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Impaired Bicarbonate Secretion causes Mucoviscidosis in Cystic Fibrosis

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ABSTRACT:

Is mucus in CF actually dehydrated? For more than 20 years it has been widely assumed that the abnormally thick mucus (mucoviscidosis) in Cystic Fibrosis (CF) is linked to the genetic defect in the CFTR Cl^- channel by assuming that mucus is "dehydrated" due to basic defects in Cl^- dependent fluid transport. This widely held explanation is inconsistent with known physiological properties and functions of affected organs in CF. During the process of releasing highly condensed mucins from intracellular granules, Ca^{++} and H^+ cations must be removed from condensed mucins to enable them to expand by as much as 1000 fold into extracellular mucus-gel networks. Over the past several years, it has become apparent that HCO_3^- transport is also defective in CF. We propose that HCO_3^- is crucial to normal mucin expansion because it complexes with these cations. Thus, because HCO_3^- secretion is defective in CF, mucins in CF target organs tend to remain aggregated, poorly solubilized, and less transportable. If the hypothesis is valid, mucus pathogenesis in CF may be due as much or more to defective HCO_3^- than to defective Cl^- transport.

Over the past half century, the term “Mucoviscidosis” has been almost completely abandoned in favor of “Cystic Fibrosis” (CF) despite the fact that the pathology of this disease begins with mucus that plugs airways, blocks gland ducts, and obstructs lumina of hollow organs (1, 2). However, the mutations causing CF are in a gene that codes irrefutably for a membrane protein that conducts Cl^-. These two facts present a long-standing conundrum in CF, which asks how a basic defect in anion transport can translate into devastatingly pathogenic mucus.

Since mucus was first implicated in CF, a number of hypotheses have been advanced to explain the characteristically thick mucus or ‘mucoviscidosis’ (1). It was first thought that CF mucus was abnormally synthesized, but numerous investigations of CF mucus have uncovered little to indicate any unique composition. Changes in sulfation and fucose to sialic acid ratios in CF mucus were reported early(3), but these changes seem most likely to be a normal response to increased infection and/or inflammation (4, 5). Additionally, mucus composition is probably not primarily abnormal in CF because different target organs predominantly express different mucins; e.g. MUC5AC in airway epithelia (6, 7), MUC5B in submucosal glands (7), MUC2 in intestine(8), MUC5B in cervix (9), etc. Yet, the physical properties of all these different mucins are abnormal in CF. Wherever they are produced in CF target organs, mucins tend to aggregate and immobilize so as to obstruct ductal and luminal passages, strongly implying that it is not mucus *per se* that is abnormal, but the manner and conditions under which mucins are released that results in this common pathogenic fate.

Presently, it is almost universally assumed that CF mucus is thick and sticky because it is “dehydrated”, and two general concepts have evolved to explain the lack of water. First, from observations in the airways and airway-derived cells, fluids are reported to be hyper-absorbed by epithelia from the media into which mucins are secreted and therefore cannot be adequately hydrated (10). While this rationale may seem consistent with airways, it is very difficult to extend to other organs such as the pancreas or biliary tree where fluid absorption does not occur in either normal or CF phenotypes.

Second, dehydration is also ascribed to hypo-secretion of fluids (11, 12). However, hypo-secretion tacitly assumes that mucins once secreted are without access to fluid, which seems doubtful since the secretory epithelia of most, if not all, affected organs are highly permeable to water. Thus, even though active fluid secretion is compromised, the Donnan forces of secreted polyanionic mucins should, in time, osmotically attract sufficient fluids to accommodate hydration. In fact, post secretory Donnan swelling may well be a significant component of the dilation seen in blocked ducts and lumens in mucus secreting glands in CF (1, 13) resulting in a kind of "bronchiectasis" of the gland. This is not to say that secreted fluids are not important to transport hydrated mucins from gland lumens and other compartments, but simply that thick mucus cannot in general be due to a lack of water.

We submit that another *factor* must be involved. A telling clue comes from the pancreas, which based on pancreatic sufficiency and insufficiency gives the best-known segregation of genotype with phenotype in terms of disease severity in CF. Notably, the pancreas is the organ of HCO_3^- secretion and almost inescapably indicts this anion in pathogenesis. While evidence is not absolute or overwhelming (likely because studies of the HCO_3^- anion have been comparatively neglected (14), there is good evidence that a HCO_3^- transport defect parallels the much more widely studied and acknowledged defect in Cl^- transport. In fact, in addition to the pancreas, a defect in HCO_3^- transport can either be demonstrated or strongly implicated in target organs of CF (15).

Still, how can a loss of HCO_3^- cause thick mucus? Normally, gel-forming mucins begin as huge, highly condensed macromolecules ($10^6 – 10^8$ Daltons) in intracellular granules that are released during exocytosis to form immensely expanded polyanionic polymers that protect the apical surfaces of epithelial tissues. These enormous molecules are compacted and stored intracellularly as granules, with very high concentrations of Ca^{++} (>200 mM) and H^+ (pH < 6). These cations shield the repulsive forces of fixed negative charges on mucins to prevent their expansion by as much as 1000 fold, as occurs in forming mucus gels. Ca^{++} also forms electrostatic divalent bridges between fixed negative charges that further stabilize mucins and oppose expansion (16-18).

To expand when the granule is secreted, the anionic sites must be unshielded by removing Ca^{++} and H^{+}. Unshielding exposes the mutually repulsive electrostatic forces of the fixed negative charges so that massive mucin expansion occurs within 1-2 sec *or less* of granule release(18, 19), demanding an extremely efficient removal of shielding cations. Current theory holds that the unshielding of mucin anions occurs as a function of exchanging Ca^{++} and H^{+} with K^{+} and Na^{+} (20). In CF, it is difficult to envision a paucity of these ions in any fluid into which mucins are released. Therefore, we propose that the *other factor* required to form normal mucus is HCO_3^{-}. That is, once the granule is released, HCO_3^{-} is critical to sequester Ca^{++} and H^{+} away from the mucin anions by complexing with them. In short, secreted HCO_3^{-} and its equilibrium form, CO_3^{2-}, react with condensed mucins to form H_2CO_3, $CaHCO_3^{+}$, and $CaCO_3$ and reduce the activity of Ca^{++} and H^{+} in the mucin solution. Once unshielded, the electrostatic anionic forces rapidly expand the mucin macromolecules. There are no other anions of significance in secreted fluids capable of effectively complexing these cations. The only other significant anion, Cl-, cannot buffer H^{+} or chelate Ca^{++} because both HCl and CaCl2 are highly dissociable. It may be important to note that both lower pH and higher Ca^{++} concentrations dramtically slow the rate of swelling of released granules; i.e., mucin expansion (18).

We hypothesize that in CF the lack of secreted HCO_3^{-} in the extracellular medium of mucins during “exocytotic birth” impairs calcium removal, prevents normal mucin expansion, and promotes stasis of mucus in the ducts or on the luminal surfaces of affected organs (Fig. 1). It is of note that the contents of pancreatic granules secreted from acinar cells require HCO_3^{-} to disaggregate (21) and that CF secretions generally contain elevated [Ca^{++}]. This is not to say that mucins in CF do not expand or that no Ca^{++} is removed from them, but simply that the process is impaired by the lack of HCO_3^{-}. We do not exclude the possibility that pH changes associated with altered HCO_3^{-} may have far-reaching and deleterious effects in CF, but we propose that excessive Ca^{++} and H+ remaining in mucus will diminish expansion and render mucins less transportable due

to increased electrostatic cross linking within extrinsic mucins in mucus gels as well as with intrinsic mucins of the cell membrane.

Validating this hypothesis should require that the absence of (secreted) HCO_3^- in the medium of normally secreted mucin granules results in 1.) slowed and incomplete expansion of mucus, 2.) abnormally viscous and tenacious mucus, and probably 3.) elevated mucus Ca^{++} content. In contrast, under conditions that would assure secreting mucins into fluid with ample HCO_3^-, CF epithelia should release virtually normal mucins with normal expansion kinetics and viscoelastic properties. It is of some concern that we do not know how pronounced these effects might be. It is clear that many exocrine and respiratory units of tissue function in CF patients over long periods (years) so that the disturbance may be subtle and benign until reaching a threshold that precipitates a pathogenic event.

A measure of mucin expansion kinetics from granules might be obtained from mucins tagged by integrating a green fluorescent protein (GFP) into the mucin gene(22). Such insertions might, however, disrupt normal mucin behavior. Alternatively, the dilution of closely associated companion molecules such as tagged trefoil peptides might be monitored to follow expansion. Since mucus properties easily change with time and condition, quantitative evaluations of viscoelastic properties will likely require study of intact mucus secreting epithelia and may well require developing new techniques to collect, quantitate, and assay micro specimens of pure mucus secreted from native tissues *ex vivo*.

If this hypothesis is valid, it should not only change our concepts of the pathogenesis of CF, but it should also provide a basis for new approaches to managing abnormal mucus in other diseases as well. For example, it is sobering to consider that new drugs now entering clinical trials that promise to correct Cl^- transport in CF may not be therapeutic if the mutant protein cannot transport HCO_3^- adequately, so that "Mucoviscidosis" would persist. Finally, the hypothesis may explain the general observation that physiologically mucins always appear to be secreted simultaneously with HCO_3^-.

Citations

1. Bodian M. Fibrocystic Disease of the Pancreas: A congenital disorder of mucus production -- mucosis. New York: Grune and Stratton,Inc.; 1953.
2. Burgel PR, Montani D, Danel C, Dusser DJ, Nadel JA. A morphometric study of mucins and small airway plugging in cystic fibrosis. Thorax. 2007 Feb;62(2):153-61.
3. Dische Z, di SA, Pallavicini C, Youlos J. Composition of mucoid fractions from duodenal fluid of children and of adults. Arch Biochem Biophys. 1959 Sep;84:205-23.
4. Leir SH, Parry S, Palmai-Pallag T, Evans J, Morris HR, Dell A, et al. Mucin glycosylation and sulphation in airway epithelial cells is not influenced by cystic fibrosis transmembrane conductance regulator expression. Am J Respir Cell Mol Biol. 2005 May;32(5):453-61.
5. Voynow JA, Gendler SJ, Rose MC. Regulation of Mucin Genes in Chronic Inflammatory Airway Diseases. Am J Respir Cell Mol Biol. 2006 Feb 2.
6. Davies JR, Herrmann A, Russell W, Svitacheva N, Wickstrom C, Carlstedt I. Respiratory tract mucins: structure and expression patterns. Novartis Found Symp. 2002;248:76-88; discussion -93, 277-82.
7. Hays SR, Fahy JV. Characterizing mucous cell remodeling in cystic fibrosis: relationship to neutrophils. Am J Respir Crit Care Med. 2006 Nov 1;174(9):1018-24.
8. Allen A, Hutton DA, Pearson JP. The MUC2 gene product: a human intestinal mucin. Int J Biochem Cell Biol. 1998 Jul;30(7):797-801.
9. Gipson IK. Mucins of the human endocervix. Front Biosci. 2001 Oct 1;6:D1245-55.
10. Boucher RC. Airway surface dehydration in cystic fibrosis: pathogenesis and therapy. Annu Rev Med. 2007;58:157-70.
11. Trout L, King M, Feng W, Inglis SK, Ballard ST. Inhibition of airway liquid secretion and its effect on the physical properties of airway mucus. Am J Physiol. 1998 Feb;274(2 Pt 1):L258-63.
12. Joo NS, Irokawa T, Robbins RC, Wine JJ. Hyposecretion, not hyperabsorption, is the basic defect of cystic fibrosis airway glands. J Biol Chem. 2006 Mar 17;281(11):7392-8.
13. Oppenheimer EH, Esterly JR. Pathology of cystic fibrosis review of the literature and comparison with 146 autopsied cases. Perspect Pediatr Pathol. 1975;2:241-78.
14. Quinton PM. The neglected ion: HCO3. Nature medicine. 2001 Mar;7(3):292-3.
15. Quinton PM, editor. HCO3- and Cystic Fibrosis. Genova: E.S. Burioni Ricerche Bibliografiche; 2001.
16. Verdugo P, Deyrup-Olsen I, Aitken M, Villalon M, Johnson D. Molecular mechanism of mucin secretion: I. The role of intragranular charge shielding. J Dent Res. 1987 Feb;66(2):506-8.
17. Perez-Vilar J, Olsen JC, Chua M, Boucher RC. pH-dependent intraluminal organization of mucin granules in live human mucous/goblet cells. J Biol Chem. 2005 Apr 29;280(17):16868-81.
18. Espinosa M, Noe G, Troncoso C, Ho SB, Villalon M. Acidic pH and increasing [Ca(2+)] reduce the swelling of mucins in primary cultures of human cervical cells. Hum Reprod. 2002 Aug;17(8):1964-72.

19. Verdugo P, Langley L, Aitken ML, Villalon M. Development of an in vitro model of primate cervical goblet cells. Biorheology. 1990;27(3-4):465-70.
20. Nguyen T, Chin WC, Verdugo P. Role of Ca2+/K+ ion exchange in intracellular storage and release of Ca2+. Nature. 1998 Oct 29;395(6705):908-12.
21. Freedman SD, Scheele GA. Acid-base interactions during exocrine pancreatic secretion. Primary role for ductal bicarbonate in acinar lumen function. Ann N Y Acad Sci. 1994 Mar 23;713:199-206.
22. Perez-Vilar J, Mabolo R, McVaugh CT, Bertozzi CR, Boucher RC. Mucin granule intraluminal organization in living mucous/goblet cells. Roles of protein post-translational modifications and secretion. J Biol Chem. 2006 Feb 24;281(8):4844-55.

Fig 1. The schematic depicts transition of compacted mucins in intracellular granules in the present of HCO_3^- into highly expanded mucus ("Normal") in contrast to the impaired expansion of mucins in the absence of HCO_3^- in CF ("Mucoviscidosis"). HCO_3^- chelates Ca^{++} from the anionic mucin sites, exposing their negative charges, which induce rapid molecular expansion by electrostatic repulsion. Without HCO_3^-, Ca^{++} removal is compromised, divalent bridging persists, negative charges remain shielded, and mucus expansion and transportability is impaired.