Mucin Glycan Expression is Individual-Specific and Governed by the *In Situ* Microenvironment

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Abstract: Mucin glycosylation and individual susceptibility to disease is linked to the *in situ* microenvironment that determines features of the individual- and disease-specific glycans. In tissue culture paradigm, the *in situ* environment is not recreated and the synthesis of signature glycans vanishes. To evaluate the impact of the *in situ*-specific gastric epithelial environment on mucin glycan synthesis we investigated gastric mucin collected from 36 individual rats subjected to the same conditions and diet. The secretion collected during 3 hours of gastric perfusion contained from 67.7 to 1096.6 nmol mucin glycans and from 42.0 to 245.8 nmol glycosphingolipids (GSL). We identified four groups, each consisting of 6 animals, that contained O-glycans interacting with apical epithelial mucin receptor comprised of N-acetylgalactosamine (GalNAc), galactose (Gal), N-acetylglucosamine (GlcNAc) and fucose (Fuc) in the ratio of 1:2:2:1, 1:2:3:1, 1:3:2:1, and 1:4:2:1. The remaining 10 rats produced glycans containing GalNAc, Gal, GlcNAc, Fuc in ratio 1:1:1:1, partially fucosylated glycans, and Fuc-free glycans consisting of GalNAc:Gal:GlcNAc in ratio of 1:4:2. The data support our hypothesis that individual-specific-gastric epithelial environment and individual-specific metabolome provide final imprint on the quantity, composition, and termination of mucin O-glycans.

Keywords: Mucin, O-glycans, *in situ* environment, individual-specific glycome.

INTRODUCTION

The MUC glycoproteins that protect and sustain homeostatic environment of lumenal epithelial surfaces, and those implicated in cell growth, fetal development, epithelial renewal, differentiation, carcinogenesis and metastasis contain numerous and variable O-glycosidically linked carbohydrate chains (glycans) [1-7]. The variable glycans have been presupposed as coded by amino acid and tandem repeats in apomucin [8-11] yet the correlations have not been proven [12-14]. Unique domains in protein backbone have not introduced any distinctive features in glycans, or coded functional relation of the glycans to health or disease [6, 7, 11, 15, 16]. Chimeric MUC (mucin) assembled from a number of tandem units derived from secretory and membrane-integrated mucin (MUC1) has not introduced novel glycans [10]. Thus, the viable inner factor that dictates distinct properties of assembled glycoprotein and custom synthesis of mucin O-glycans is still not revealed.

We reason that the research on mucin complexity is skewed by the conceptual framework that relies on the *in vitro* paradigms. In cell culture, the elements of milieu are established to sustain cell viability and growth, but the specific homeostatic or aberrant environment is not created and the inner cellular controls that dictate custom synthesis and fidelity of the glycan required for homeostatic reproduction, or register pathological signals created in disease are not reproduced. Hence, to identify normal spectrum of mucin O-glycans in health and to uncover the critical changes in disease, we must identify the glycans expressed in the *in situ* individual-specific unmodified microenvironment, and then assess the changes in disease modified foreign milieu [17-19]. In our earlier project, we focused on diet-induced specific changes in O-glycan production by monitoring expression of wheat germ agglutinin (WGA) binding O-glycans in gastric mucin [18]. We found that the carbohydrate-low diet, in individual specific mode, impacted expression of WGA ligands and the quantity and the length of glycans.

In the study communicated here, we reassess these findings by employing larger cohort of animals exposed to identical conditions and diet, and in each animal we evaluate gastric mucin secretion, glycan quantity and composition, identify spectrum of glycans responsible for mucin interaction with apical epithelial mucin receptor, and gastric epithelial cells turnover. The results allow us to conclude that in each animal the cell metabolome created by the same isocaloric diet impacts differently posttranscriptional events. In gastric mucosal tissue this is manifested in animal-specific posttranslational O-glycosylation, expression of different O-glycans on mucin, and the individual-specific epithelial cell turnover.

MATERIALS AND METHODOLOGY

Male Sprague-Dawley rats of the same age and weight of 180-200g were subjected to isocaloric normal rat liquid diet prepared as detailed earlier [18, 20] and supplied by Bio-Serv, Frenchtown, NJ as Liquid Rat Diet, LD'82. Since on the average the animals consumed 100 ml of normal liquid diet/day, each animal received 100 ml of the diet per day for 7days. On 8th day the animals were fasted overnight with a free access to water and prepared for gastric intubation. The

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tubing inserted into the stomach through the esophagus was used for filling and perfusion of the stomach, while second catheter was used for collection. First, the stomach was washed three times with saline by filling to capacity (2.5-3.5ml) and discarding the aliquots. The perfusion with saline containing 10 nM sodium cholate (saline-cholate) was carried out in thermostatically controlled chamber at 37°C for 3 h. The perfusion rate was adjusted to the capacity of the individual rat stomach so that perfusate was exchanged 4 times. After perfusion was concluded, the rat was subjected to overdose of urathane, and the stomach used for harvesting of gastric mucosal tissue. The aliquots collected during 3h perfusion were dialyzed, internal standard for quantitation of carbohydrate was incorporated, the samples lyophilized, weighed, reconstituted in same volume of water, and aliquots taken for carbohydrate gas chromatography (GC). The remaining portions of the samples were subjected to lipid extraction, exclusion column chromatography, and CsCl equilibrium density centrifugation in 38% w/w CsCl [21, 22]. Purified mucin samples were subjected to β-elimination and O-glycan compositional analysis [23]. The aliquots drawn for the initial carbohydrate quantitation, for estimation of carbohydrate in purified mucin samples, and lipid extracts used for glycosphingolipid quantitation were admixed with 20 nmol internal standard of mannitol.

Mucin Purification and Carbohydrate Analysis

The materials collected from individual perfusates were purified by CsCl gradient centrifugation following previously published procedures [21, 22]. Briefly, the material was prepared for gradient centrifugation by subjecting the individual rat stomach perfusates to DNase treatment, dialysis, lipid extraction with chloroform/methanol (2:1) and then CsCl to initial density of 1.43g/ml was added. The solution was centrifuged for 48h, at 15°C, at 46 000 rpm. Each tube was fractionated into 15 fractions, assessed for carbohydrate, and protein content, and the fractions containing mucin were combined, dialyzed, and used for SDS gel electrophoresis, monosaccharide quantitation, β-elimination and HPTLC separation of the released O-glycans. The major fractions corresponding to octasaccharide standard were extracted from thin layer gel and subjected to GC in the presence of the internal standard of mannitol.

Carbohydrate Analysis of Mucin- and Glycolipid-Linked Glycans

Separation of glycosphingolipids from lipid extracts of the individual perfusates was performed on micro-thin layer plates (2.5 x 2.5 cm) [24]. The lipid extracts were subjected to alkaline degradation, the phospholipids-derived fatty acid and cholesterol of each sample were chromatographed to upper portion of the plate with low polarity solvent consisting of hexane/ acetic acid (7:1) and then the plate developed with chloroform/methanol/water (30:60:8). Thus separated fractions of glycosphingolipids were extracted from the plates, subjected to acid methanolysis, and quantitated by GC estimation of glucose in the presence of the internal standard of mannitol.

The thin layer chromatography (HPTLC) was also used to separate glycans released from mucin by β-elimination reaction [19]. The glycans were applied to 20x20 cm plate and subjected to chromatography in solvent system consisting of n-butanol/acetone/water (5:3:2). The glycans corresponding to octasaccharide standard, the fraction previously identified as one interacting with mucin epithelial receptor, the mucin binding protein (MBP) of rat gastric mucosa were collected by extracting silica gel with ethanol/water (2:1). The fractions were subjected to acid methanolysis, carbohydrate analysis and quantitation using internal standard of mannitol. The size of glycans was determined by establishing molar ratio of monosaccharides to that of N-actylgalactosaminitol (GalNAc-ol).

OTHER PROCEDURES

The individual stomachs were inspected for the presence of erosions and hemorrhagic foci. Histological examination consisted of hematoxylin-eosin, periodic acid-Schiff and Alcian blue stained specimens [25]. The dialyzed, concentrated and adjusted to the same volume perfusates, were assayed for protein, immunoreactive albumin and hemoglobin [24]. The samples recovered from the density gradient sepa-

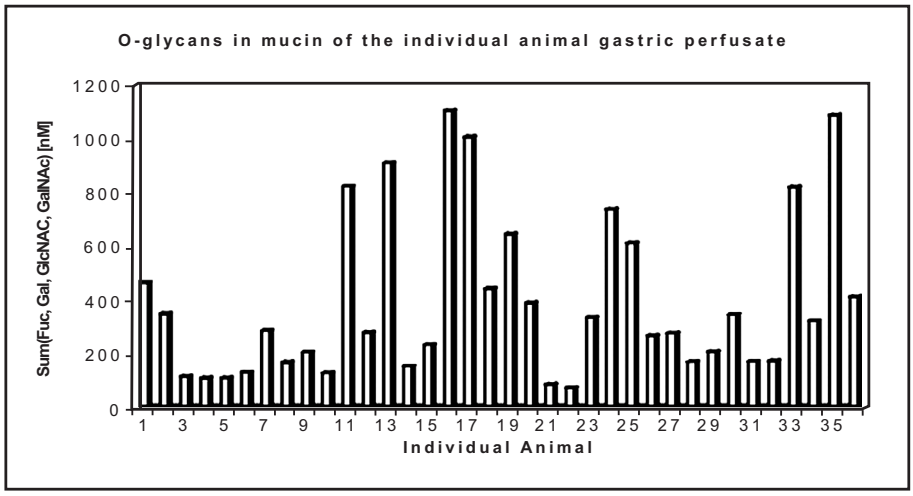

Fig. (1). Output of gastric mucin O-glycans varies in individual animals subjected to the same conditions and isocaloric diet. The columns represent the quantitative analysis of mucin-bound glycans derived from 3h gastric perfusion described in the Materials and Methodology section.

ration were subjected to SDS-PAGE, western blotting and glycoprotein visualization with Glyco stain [19].

RESULTS

Perfusion of the rat stomachs with saline-cholate was used to estimate the potential of individual animals to synthesize and secrete mucin. The perfusion was performed on the healthy animals of the same age and weight that were kept in individual metabolic cages and fed for 7 days isocaloric, normal liquid diet. The results gathered illustrate quantitative analyses of the protein-bound carbohydrate collected individually during perfusion of the 36 rat stomachs. The 3 hours perfusion with 4 stomach volumes of liquid afforded material containing from 118.2 to 1171.0 nmol of carbohydrate/rat stomach. The samples collected from rats #19,24,25,33,35 contained serum-derived albumin, and the rats #11,13,16,17 contained elevated contents of glycosphingolipid-derived glucose (Glc), which were indicative of serum transudation and increased epithelial turnover, respectively. The amounts of mucin glycans and glycosphingolipids recovered from the 36 individual samples corresponding to three hours output are shown in Fig. (**1** and **2**).

As demonstrated in the above shown figures, the large variability in mucin release was not caused or paralleled by epithelial turnover. Also, similar variation in the recovered mucin-bound glycans was detected in the samples collected by perfusing saline or saline-cholate. With saline only, the amount of mucin recovered from rats #5,9,21,29 was in the range of values found for saline-cholate samples collected from animals #3,4,8,10,28,30,34. Thus, based on analyses of the group of 26 samples which was not contaminated with serum transudate (#19,24,25,33,35) or contained elevated Glc (#11,13,15,16,17), we deduced that in spite of identical conditions, diet and caloric intake, the synthesis of mucin by individual animals differed at least 6.7 fold while glycosphingolipids varied 2.5 fold.

COMPOSITIONAL SIMILARITIES AND DIFFERENCES IN RAT GASTRIC MUCIN GLYCANS

Estimation of Galactose Incorporation into O-Glycans of Individual Animals

Mucin samples analyses revealed differences in the amount of Nacetylgalactosamine (GalNAc), Galactose (Gal), Nacetylgalactosamine (GlcNAc) and Fucose (Fuc) (Figs. **3** and **4**). As shown in Fig. (**3**), the ratio of Gal/GalNac varied from 1.0 to 10.0 Gal residues. On the average, glycan chain in four animals (# 4,11,14,26) contained 1.0-2.0 Gal residues, in thirteen animals (#1,2,13,15,16,17,18,23,24,28,30,33,36) contained 2.0-3.0 Gal residues, in eight (#3,8,10,12,29, 32,34) contained 3.0-4.0 Gal residues, in four (#7,9,19,35) contained 4.0–5.0 Gal residues, in three (#20,21,25) contained 5.0-6.0 Gal, and in four (# 5,6,22,27) contained 6.0-10.0 Gal residues.

The O-glycan size variability was observed in all samples including those collected with saline only. The saline perfusates (# 5,9,21,29) were found to represent glycans in four groups and ranged in size from 3.0-10.0 Gal residues/glycan. Thus, we concluded that each animal displayed different potential for the synthesis of O-glycans. The variability occurred in spite of highly controlled housing conditions, diet composition, caloric intake and mode of collection. The saline-collected samples, which presumably were enriched in surface or unattached mucin, reflected the same type of O-glycans variability as the complete spectrum of mucin collected with saline-cholate perfusion. Clearly, the quantitative studies on Gal incorporation into mucin glycans demonstrate that each animal displays different ability to synthesize mucin glycans, even when the nutrients are provided in equal and highly controlled manner.

Termination of Mucin O-Glycans with Fucose

On the average, O-glycans contained one Fuc residue/GalNAc in mucin isolated from 14 rat stomach per-

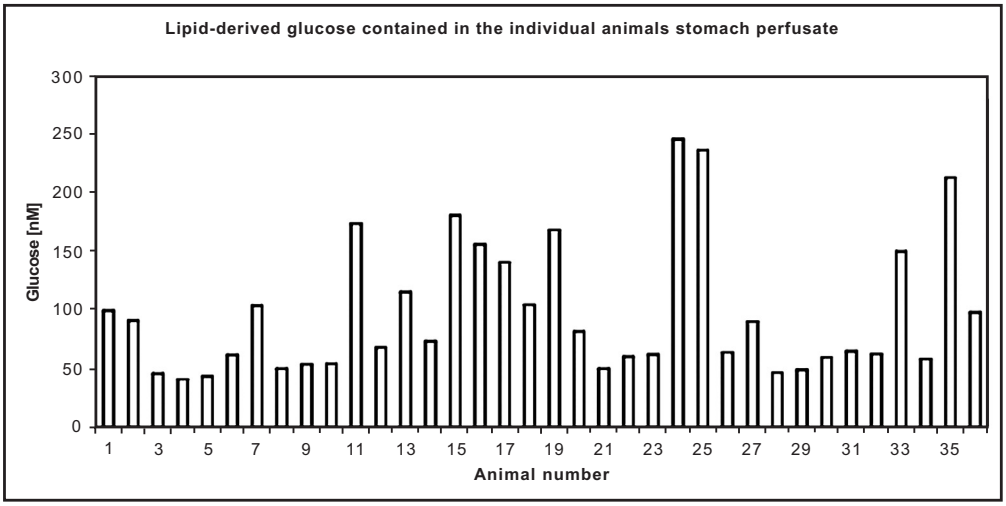

Fig. (2). Gastric epithelial cells turnover in the individual animals subjected to the same conditions and isocaloric diet. The individual columns represent amount of glycosphingolipids-derived glucose (Glc) from the 3h gastric perfusion. The details of the perfusion and lipid isolation are described in Materials and Methodology section.

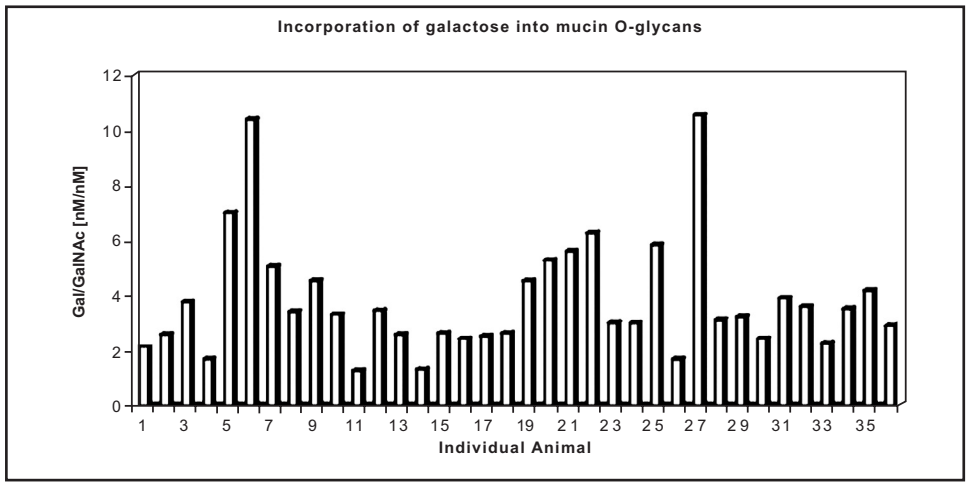

Fig. (3). Incorporation of galactose (Gal) into mucin O-glycans is individual-animal specific. The columns reflect the molar ratio of Gal to Nacetylgalactosamine (GalNAc) established by quantitative gas chromatographic analysis of the purified mucin from individual gastric perfusions from the animals subjected to the same conditions and isocaloric diet.

fusates (Fig. **4**), (#1,2,4,11,12,13,14,15,21,22,25,26,28,36), 1,0-1.5 Fuc/GalNAc was found in mucin O-glycans isolated from 9 animals (#3,5,8,9,17,18,30,32,33,36) and up to 3 Fuc residues/GalNAc were found in O-glycans isolated from mucin of 13 animals (#6,7,9,10,16,19,20,23,24,27,29,34, 35).

The samples with low content of Gal in O-glycans (#4,11,14,26) contained only one Fuc residue, but those with 2-3 Gal/GalNAc contained 1-3 Fuc residues. The mucin O-glycans isolated from animal #17,18,30,33,36 contained on the average 1-1.5 Fuc/2-3 Gal/GalNAc, whereas #16,23,24 contained 2 or more Fuc/2-3 Gal/GalNAc. Thus again, the termination of O-glycan chains varied depending on the animal and was not associated with the average length of glycan chains. In our interpretation, this indicated that individual animals contained different levels of Fuc substrate to terminate mucin glycans.

Expression of O-Glycans Responsible for Mucin Association with Gastric Apical Epithelial Surface. Quantitative and Compositional Differences

Our next question was to ascertain whether variations in the amount and composition of glycans were reflected in glycans carrying specific function that imparted protection of epithelial cells from lumenal gastric contents and ensured homeostatic interaction of mucin with apical membrane. Thus, we concentrated our efforts on the identification and quantitation of the O-glycans which in earlier study were found responsible for the interaction of mucin with mucin receptor in gastric apical membrane, the mucin binding

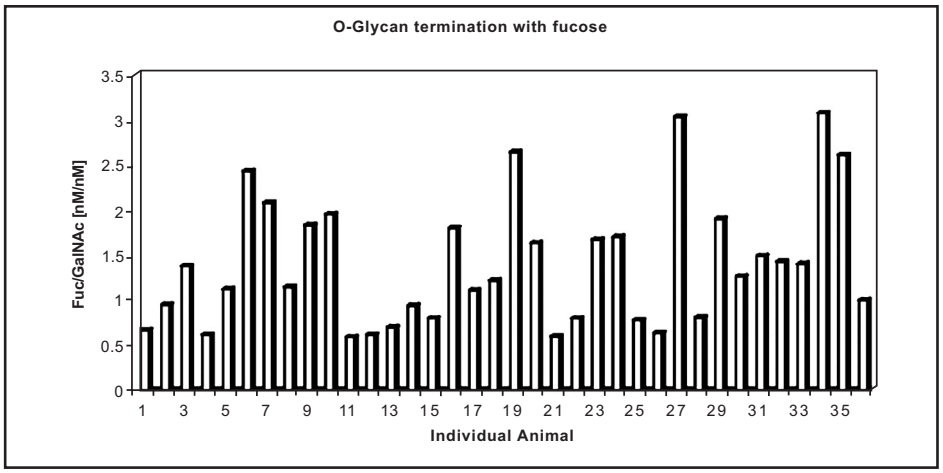

Fig. (4). Mucin O-glycan termination with fucose (Fuc) is individual animal specific. The columns represent the molar ratio of Fuc to GalNAc in mucin O-glycans isolated from gastric perfusates collected from individual animals subjected to the same conditions and isocaloric diet.

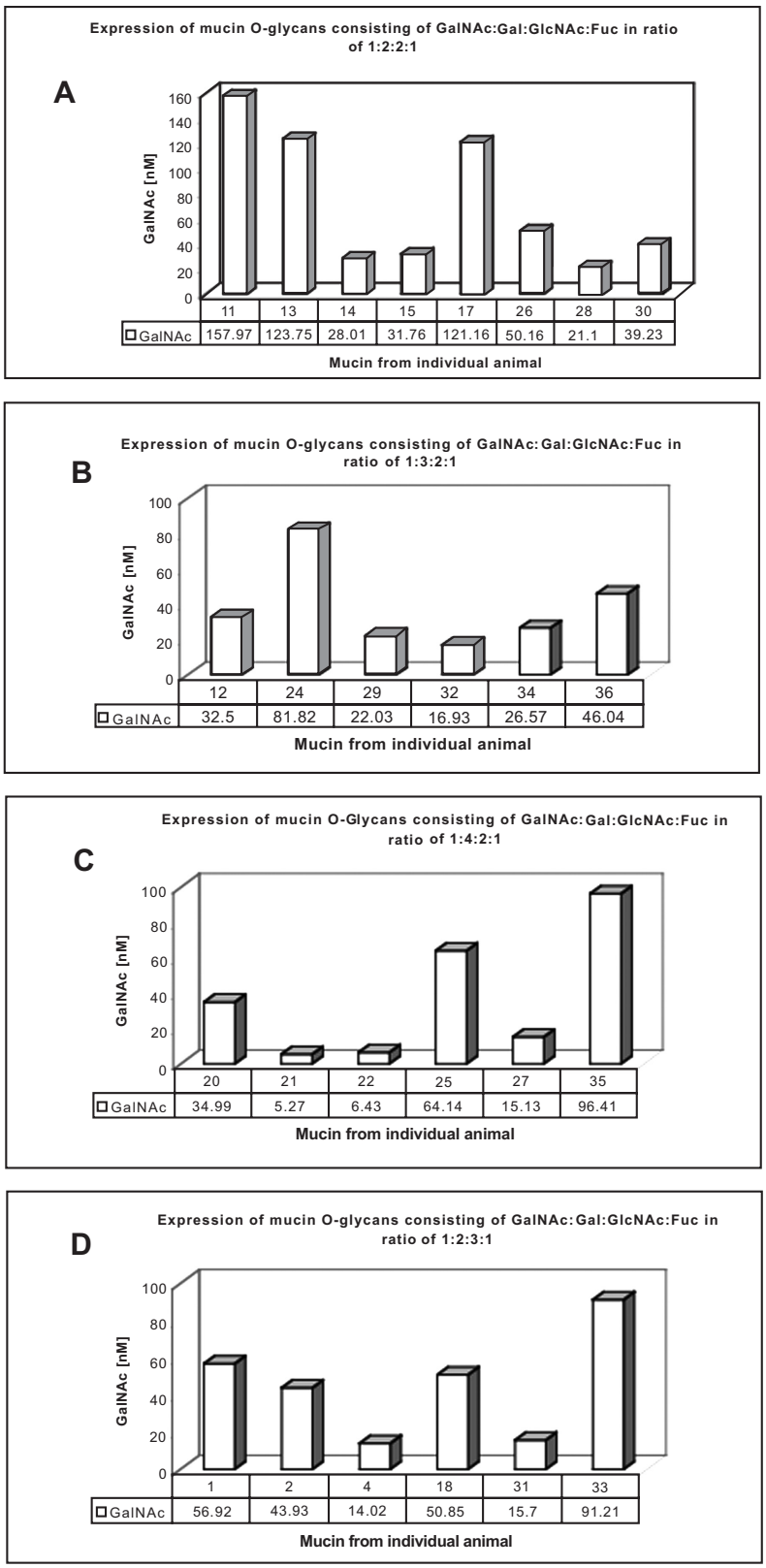

Fig. (5) A-D. Qualitative and quantitative expression of the O-glycans responsible for mucin attachment to apical epithelial mucin receptor, the Mucin Binding Protein (MBP) is different in the individual animals subjected to the same condition and isocaloric diet. The columns represent quantitative analysis of the specific O-glycans whose carbohydrate composition and molar ratio to GalNAc is shown in each figure. The quantitative expression of the specific glycans in the individual animals is shown in nmol/mucin/perfusate. In each figure under the columns, the first row of numbers refers to the individual animal, and the second row shows the amount of the specific glycans quantitated in terms of nmol GalNAc.

protein (MBP) [19]. As we demonstrated, the MBP receptor was interacting with O-glycans migrating in the range of octasaccharide standard. Taking this as a template, we have isolated O-glycans from mucin of individual animals and from each we isolated the fraction corresponding to octasaccharide-MBP binding glycans, and determined their carbohydrate composition. Based on the complete β-elimination-conversion of GalNAc to N-acetylgalactosaminitol (GalNAc-ol), the GalNAc was assigned the glycan-initiating position only, and thus the O-glycan size, molar composition and molar ratio of Gal, GlcNAc and Fuc were established from their ratio to GalNAc-ol following GC in the presence of the internal standard of mannitol (Man-ol). The results of the studies are shown in Fig. (**5A-D**).

The presented data reflect individual-specific variability in quantity, length and composition of the O-glycans corresponding to the octasaccharide standard. The O-glycans isolated from mucin of eight animals (Fig. **5A**) were characterized as hexasaccharides that consisted of GalNAc:Gal:GlcNAc:Fuc in the ratio of 1:2:2:1. As determined, the amount of these oligosaccharides varied up to 8 fold, from 13.1 nmol in mucin collected from rat #28 to 121.8 nmol in mucin from #11. The O-glycans isolated from six animals (Fig. **5B**) were characterized as heptasaccharides that consisted of GalNAc:Gal:GlcNAc:Fuc in the ratio of 1:3:2:1, and their expression varied up to 5 fold; from 9.3 nmol in mucin from rat #32 to 40.7nmol in mucin from rat #24.

The fraction corresponding to octasaccharide standard isolated from animals grouped in Fig. (**5C**), contained O-glycans consisting of GalNAc:Gal:GlcNAc:Fuc in the ratio of 1:4:2:1 and their expression varied 18 fold; from 2.2 nmol in mucin from rat #21 to 46.8 nmol in rat #35. The O-glycans corresponding to octasaccharide standard grouped in Fig. (**5D**) consisted of GalNAc:Gal:GlcNAc:Fuc in the ratio of 1:2:3:1. Their expression in mucin isolated from the individual rats varied 6.5 fold, from 9.9 nmol in mucin from rat #4 to 52.2 nmol in rat #33. The fractions of O-glycans isolated from the remaining ten mucin samples were represented by the mixture of oligosaccharides which could not be resolved into homogeneous fractions by thin layer chromatography and recovered in sufficient quantity for GC analysis. Based on the compositional studies, they contained glycans with more than two GlcNAc and two Fuc residues, but we could not determine their molar composition and expression in mucin of the aforementioned individual animals.

Based on the results presented in the Fig. (**5A-D**), we affirmed further that each animal, although subjected to the same conditions and same isocaloric diet, expressed mucin with different O-glycan pattern. Importantly, we provided evidence that inherently due to variable expression of the specific glycans, the interaction of mucin with apical epithelial receptor varies from one animal to the next and this contributes to different protection of gastric apical epithelium. By extrapolation, it is tempting to suggest that the same scenarios are developing in humans, and that in some individuals carbohydrate-low diet may be responsible for deterioration of the protective gastric mucous barrier.

DISCUSSION

Our ultimate goal in mucus glycoproteins investigation is an identification of glycan-specific signature on gastric mucin in health and disease [3, 17-19]. In contrast to studies by others, that focused on mucin producing cell lines and glycosylation impacted by mucin differences in amino acid composition, size and number of tandem repeats [10, 11], we searched for innate and intracellular factors that support carbohydrate metabolism and mucin glycosylation [17, 26]. Thus, we found that cytosol derived from healthy rats produced mucin with denser O-glycans substitution than diluted ATP-enriched cytosol, or the cytosol derived from animals subjected to low carbohydrate diet [18]. Together, the results allowed us to surmise that intracellular milieu, the individual-specific metabolic environment and the dietary carbohydrates, impact glycosylation density and the organ-specific synthesis of mucin glycans.

In continuation, we focused on the individual-specific and function-specific glycans in the paradigm employing rats subjected to normal and low-carbohydrate isocaloric diet [18]. The study on rats pair-fed isocaloric diet demonstrated diet-dependent and individual-specific expression of WGA determinant. In addition to quantitative dissimilarity, the mucin collected from animals on carbohydrate-low diet contained progressively lower amount of WGA-binding glycans, whereas animals on normal diet continued to produce the same amount of the specifically terminated glycans.

In the study presented here, we continued our search for the signature glycan in healthy rats subjected to same conditions and same isocaloric diet. Analysis of gastric mucin secreted during 3h of continuous infusion of saline or saline-cholate revealed quantitative and qualitative differences in mucin and gastric apical epithelial cells turnover. The 16-fold spectrum in mucin glycan production and 6 fold spectrum in epithelial cell turnover was documented in group of 36 rats. It is quite possible that such disparity in the individual-specific mucin synthesis contributes to a different sensitivity of the individual animals to low-carbohydrate diet, as that some rats showed its impact within one week, whereas in other the carbohydrate deficiency was developed within 6 weeks [18]. The significant findings of these studies are that each individual animal responded differently to the same diet, produced different quantity of mucin and, manifested different apical epithelial cells turnover.

Our aim to identify specific glycan whose expression is reflecting mucin interaction with apical epithelial mucin receptor lead to identification of several glycans consisting of GalNAc, Gal, GlcNAc and Fuc that varied in quantity, length, amount of Gal, GlcNAc and Fuc residues. Based on the general principle in mucin glycan synthesis, these functionally linked products consisted of linear and branched chains and differed in the amount of terminal Fuc residues. Thus, fundamental part of the concept that require further investigation is whether Fuc is the required part of the terminal glycan configuration determining mucin retention and interaction with apical mucin receptor and is controlling factor in apical epithelium turnover. After analysis of individual cases, it appears significant that expression of certain types of glycans, as those terminated with Fuc is particularly sensitive and should be addressed through dietary supplementation [27-29].

Our study do not provide direct examples of dietary impact and individual-specific synthesis on human mucin glycans, but the study of Xia *et al*. [16] demonstrate the phe-

nomenon in CF and non-CF mucin. They found that different O-glycans reside on the same MUC protein backbone isolated from organs but not from organ-derived cell lines. Both, our study on rat-derived gastric mucin and their from human lungs imply that genetic factors, microenvironment of the organ and the metabolic resource provide different outcome in each individual, and we must learn under what circumstances the interaction of these factors determines normalcy or disease, and how we can manipulate metabolites to restore normal homeostatic controls in glycan expression. Thus, it is conceivable that diet-intrusive effect on O-glycan elongation and density could be used to our benefit and to pave the way for new strategies to modify nutrients and derail formation of the pathogen binding glycans. Consequently, it is tempting to suggest that by using specific dietary supplements, we could prevent microbial colonization of unprotected epithelial surfaces and thus minimize occurrence of many diseases, and intervention with antibiotics.

The results of our investigation demonstrate that determining factor in the synthesis of mucin glycans is organ environment-specific and the *in situ* conditions provide metabolic and individual-specific imprint on quantity and structure of the glycans. It is, therefore, highly significant that the investigation of glycosylation in normal or diseased tissue is scrutinized in the environment reflecting the precise conditions of that organ. We believe that the investigations of posttranscriptional, cell metabolite-impacted events carried out in an artificial and uniform paradigm that neither mimics normal homeostatic milieu nor disease-associated environment provide skewed picture. Such fixed environment produces uniformity in glycans synthesis and erases contribution of the organ-specific conditions. Thus, the aberrations in posttranscriptional events in CF, carcinoma-associated glycoproteins, and modified expression of glycoproteins and their glycans in organs cannot be deciphered in the *in vitro* cultured cells. As for non-CF, non-cancerous cells or gastrointestinal apical epithelial cell lines, the culture paradigm provides culture-specific but foreign to the true site milieu. The consequence of foreign cocktail of substrates, including carbohydrate components, provided with empirically established culture medium, produces cells that do not reflect the intrinsic manifestation of the relationship between disease-induced protein expression and the environment that dictates aberrant posttranslational modification. Therefore, it is questionable whether cancer-derived cell lines produce the same glycans in culture as those in the cells mass that determine destination of the released cells from solid tumor and metastasis [30-32].

Our findings of the dietary impact on the synthesis of mucin carbohydrate chains and their expression in secreted mucin and membrane glycosphingolipids, thus legitimize nutritional approach to seek for signature glycans that reflect health status of the individual and to reevaluate the conclusions based on the results derived from *in vitro* cultured cells.

REFERENCES

[1] Slomiany BL, Slomiany A. Role of mucus in gastric mucosal protection. J Physiol Pharm 1991; 42: 147-61.

[2] Slomiany BL, Murty VLN, Piotrowski J, *et al*. Salivary mucins in oral mucosal defense. Gen Pharmac 1996; 27: 761-71.

[3] Slomiany A, Kasinathan C, Slomiany BL. Glycosylation patterns in mucus glycoprotein. Adv Macrobiol Carbohydr Res 1997a; 1: 177-211.

[4] Aebi C, Hennet T. Congenital disorders of glycosylation: Genetic model systems lead the way. Trend Cell Biol 2001; 11: 136-41.

[5] Byrd JC, Bresalier RS. Mucins and mucin binding proteins in colorectal cancer. Cancer Metastasis Rev 2004; 23: 77-99.

[6] Rose MC, Voynow JA. Respiratory tract mucin genes and mucin glycoproteins in health and disease. Physiol Rev 2006; 86: 245-78.

[7] Wopereis S, Lefeber DJ, Morava E, *et al*. Mechanisms in protein O-glycan biosynthesis and clinical and molecular aspects of protein O-glycan biosynthesis defects. Clin Chem 2006; 52: 574-600.

[8] Perez-Vilar J, Hill RL. The structure and assembly of secreted mucins. J Biol Chem 1999; 274: 31751-4.

[9] Lowe JB, Marth JD. A genetic approach to mammalian glycan function. Annu Rev Biochem 2003; 72: 643-91.

[10] Silverman HS, Sutton-Smith M, McDermott K, *et al*. The contribution of tandem repeat number to the glycosylation of mucins. Glycobiology 2003; 13: 265-77.

[11] Holman JM, Karlsson NG, Abdullah LH, *et al*. Mucins and their O-glycans from bronchial epithelial cell cultures. J Physiol Lung Cell Mol Physiol 2004; 287: L824-34.

[12] Muller S, Goletz S, Packer N, *et al*. Localization of O-glycosylation sites on fragments from lactation-associated MUC1. All putative sites within the tandem repeat are glycosylation targets *in vivo*. J Biol Chem 1997; 272: 24780-93.

[13] Karlsson NG, Herrmann A, Karlsson H, *et al*. The glycosylation of rat intestinal MUC2 mucin varies between rat strains and small and large intestine. A study of O-linked oligosaccharides by mass spectrometric approach. J Biol Chem 1997; 272: 27025-34.

[14] Hanisch FG, Muller S, Hassan H, *et al*. Dynamic epigenetic regulation of initial O-glycosylation by UDP-N-acetylgalactosamine: peptide Nacetylgalactosaminyl transferase. J Biol Chem 1999; 274: 9946-54.

[15] Burdick MD, Harris A, Reid CJ, *et al*. Oligosaccharides expressed on MUC1 produced by pancreatic and colon cell lines. J Biol Chem 1997; 272: 24198-202.

[16] Xia B, Royall JA, Damera G, *et al*. Altered O-glycosylation and sulfation of airway mucin associated with cystic fibrosis. Glycobiology 2005; 15: 747-75.

[17] Slomiany A, Morita M, Sano S, *et al*. Effect of ethanol on gastric mucus glycoprotein synthesis, translocation, transport, glycosylation and secretion. Alcohol Clin Exp Res 1997b; 21: 417-23.

[18] Slomiany A, Piotrowski J, Piotrowski E, *et al*. Impact of ethanol on innate protection of gastric epithelial surfaces and the risk of injury. J Physiol Pharmac 2000; 51: 433-47.

[19] Slomiany A, Grabska M, Slomiany BL. Essential components of antibacterial gastrointestinal epithelial barrier: Specific interaction of mucin with with integral apical membrane protein of gastric mucosa. Mol Med 2001; 7: 1-10.

[20] Lieber CS, de Carli LM. The feeding of alcohol in liquid diets: Two decades of application and 1982 update. Alcohol Clin Exp Res 1982; 6: 523-31.

[21] Slomiany BL, Nadziejko C, Mizuta K, *et al.* Role of associated and covalently bound lipids in the physicochemical properties of mucus glycoprotein. In: Mastella G, Quinton PM, Eds. Cellular and molecular basis of Cystic Fibrosis. San Francisco Press, Inc. San Francisco 1988; 263-79.

[22] Slomiany A, Tamura S, Grzelinska E, *et al*. Mucin complexes: Characterization of the "link" component of submandibular mucus glycoprotein. Int J Biochem 1992b; 24: 1003-15.

[23] Slomiany A, Zdebska E, Slomiany BL. Structure of neutral oligosaccharides isolated from A-active human gastric mucin. J Biol Chem 1984; 259: 14743-9.

[24] Slomiany A, Patkowska MJ, Slomiany BL, *et al.* The effect of ethanol on the constituents of the gastric mucous barrier. Int J Biol Macromol 1979; 1: 165-70.

[25] Slomiany BL, Slomiany A. Mucus and gastric mucosal protection. In: Domschke W, Konturek SJ, Eds. The stomach. New York: Springer-Verlag 1993; 116-43.

[26] Slomiany A, Grzelinska E, Grabska M, *et al*. Intracellular processes associated with glycoprotein transport and processing. Arch Biochem Biophys 1992a; 298: 1021-8.

[27] Lubke T, Marquardt T, von Figura K, *et al*. A new type of carbohydrate deficient glycoprotein syndrome due to decreased import of GDP-fucose into Golgi. J Biol Chem 1999; 274: 25986-9.

[28] Marquardt T, Luhn K, Srikrishna G, *et al*. Correction of leukocytes adhesion deficiency type II with oral fucose. Blood 1999; 94: 3976-85.

[29] Becker DJ, Lowe JB. Fucose: Biosynthesis and biological function in mammals. Glycobiology 2003; 13: 41R-53R.

[30] Capon C, Wieruszewski JM, Lamoine J, *et al*. Sulfated Lewis X determinants as major structural motif in glycans from LS174T-HM7 human colon carcinoma mucin. J Biol Chem 1997; 272: 31957-68.

[31] Muramatsu T. Carbohydrate signals in metastasis of human carcinoma. Glycobiology 1993; 3: 294-96.

[32] Lawrie CH, Marafioti T, Hatton SSR, *et al*. Cancer-associated carbohydrate identification in Hodkin's lymphoma by array profiling. Int J Cancer 2006; 118: 3161-6.

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