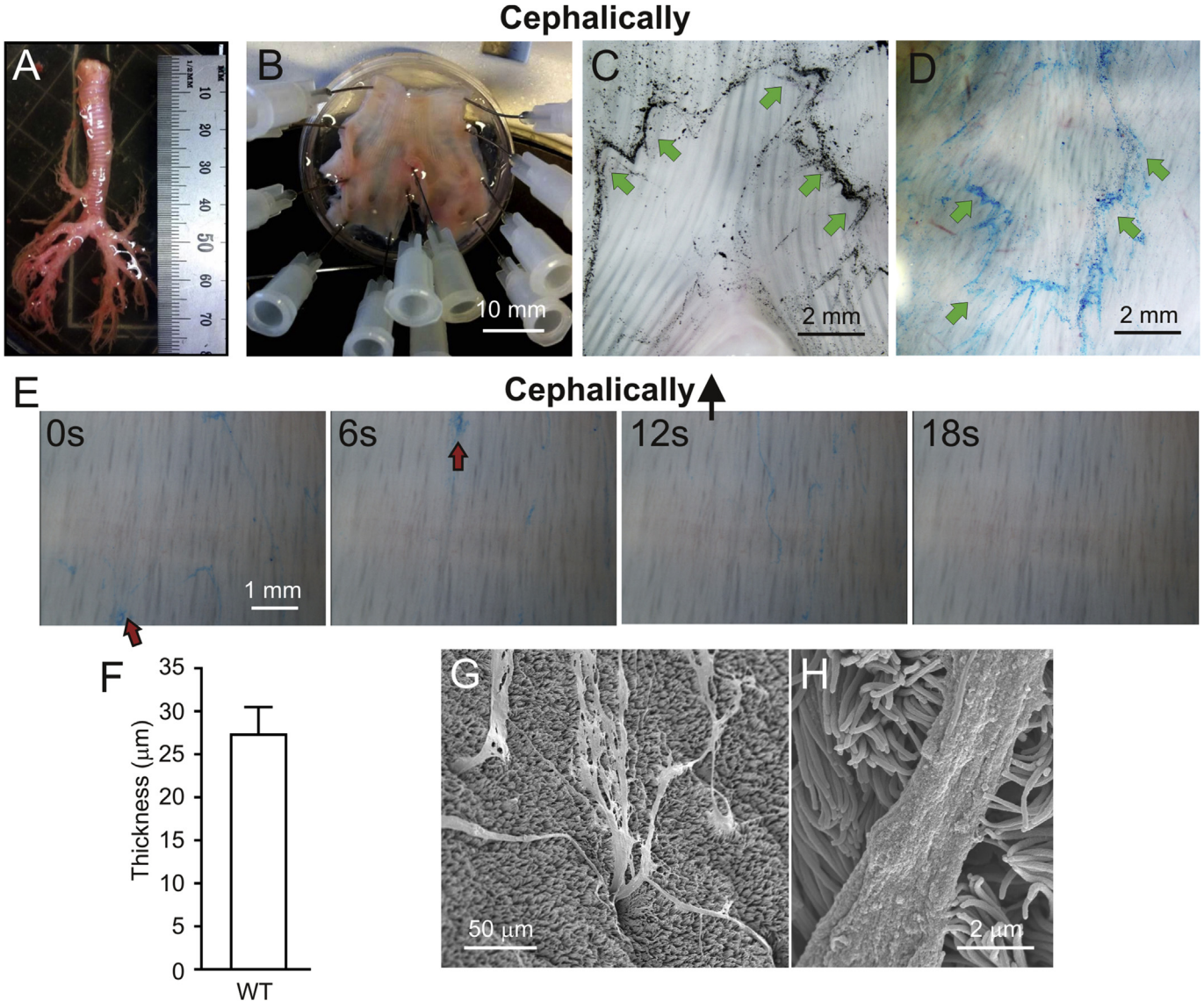

Fig. 1. Alcian blue-stained bundles from piglet submucosal glands are transported cephalically. A. Pig trachea. B. Distal trachea and primary bronchi mounted in the experimental chamber. C. Dorsal side of piglet trachea with bifurcation and charcoal collected in the mucus. Mucus was moved cephalically and outwards as indicated by the green arrows. D. The ventral side of the trachea with Alcian blue-stained bundles moving according to the arrows, up and ventrally. E. Pig trachea mounted as in B with Alcian blue-stained bundles moving upwards over the surface in Movie S1. Images at 0, 6, 12 and 18 s from the same surface of a time-lapse recording at 16 times the original speed. Red arrows point to the same mucus bundles. F. Diameter of Alcian blue-stained bundles in airways, mean of 8 bundles from 3 pigs. G. SEM image of a pig trachea with bundles coming out of submucosal glands. H. Mucus bundle and cilia appear to interact.