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Human respiratory mucins

G. Lamblin, J.P. Aubert, J.M. Perini, A. Klein, N. Porchet, P. Degand, P. Roussel

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ABSTRACT: Human respiratory mucins are secreted by goblet cells and mucous glands of the respiratory mucosa. They consist of a broad family of complex glycoproteins with different peptides, or apomucins, corresponding to several genes located on at least three different chromosomes.

Glycosylation, the major posttranslational phenomenon, is responsible for about 70–80% of the weight of mucins: it produces an extraordinary diversity of O-glycosidically linked carbohydrate chains which are expressed as several hundreds of different chains in the mucins of a single individual.

The variety of mucin peptides and the diversity of carbohydrate chains probably allows many interactions, especially with microorganisms: this may be an essential factor in the defence of the underlying respiratory mucosa.

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Unité INSERM N° 16, place de Verdun, Lille, France.

Correspondence: Unité INSERM N° 16, place de Verdun, 59045 Lille, France.

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For more than a century the concept of mucin was associated with material secreted in mucus. Mucins (including blood group substances), the main constituents of most mucus, were probably the first type of compounds to be clearly recognized as glycoproteins. However, the precise definition of mucins in general, as well as that of respiratory mucins, is still evolving due to the complexity of these molecules [1].

Initially, the definition of mucins was based on the chemical composition (from 50–80% carbohydrate) and molecular mass (from several hundred to several thousand kDa). In the typical chemical composition of human respiratory mucins, there is more than three times more carbohydrate than peptide. The proportion of hydroxylated amino acid is high (from 30–35 serine and threonine residues per 100 amino acid residues) as are the proportions of all the sugars usually found in mucins, *i.e.* fucose, galactose, N-acetylglucosamine, N-acetylgalactosamine, sialic acid. Respiratory mucins also contain sulphate but no uronic acid and perhaps a small quantity of mannose which will be discussed later.

The physical polydispersity and several other lines of evidence clearly indicate that there is not a single mucin, such as a unique respiratory mucin, but a very large family of mucin molecules, differing from each other at the peptide and at the carbohydrate levels.

In the present review, we will describe the evidence which suggests that human respiratory mucins are a broad family of different glycoproteins which seems to stem from two events, firstly the expression of mucin genes into multiple apomucins, then a diversity of posttranslational phenomena, mainly O-glycosylation leading to carbohydrate chains with a vast microheterogeneity. We will also try to speculate on the possible biological significance of such a diversity.

Cellular origins of human respiratory mucins

The goblet cell found in the human respiratory epithelium is a very good example of a mucin synthesizing cell. At the basal part, it has a nucleus surrounded by rough endoplasmic reticulum and an apical part filled with mucin granules intensely stained by periodic acid-Schiff (PAS). The Golgi apparatus is between these two cell compartments.

The cells which form the mucous glands of the submucosa have a similar shape. They also synthesize mucins and greatly outnumber the goblet cells of the surface [2]. These different cells may differ in their staining intensity with different dyes as well as their affinity for different lectins [3, 4].

The mucin peptides are thought to be translated in the rough endoplasmic reticulum as apomucins and most of the glycosylation process occurs in the Golgi apparatus, which delivers mucus granules accumulating at the apical part of the mucin synthesizing cells before secretion.

Physicochemical evidence for the heterogeneity of human respiratory mucins

One of the main difficulties in working on mucin is related to the viscoelastic properties of respiratory mucus. Mucus has to be solubilized before the

purification of mucins. For this purpose, various mucolytic procedures have been used but some of them, such as proteolytic enzymes or reducing agents, will simultaneously produce some degradation of the mucin molecules [5–7]. Mild agitation in dissociating agents or after water dilution leads to disentanglement of mucin molecules which are then ready for chemical or physical analyses [1, 8].

Based on its peptide and carbohydrate composition and its susceptibility to alkali, the usual representation of a respiratory mucin is that of a "bottle-brush" with hundreds of carbohydrate chains attached to serine and threonine residues of the mucin peptide (fig. 1).

Proteolytic cleavage of mucins produces small peptides and glycopeptides (fig. 1). There is a partial degradation of the "naked" regions of the mucin peptide, which is more or less devoid of carbohydrate chains, leaving "highly glycosylated" regions resistant to proteolysis (or mucin glycopeptides) [9]. The exact distribution of the naked regions is completely unknown.

The estimation of the molecular mass of respiratory mucin is usually very difficult and still a matter of debate. There are large differences according to the method used: in the range 1–8 × 1,000 kDa with sedimentation equilibrium [1, 7, 10, 11], 10–20 × 1,000 kDa with light scattering [1, 12, 13]. Most groups agree on the large polydispersity of these molecules.

A major advance in the understanding of mucin conformation occurred with the introduction of electron microscopy for studying mucin molecules. Human respiratory mucins appeared as polydisperse, linear and apparently flexible threads [11, 14–16] (fig. 2: [11]). However, there are some discrepancies between laboratories with regard to the width of distribution: in most data, reported so far, the distribution of respiratory mucins filaments ranged between two or three hundred nm and about 1,500 nm [11, 14, 15]; larger species have been observed by SHEEHAN *et al.* [16] but, in that study, the size of more than 80% of the filaments was less than 2 μm. Frequently, electron microscopy also shows aggregates and it is difficult to establish firmly

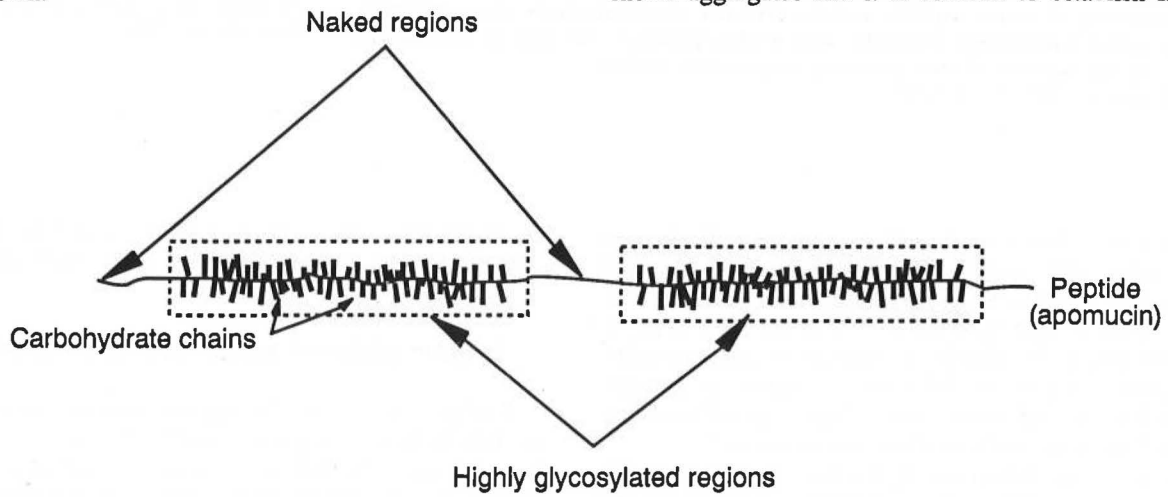

Fig. 1. – Schematic representation of human respiratory mucins and mucin glycopeptides (highly glycosylated regions) obtained by proteolysis.

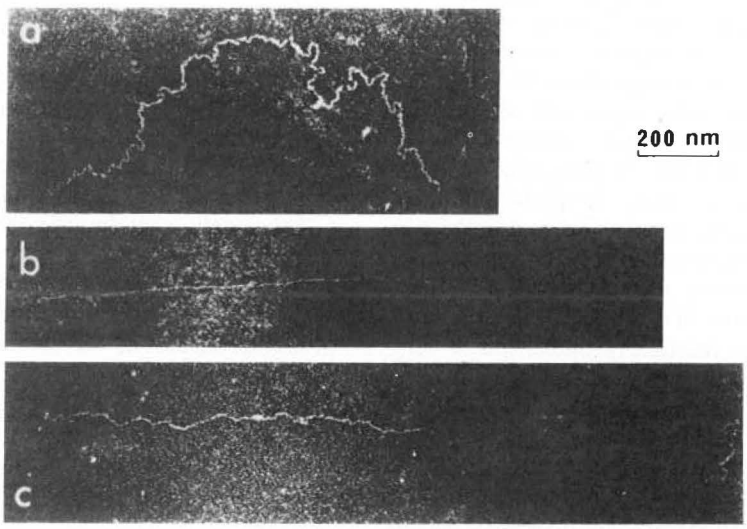

Fig. 2. – Electron micrographs of tungsten replicas of human respiratory mucin. Mucin molecules may be kinked (a) or extended (b). Some pictures, as for instance in (c) are more difficult to interpret: they may correspond either to an overlap of several mucin molecules or to longer species. The micrographs were provided by the courtesy of Dr H.S. Slayter (Harvard Medical School, Boston) [11].

whether the longest filaments correspond to one mucin molecule as suggested by SHEEHAN *et al.* [16] and THORNTON *et al.* [17] or to tangled units. In fact, respiratory mucins have lipid-binding [1, 18] and hydrophobic properties which can contribute to their polymeric structure through noncovalent interactions [19].

Mucin glycopeptides obtained by direct reduction of mucus [20] or by proteolysis of purified mucins [17] appear also to be polydisperse, although as shorter rods with a distribution of sizes ranging from 50–250 nm.

The question of disulphide bridges linking respiratory mucin subunits is still a matter of controversy. Reducing agents act on the longer species to produce shorter species [20] but whether their exact role is reduction of disulphide bridges linking mucin subunits [9] or proteolysis activation [7] is not perfectly clear. In other mucins, "link proteins" covalently attached to mucin subunits have been reported [21]. The presence of such a link has not yet been firmly proved, although the association of mucins to a 65 kDa protein has been reported [22].

Biological evidence for a wide diversity of the peptides of human respiratory mucins

Human respiratory apomucins

Since human respiratory mucins appeared as polydisperse glycoproteins, even when collected directly from healthy areas of the bronchial tree, several experiments were designed in order to characterize the size of the apomucins, or peptide precursors, in the rough endoplasmic reticulum, before glycosylation in the Golgi apparatus.

Several antibodies were prepared against deglycosylated products of "highly glycosylated" regions isolated from human respiratory mucins [23, 24]. These antibodies, which recognized uncovered mucin peptides, or apomucins, were used to immunoprecipitate radiolabelled mucin precursors synthesized in explants of human bronchial mucosa during pulse-labelling experiments with [^{3}H] threonine. They demonstrated the existence of a population of peptide precursors in the range of 200–400 kDa [25].

The same antibodies have also been used to characterize the respiratory mucin precursors obtained during *in vitro* translation experiments of messenger ribonucleic acids (mRNAs) purified from human tracheobronchial mucosa [26]: these precursors also appeared as a polydisperse population of peptides in the range 100 to >400 kDa. Such values of Mr are quite compatible with the size of mucin filaments observed by electron microscopy [11].

Preparation of respiratory mucin complementary deoxyribonucleic acids (cDNAs)

Is it possible to correlate the diversity of the peptide moiety to the diversity of ribonucleic acid (RNA) messages? To answer this question, a cDNA library was constructed in λgt11 vector and screened with a polyclonal antiserum directed against tracheobronchial apomucins [27]. The experimental approach which has been described elsewhere [27–30] is schematized in figure 3.

Twenty positive clones were obtained and were sequenced [28]. The positivity of each clone was controlled by immuno-histochemical studies. "Monoclone" antibodies were purified by adsorption of specific antibodies from the total antiserum on the fusion protein from each positive clone. Every "monoclone" antibody was able to recognize either both goblet cells and mucous cells, or only goblet cells, demonstrating the cellular specifity of these immunological probes (fig. 3).

cDNA sequences

Very little is known about the peptide sequence of secreted mucins as opposed to mucin-like glycoproteins. The mucin-like glycoproteins are transmembrane molecules expressed in cancer cell lines such as human breast tumour and pancreatic carcinoma.

The complete sequence of episialin, a mucin-like glycoprotein, has recently been described [31, 32]. In contrast, only partial cDNA sequences have been reported for secreted mucins such as intestinal mucins: these sequences were characterized by the presence of "tandem repeats" of 23 and 17 amino acids, respectively, [33, 34].

In the case of human airway mucins, repetitive sequences of 8 and 16 amino acids were found. These incomplete sequences were not homologous and differed from the sequences described previously [27, 30]. Recently, a mucin cDNA obtained from a patient suffering from cystic fibrosis has been reported: the sequence is homologous with a sequence reported for colonic mucins. Other families of bronchial mucin cDNAs were also observed [28].

Several cDNAs coded for stretches of peptides with an amino acid composition characteristic of human bronchial mucins. Among them, small sequences were found to be identical with previously reported tracheal mucin peptide sequences that were determined chemically [35].

Finally, the sequences of other cDNA clones corresponded to a perfect alternation of potentially glycosylated regions and naked regions. These deduced amino acids zones were varying in length but always organized according to the same scheme.

By nucleotide sequence comparisons, it seems that exons of respiratory mucin genes are small and that primary mRNAs are submitted to a very complex alternative splicing system.

All these deduced amino acid sequences emphasize the heterogeneity of the protein cores of human airway mucins: five out of the 20 positive clones sequenced possess a C-terminal amino acid. The same thing is observed with clones derived from the λgt10 cDNA

library. In some cases, the code sequence Asn-X-Ser/Thr was found. This sequence is potentially N-glycosylated and might explain the presence of mannose traces in secreted respiratory mucins [11].

These results corroborate those obtained by *in vitro* translation experiments.

Northern blot analysis

Human airway mucin probes hybridized with mucin mRNAs as very polydisperse signals characterized in electrophoresis as a large smear with a size ranging from 0.4 to about 20 Kb [28]. Usual controls were done and no RNA degradation was observed. The same observations have recently been obtained in colonic mucosa [34, 36, 37].

Expression of mucin mRNAs from different human mucosae and from two human breast tumour cell lines was examined with all the probes in our possession. Only a few probes were able to recognize all the tissues tested [28], suggesting a tissue-specific regulation of the expression of at least some human mucin genes.

Chromosomal localization of human airway mucin genes

At present, five different chromosomes seem to be implicated in the synthesis of human mucins and mucin-like glycoproteins. A human urinary mucin gene, Muc1, was localized in the q21–q24 region of chromosome 1 [38]. Two human intestinal mucin genes, Muc2 and Muc3, were mapped on the p15 band of chromosomes 11 [39] and 7 [34], respectively. For human tracheobronchial mucins, we have identified three genes on chromosome 11 in p15 (Muc6), 13 (Muc6L) [29] and 3 (Muc4) [30]. The mucin gene located on chromosome 11 was also recently identified in cDNA library from a patient with cystic fibrosis [36].

Moreover, since the cDNA clones described for respiratory [27] and intestinal mucins [33] are incomplete, we do not know if there is a single mucin gene on chromosome 11 in p15 expressed in human tracheobronchial as well as in intestinal mucosae or if there are several genes.

The results obtained from the study of a gene library suggest a genomic organization with many small exons with about 90% of sequence homologies therefore generating a broad spectrum of mRNA.

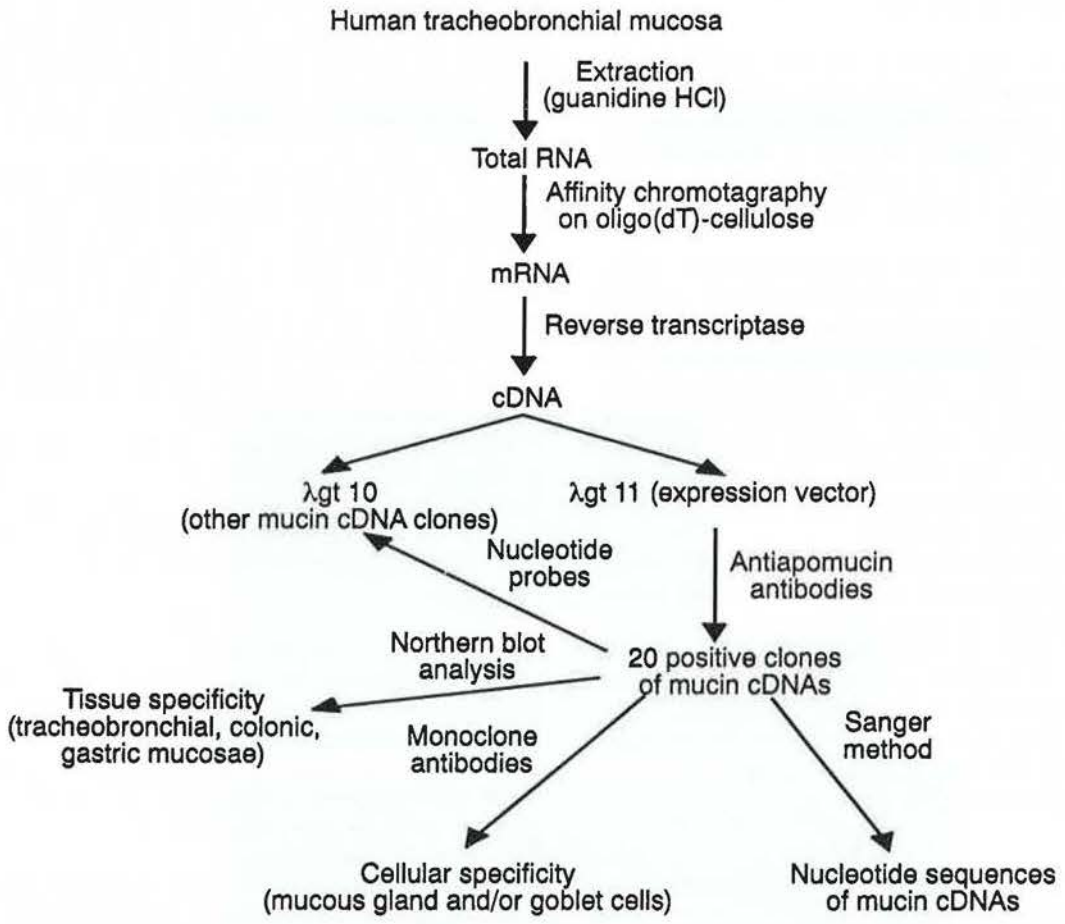

Fig. 3. – Preparation of human mucin complementary deoxyribonucleic acid (cDNA) clones. Two cDNA libraries have been prepared: i) in λgt 11; and ii) in λgt 10. The λgt 10 library has been screened with the nucleotide probes from the λgt 11 library. RNA: ribonucleic acid; mRNA: messenger RNA.

Besides the heterogeneity of the RNA messages coding for tracheobronchial mucins in a given individual, additional complexity might result from polymorphisms leading to sequence differences from individual to individual [30, 40].

In summary: 1) pulse/chase experiments have revealed a wide range of apomucins; 2) the mucin mRNAs are polydisperse and *in vitro* translation experiments give multiple translation products; 3) there are certainly several (how many?) mucin genes located on at least three different chromosomes.

Posttranslational modifications: the diversity of O-glycan chains

The wide diversity of carbohydrate chains

Mucin oligosaccharides are joined to the protein core through N-acetylgalactosamine (GalNAc) in an α-O-glycosidic linkage to the hydroxyl oxygen of serine or threonine [41, 42]: they correspond to O-glycans. In addition to GalNAc, fucose (Fuc), galactose (Gal), N-acetylglucosamine (GlcNAc) and N-acetylneuraminic acid (NeuAc) are also found in mucins. Human respiratory mucins may also contain sulphate group and a small quantity of mannose (Man) [11]. No uronic acid is found in mucins [1].

Although five types of monosaccharide residues are commonly found in respiratory mucins (and in most mucins), the biosynthetic assemblage leads to a wide spectrum of oligosaccharide structures, varying in composition, length, branching and acidity [42, 43]. This broad diversity has been a tremendous obstacle to the structural elucidation of the carbohydrate chains of human respiratory mucins.

During the seventies, structure analysis of oligosaccharides was time- and material-consuming. Due to the large amount of mucins required to purify a few micrograms of a given oligosaccharide, most of the structural studies have been performed with pools of mucins (secreted by blood group O patients suffering from chronic bronchial hypersecretion, either cystic fibrosis or chronic bronchitis).

However, major progress has been made within the last ten years with the application of modern high performance liquid chromatography (HPLC) for the isolation of purified oligosaccharides and the development of gas chromatography-mass spectrometry (GC-MS), high resolution proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) and fast atom bombardment mass spectrometry (FAB-MS) for the structure elucidation.

No endo-N-acetylgalactosaminidase capable of removing all carbohydrate chains from the peptides exists, except endo-N-acetylgalactosaminidase from *Streptococcus pneumoniae*, the action of which on respiratory mucins is very limited [8]. Therefore, the only method available to release oligosaccharides from the peptide core (even if the release is not complete) is reductive alkaline cleavage, which produces a mixture of oligosaccharide-alditols and glycopeptides [42, 43].

This mixture can then be fractionated by ion-exchange chromatography according to acidity and four pools of oligosaccharide-alditols are obtained, one consisting of neutral oligosaccharide-alditols, another of sialylated oligosaccharide-alditols and two pools of sulphated oligosaccharide-alditols [42, 43]. Each fraction can be subdivided according to molecular size by gel-filtration chromatography into three subfractions leading to a total of 12 subfractions ranging in size from 1–20 sugar-residues.

Only three subfractions (two neutral and the smallest sialylated fraction) have been extensively studied so far. These three subfractions have been treated by several steps of HPLC and the structure of the purified oligosaccharides-alditols determined by a combination of sugar analysis, ^{1}H-NMR spectroscopy and GC-MS. Tremendous heterogeneity of structure has been observed [42–48] which might have been due to differences in carbohydrate chains of the different individual mucins contained in the pools.

To rule out the possibility that different glycosylation genotypes (Lewis, Secretor System...) were responsible for the heterogeneity between the mucins of the different patients, the carbohydrate chains from the respiratory mucins of a single patient suffering from bronchiectasis were prepared: marked heterogeneity was also found and at least 80 different carbohydrate structures have been observed in the three subfractions studied so far [49–53]. These three subfractions make up only about 20% of the entire carbohydrate chains.

Therefore, one may deduce that the respiratory mucins of a single individual may contain several hundred different carbohydrate chains.

Carbohydrate-peptide linkage and cores of carbohydrate chains

The only structural element shared by all respiratory mucin carbohydrate chains is the GalNAc linked to the peptide.

Mucin oligosaccharides are initiated by the action of a very specific enzyme, UDP-GalNAc-polypeptide-α-N-acetylgalactosaminyl-transferase, on the apomucins [54]. The exact intracellular localization of the addition of the first GalNAc residues on the respiratory apomucins is still unknown.

The linkage GalNAc and the sugar(s) directly attached to it constitute the core region of the mucin oligosaccharides [55]. This GalNAc can be substituted on C3 hydroxyl either by a Galβ(1-3) or a GlcNAcβ (1-3) to give, respectively, core 1 and core 3 (cores have been numbered according to the sequence of their discovery [54, 55]). Addition of GlcNAc in β(1-6) linkage to core 1 and core 3 produces two other cores, core 2 and 4 (fig. 4). Two more cores can be obtained by substituting the GalNAc residue of cores 1 and 2 by N-acetylneuraminic acid in α2-6 linkage.

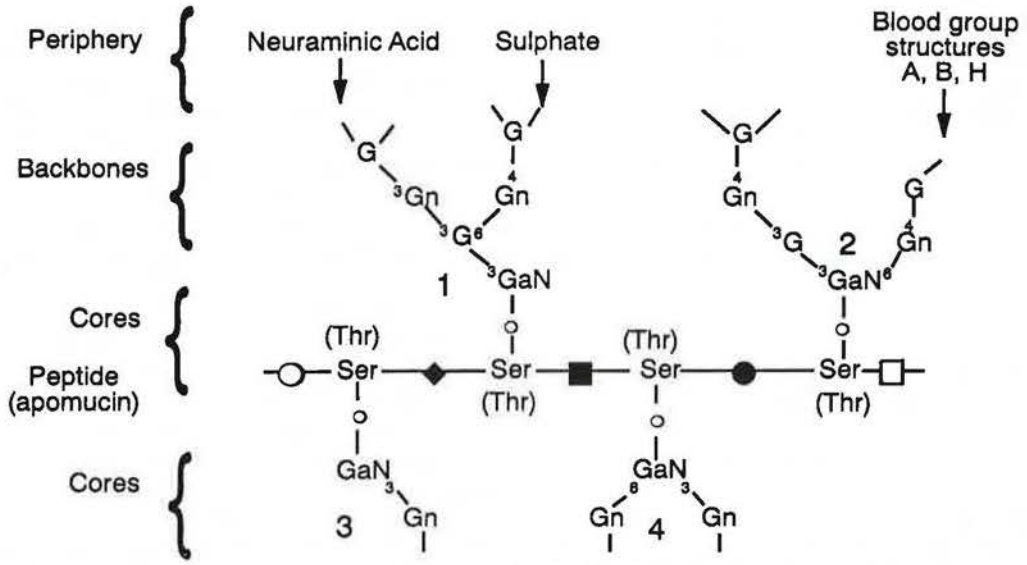

Fig 4. – Schematic representation of O-glycans, *i.e.* carbohydrate chains O-glycosidically linked to human respiratory peptide by linkages involving N-acetylgalactosamine (GaN) and hydoxyamino acid (serine (Ser) or threonine (Thr)). Each carbohydrate chain can be described with a core, a backbone and a periphery.

These six different cores which result from the action of several glycosyltransferases can be found in the different oligosaccharides of the mucins secreted by a single individual [49–53].

Carbohydrate chains elongation

The synthesis of the backbones of the different carbohydrate chains results from the action of successive glycosyltransferases allowing the transfer of galactose or N-acetylglucosamine into a determined position and anomeric linkage. Respiratory mucin carbohydrate backbones are made of disaccharides formed by alternating galactose and N-acetylglucosamine residues, always β-linked, with two types of linkages: Galβ1-3 GlcNAc (type 1 disaccharide) or Galβ1-4 GlcNAc (type 2 disaccharide) [54].

During elongation of the carbohydrate chains, these two disaccharide units can start from each of the cores or be linked β1-3 and/or β1-6 on a more internal galactose residue of the backbone to give branched or linear backbone structures (fig. 4).

The periphery of carbohydrate chains

The periphery of the respiratory mucin oligosaccharide chains is characterized by the presence of sugars such as Fuc, Gal, GalNAc, NeuAc, always in α anomeric configuration. Sulphate can also occur in the periphery [56, 57]. These sugars added by different glycosyltranferases, genetically controlled, may confer blood group antigenic activities to the mucin (ABH, Secretor, Lewis...) [54, 58, 59]. The resulting blood group antigenic structures found in respiratory mucins are listed in figure 5.

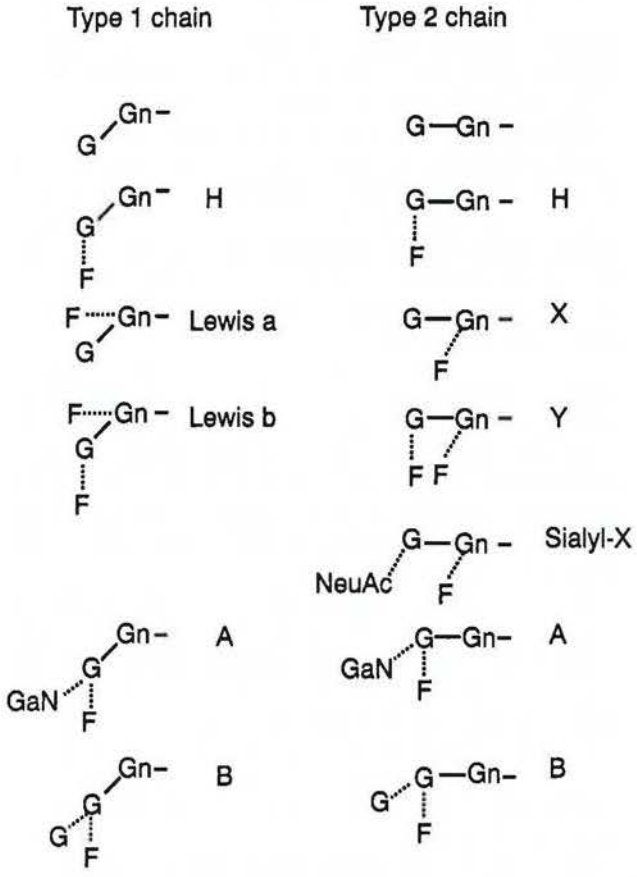

Fig. 5. – Different types of peripheral regions that have been identified so far in O-glycans from human respiratory mucins. GaN, Gn and G correspond to N-acetylgalactosamine, N-acetylglucosamine and galactose, respectively; F: fucose; NeuAc: N-acetylneuraminic acid. Glycosidic linkages are represented as follows: / = 1-3 linkage (or 2-3 in the case of NeuAc); - = 1-4 linkage; \ = 1-6 linkage; | = 1-2 linkage; solid lines correspond to β linkage and dashed lines to α linkages.

Recently, oligosaccharides containing fucose residues linked α1-2 to the galactose of a type 2 disaccharide in an internal position in the backbone have been isolated: these fucose residues are responsible for new structures called "internal H" [53].

Numerous carbohydrate chains carry acidic groups, either neuraminic acid or sulphate, responsible for the polyanionic character of mucins.

Different types of structures have already been identified where sialic acid is linked either to the N-acetylgalactosamine of the carbohydrate-peptide linkage or to a terminal galactose:

i) NeuAcα2→6GalNAc→peptide
ii) NeuAcα2→3 Galβ1→<3GalNAc→peptide
(sialylated core 1)
iii) NeuAcα2→3 Galβ1→4GlcNAc→R
iv) NeuAcα2→6 Galβ1→4GlcNAc→R
v) NeuAcα2→3 Galβ1→4(Fucα1<3)GlcNAc-R

This last structure is a well-known antigenic activity (fig. 5).

Some sulphated carbohydrate chains have been identified where sulphate groups are attached to galactose residues, either in 3 [57] or in 6 [56]. The simultaneous presence of sulphate and sialic acid on the same chains have not yet been reported.

In addition to the hundreds of O-glycans chains contained in respiratory mucins, one should note the possible occurrence of a few N-glycans. Low amounts of mannose, a sugar residue found in N-glycans, are frequently observed in the chemical composition of human respiratory mucins [11]. Due to the difficulties encountered in purifying mucins, this mannose has often been attributed to contamination of mucins by N-linked glycoproteins. However, the recent discovery of possible sites of attachment for N-glycans in the amino acid sequence deduced from apomucin cDNAs [27, 33], suggests that N-glycosylation may also occur in respiratory mucins, although to a small extent.

Finally, one should stress that only a small part of the oligosaccharides has been identified so far and that each oligosaccharide from the backbone may be substituted in many ways. Therefore, one can easily imagine that there are certainly hundreds of different carbohydrate chains in human respiratory mucins.

The reasons for this remarkable heterogeneity of carbohydrate chains in respiratory mucins are puzzling. A first explanation would be that it results from differences in glycosyltransferase activities or sugar nucleotide availability from cell to cell. There might be differences in glycosyltransferases expression from one mucin secreting cell to another. This is true for sialylation: limulin which recognizes some sialylated structures has more affinity for the goblet cells than for the mucous glands [3].

Mucin specificity and pathological status

So far, all the studies of mucin chains have been performed with mucins secreted by patients suffering from various bronchial disorders.

Information on the oligosaccharides of "normal mucins" is very limited. Glycopeptides obtained from bronchial lavages performed in normal areas of the respiratory tree were slightly heterogeneous with regard to acidity and mainly, but not solely, sialylated [60].

The diversity of carbohydrate chains has been observed in mucins obtained from various patients suffering from cystic fibrosis [42, 45–48], chronic bronchitis [43, 44] or bronchiectasis [49–53] and, as already mentioned, this diversity is most probably a general feature of human respiratory mucins.

When mucins from patients with different diseases were compared, those from patients with cystic fibrosis appeared to be shorter molecules and relatively more sulphated [61]. However, at the present time, our knowledge of carbohydrate chains is still very limited and the possibility of subtle but specific modifications of mucin glycosylation in certain diseases is open.

The mucins contained in bronchogenic cysts represent interesting cases. The chemical composition of several of them has been studied [62] and large variations have been observed. In some cysts, mucins were neutral or highly sialylated; in others, they were highly sulphated and the extent of sulphation may be much higher than is observed for secreted mucins obtained from sputum. Moreover, the N-acetylglucosamine/N-acetylgalactosamine ratio may be very different from secreted bronchial mucins and this is also true for the amino acid content (there may be more serine than threonine). These observations raise the question of the synthesis of oligoclonal populations of mucins in certain cysts.

Searching for the biological roles of respiratory mucins

The main feature of the mucins is their extraordinary diversity, at the carbohydrate and at the peptide levels. They are usually considered as high molecular weight glycoproteins but recent studies in guinea-pig tend to suggest that some mucins might have lower molecular weight [63].

Mucins contain hydrophobic and hydrophilic parts. They may be uncharged or polyanionic. Their diversity might facilitate all sorts of molecular interactions with other mucous components, with inhaled particles or microorganisms; why not with cilia?

The sites recognized by surface adhesins or haemagglutinins of microorganisms are frequently carbohydrates. When expressed on the surface of host cells, they are possible sites of attachment and colonization for these microorganisms [64–73]. Several potential carbohydrate sites, analogous with mucin carbohydrate chains, have been identified for different microorganisms, such as *Mycoplasma pneumoniae* [70–72], *Streptococcus pneumoniae* [68], *Pseudomonas aeruginosa* [69], influenza viruses [73] and a variety of E. *coli* [67] adhesins.

In human respiratory mucins the multiple carbohydrate chains that cover mucin molecules may also

represent a mosaic of potential sites for the attachment of bacteria and viruses, allowing their trapping on the mucus blanket and their removal by mucociliary clearance. Recent studies have, for instance, characterized carbohydrate chains responsible for the attachment of *Pseudomonas aeruginosa* [69] and differences in adhesion between mucin glycopeptides from different patients [74]. Therefore, the diversity of mucin carbohydrate chains most probably play an important role in the defence of the respiratory mucosa. If so, the chemical study of respiratory mucins might pave the way for new strategies against respiratory infection.

Mucins may interact with other molecules of the mucus: lipids [18], proteins such as lysozyme [75] or mucus protease inhibitor [76] are frequently strongly bound to mucins, although through non-covalent but still strong interactions. The results of these interactions are probably very important for the rheological properties of the mucus and the efficiency of the mucociliary system, and also for the protection and life-time of some of these molecules.

Mucins may also interact with exogeneous molecules. They may be useful to eliminate inhaled particles. However, acidic mucins (and nucleic acids abundant in cystic fibrosis sputum) may bind aminoglycosides and, to a certain extent, block their antibiotic properties on bacteria [77].

Finally, one may wonder if carbohydrate chains of mucins interact with cilia and play a role in the efficacy of the ciliary beating.

References

1. Roussel P, Lamblin G, Lhermitte M, Houdret N, Lafitte JJ, Perini JM, Klein A, Scharfman A. – The complexity of mucins. *Biochimie,* 1988; 70: 147 1471–1482.
2. Reid L. – Measurement of the bronchial mucous gland layer: a diagnostic yardstick in chronic bronchitis. *Thorax,* 1960; 15: 132–141.
3. Mazzuca M, Lhermitte M, Lafitte JJ, Roussel P. – Use of lectins for detection of glycoconjugates in the glandular cells of the human bronchial mucosa. *J Histochem Cytochem,* 1982; 30: 956–966.
4. Spicer SS, Schulte BA, Thomopoulos GN. – Histochemical properties of the respiratory tract epithelium in different species. *Am Rev Respir Dis,* 1983; 128: S20–S26.
5. Brogan TD. – The carbohydrate complexes of bronchial secretion. *Biochem J,* 1959; 71: 125–131.
6. Sheffner AL – The reduction *in vitro* in viscosity in mucoprotein solution by a new mucolytic agent, N-acetylcysteine. *Ann NY Acad Sci,* 1963; 106: 298–310.
7. Houdret N, Le Treut A, Lhermitte M, Lamblin G, Degand P, Roussel P. – Comparative action of reducing agents on fibrillar human bronchial mucus under dissociating and non-dissociating conditions. *Biochim Biophys Acta,* 1981; 668: 413–419.
8. Feldhoff PA, Bhavanandan VP, Davidson EA. – Purification, properties and analysis of human asthmatic bronchial mucin. *Biochemistry,* 1979; 18: 2430–2436.
9. Roberts GP. – The role of disulfide in maintaining the gel structure of bronchial mucins. *Arch Biochem Biophys,* 1976; 173: 528–537.
10. Creeth JM, Bhaskar R, Horton JR, Das I, Lopez-Vidriero MT, Reid L. – The separation and characterization of bronchial glycoproteins by density-gradient methods. *Biochem J,* 1977; 167: 557–569.
11. Slayter HS, Lamblin G, Le Treut A, Galabert C, Houdret N, Degand P, Roussel P. – Complex structure of human bronchial mucus glycoprotein. *Eur J Biochem,* 1984; 142: 209–218.
12. Carlstedt I, Sheehan JK. – Is the macromolecular architecture of cervical, respiratory and gastric mucins the same? *Biochem Soc Trans,* 1984; 12: 615–617.
13. Gupta R, Jentoft N, Jamieson AM, Blackwell J. – Structural analysis of purified human tracheobronchial mucins. *Biopolymers,* 1990; 29: 347–355.
14. Rose MC, Voter WA, Brown CF, Kaufman B. – Structural features of human tracheobronchial mucus glycoprotein. *Biochem J,* 1984; 222: 371–377.
15. Mikkelsen A, Stokke BT, Christensen BE, Elgsaeter A. – Flexibility and length of human bronchial mucin studied using low-shear viscometry, birefringence relaxation analysis, and electron microscopy. *Biopolymers,* 1985; 24: 1683–1704.
16. Sheehan JK, Oates K, Carlstedt I. – Electron microscopy of cervical, gastric and bronchial mucus glycoproteins. *Biochem J,* 1986; 239: 147–153.
17. Thornton DJ, Davies JR, Kraayenbrink M, Richardson PS, Sheehan J, Carlstedt I. – Mucus glycoproteins from "normal" human tracheobronchial secretion. *Biochem J,* 1990; 265: 179–186.
18. Houdret N, Perini JM, Galabert C, Scharfman A, Humbert P, Lamblin G, Roussel P. – The high lipid content of respiratory mucins in cystic fibrosis is related to infection. *Biochem Biophys Acta,* 1986; 880: 54–61.
19. Shankar V, Naziruddin B, Reyes de la Rocha S, Sachdev G. – Evidence of hydrophobic domains in human respiratory mucins. Effect of sodium chloride on hydrophobic properties. *Biochemistry,* 1990; 29: 5856–5864.
20. Lamblin G, Lhermitte M, Degand P, Roussel P, Slayter HS. – Chemical and physical properties of human bronchial mucus glycoproteins. *Biochimie,* 1979; 61: 23–43.
21. Roberton AM, Mantle M, Fahim REF, Specian RD, Bennick A, Kawagishi S, Sherman P, Forstner JF. – The putative "link" glycopeptide associated with mucus glycoproteins. *Biochem J,* 1989; 261: 637–647.
22. Ringler NJ, Selvakumar R, Woodward HD, Bhavanandan VP, Davidson EA. – Protein components of human tracheobronchial mucin: partial characterization of a closely associated 65-kiloDalton protein. *Biochemistry,* 1988; 27: 8056–9063.
23. Marianne T, Perini JM, Houvenaghel MC, Tramu G, Lamblin G, Roussel P. – Action of trifluoromethanesulfonic acid on highly glycosylated regions of human respiratory mucins *Carbohydr Res,* 1986; 151: 7–19.
24. Perini JM, Marianne T, Lafitte JJ, Lamblin G, Roussel P, Mazzuca M. – Use of an antiserum against deglycosylated human mucins for cellular localization of their peptide precursors: antigenic similarities between bronchial and intestinal mucins. *J Histochem Cytochem,* 1989; 37: 869–875.
25. Marianne T, Perini JM, Lafitte JJ, Houdret N, Pruvost FR, Lamblin G, Slayter HS, Roussel P. – Peptides of human bronchial mucus glycoproteins-size determination by electron microscopy and by biosynthetic experiments. *Biochem J,* 1987; 248: 189–195.
26. Perini JM, Vandamme-Cubadda N, Aubert JP, Porchet N, Mazzuca M, Lamblin G, Herscovics A, Roussel P. – Multiple apomucin translation products from human respiratory mucosa mRNA. *Eur J Biochem,* 1991; 196: 321–328.

27. Aubert JP, Porchet N, Crépin M, Duterque-Coquillaud M, Vergnes G, Mazzuca M, Debuire B, Petitprez D, Degand P. – Evidence for different human tracheobronchial mucin peptides deduced from nucleotide cDNA sequences. *Am J Respir Cell Mol Biol,* 1991; 5: 178–185.
28. Crépin M, Porchet N, Aubert JP, Degand P. – Diversity of the peptide moiety of human airway mucins. *Biorheology,* 1990; 27: 471–484.
29. Nguyen Van Cong, Aubert JP, Gross MS, Porchet N, Degand P, Frezal J. – Assignment of human tracheobronchial mucin gene(s) to 11p15 and a tracheobronchial mucin-related sequence to chromosome 13. *Hum Genet,* 1990; 86: 167–172.
30. Porchet N, Nguyen Van Cong, Dufossé J, Audié JP, Guyonnet Dupérat V, Gross MS, Denis C, Degand P, Bernheim A, Aubert JP. – Molecular cloning and chromosomal localization of a novel human tracheobronchial mucin cDNA containing tandemly repeated sequences of 48 base pairs. *Biochem Biophys Res Commun,* 1991; 175: 414–422.
31. Gendler SJ, Lancaster CA, Taylor-Papadimitriou J, Duhig T, Peat N, Burchell J, Pemberton L, Lalani EN, Wilson D. – Molecular cloning and expression of human tumor-associated polymorphic epithelial mucin. *J Biol Chem,* 1990; 265: 15286–15293.
32. Wreschner DH, Hareuveni M, Tsartafy I, Smorodinsky N, Horev J, Zaretsky J, Kotkes P, Weiss M, Lathe R, Diam A, Keydar I. – Human epithelial tumor antigen cDNA sequences. *Eur J Biochem,* 1990; 189: 463–473.
33. Gum JR, Byrd JC, Hicks JW, Toribare NW, Lamport DTA, Kim YS. – Molecular cloning of human intestinal mucin cDNAs. *J Biol Chem,* 1989; 264: 6480–6487.
34. Gum JR, Hicks JW, Swallow DM, Lagace RL, Byrd JC, Lamport DTA, Siddiki B, Kim YS. – Molecular cloning of cDNAs derived from a novel human intestinal mucin gene. *Biochem Biophys Res Comm,* 1990; 171: 407–415.
35. Rose MC, Kaufman B, Martin BM. – Proteolytic fragmentation and peptide mapping of human carboxyamidomethylated tracheobronchial mucin. *J Biol Chem,* 1989; 264: 8193–8199.
36. Gerard C, Eddy RL, Shows TB. – The core polypeptide of cystic fibrosis tracheal mucin contains a tandem repeat structure. *J Clin Invest,* 1990; 86: 1921–1927.
37. Jany BH, Gallup MW, Yan PS, Gum JR, Kim YS, Basbaum CB. – Human bronchus and intestine express the same mucin gene. *J Clin Invest,* 1991; 87: 77–82.
38. Swallow DM, Gendler S, Griffiths B, Kearney A, Povey S, Sheer D, Palmer RW, Taylor-Papadimitriou J. – The hypervariable gene locus PUM which codes for the tumor associated epithelial mucins is located on chromosome 1, within the region 1q21–24. *Ann Hum Genet,* 1987; 51: 289–294.
39. Griffiths B, Gum JR, West LF, Povey S, Swallow DL, Kim YS. – Mapping of the gene coding for intestinal mucin to chromosome 11p15. *Cytogenet Cell Genet,* 1989; 51: 1008.
40. Griffiths B, Matthews DJ, West L, Attwood J, Povey S, Swallow DL, Gum JR, Kim YS. – Assignment of the polymorphic intestinal mucin gene (Muc 2) to chromosome 11p15. *Ann Hum Genet,* 1990; 54: 277–285.
41. Carlson DM. – Structure and immunochemical properties of oligosaccharides isolated from pig submaxillary mucins. *J Biol Chem,* 1968; 243: 616–626.
42. Roussel P, Lamblin G, Degand P, Walker-Nasir E, Jeanloz RW. – Heterogeneity of the carbohydrate chains of sulfated bronchial glycoproteins isolated from a patient suffering from cystic fibrosis. *J Biol Chem,* 1975; 250: 2214–2222.
43. Lamblin G, Humbert P, Degand P, Roussel P. – Hétérogénéité des chaînes glycanniques des mucines bronchiques acides isolées à partir de l'expectoration de deux sujets atteints de bronchite chronique. *Clin Chim Acta,* 1977; 79: 425–436.
44. Lamblin G, Lhermitte M, Boersma A, Roussel P, Reinhold V. – Oligosaccharides of human bronchial glycoproteins: neutral di- and trisaccharides isolated from a patient suffering from chronic bronchitis. *J Biol Chem,* 1980; 255: 4595–4598.
45. Van Halbeek H, Dorland L, Vliegenthart JFG, Hull WE, Lamblin G, Lhermitte M, Boersma A, Roussel P. – Primary-structure determination of fourteen neutral oligosaccharides derived from bronchial-mucus glycoproteins of patients suffering from cystic fibrosis employing 500 MHz ^{1}H-NMR spectroscopy. *Eur J Biochem,* 1981; 127: 7–20.
46. Lamblin G, Boersma A, Klein A, Roussel P, Van Halbeek H, Vliegenthart JFG. – Primary structure determination of five sialylated oligosaccharides derived from bronchial mucus glycoproteins of patients suffering from cystic fibrosis. *J Biol Chem,* 1984; 259: 9061–9068.
47. Lamblin G, Boersma A, Lhermitte M, Roussel P, Mutsaers JHGM, Van Halbeek H, Vliegenthart JFG. – Further characterization by a combined HPLC/^{1}H-NMR approach of the heterogeneity displayed by the neutral carbohydrate chains of human bronchial mucus. *Eur J Biochem,* 1984; 143: 227–236.
48. Breg J, Van Halbeek H, Vliegenthart JFG, Lamblin G, Houvenaghel MC, Roussel P. – Structure of sialyl-oligosaccharides isolated from bronchial mucus glycoproteins of patients (blood group O) suffering from cystic fibrosis. *Eur J Biochem,* 1987; 168: 57–68.
49. Klein A, Lamblin G, Lhermitte M, Roussel P, Breg J, Van Halbeek H, Vliegenthart JFG. – Primary structure of neutral oligosaccharides derived from respiratory-mucus glycoproteins of a patient suffering from bronchiectasis, determined by combination of 500-MHz ^{1}H-NMR spectroscopy and quantitative sugar analysis. 1. Structure of 16 oligosaccharides having the Galβ (1→3) GalNAc-ol core (type 1) or the Galβ(1→3)[GlcNAcβ1→6]GalNAc-ol core (type 2). *Eur J Biochem,* 1988; 171: 631–642.
50. Breg J, Van Halbeek H, Vliegenthart JFG, Klein A, Lamblin G, Roussel P. – Primary structure of neutral oligosaccharides derived from respiratory-mucus glycoproteins of a patient suffering from bronchiectasis, determined by combination of 500-MHz ^{1}H-NMR spectroscopy and quantitative sugar analysis. 2. Structure of 16 oligosaccharides having the GlcNAcβ(1→3)GalNAc-ol core (type 3) or the GlcNAcβ(1→3)[GlcNAcβ1→6]GalNAc-ol core (type 4). *Eur J Biochem,* 1988; 171: 643–654.
51. Van Halbeek H, Breg J, Vliegenthart JFG, Klein A, Lamblin G. Roussel P. – Isolation and structural characterization of low-molecular-mass monosialyl oligosaccharides derived from respiratory-mucus glycoproteins of a patient suffering from bronchiectasis. *Eur J Biochem,* 1988; 177: 443–460.
52. Klein A, Carnoy C, Lamblin G, Roussel P, Van Kuik A, De Waard P, Vliegenthart JFG. – Isolation and structural characterization of novel neutral oligosaccharide-alditols from respiratory mucus glycoproteins of a patient suffering from bronchiectasis: 1. Structure of 11 oligosaccharides. *Eur J Biochem,* 1991; 198: 151–168.
53. Van Kuik A, De Waard P, Vliegenthart JFG, Klein A, Carnoy C, Lamblin G, Roussel P. – Isolation and structural characterization of novel neutral oligosaccharide-alditols from respiratory-mucus glycoproteins of a patient suffering from bronchiectasis: 2. Structure of 12 hepta-to-nonasaccharides. *Eur J Biochem,* 1991; 198: 169–182.

54. Schachter H, Brockhausen I. – The biosynthesis of branched O-glycans. *In*: Mucus and related topics. E. Chantler, N.A. Ratcliffe eds, Symposia of the Society for Experimental Biology. 1989; pp. 1–26.
55. Hounsell EF, Feizi T. – Gastrointestinal mucins: structures and antigenicities of their carbohydrate chains in health and disease. *Med Biol,* 1982; 60: 227–236.
56. Mawhinney TP, Adelstein E, Morris DA, Mawhinney AM, Barbero GJ. – Structure determination of five sulfated oligosaccharides derived from tracheobronchial mucus glycoproteins. *J Biol Chem,* 1987; 262: 2994–3001.
57. Lamblin G, Rahmoune H, Wieruszeski JM, Lhermitte M, Strecker G, Roussel P. – Structure of two sulfated oligosaccharides from respiratory mucins of a patient suffering from cystic fibrosis: a fast atom bombardment mass spectrometric and ^{1}H-NMR spectroscopic study. *Biochem J,* 1991; 275: 199–206.
58. Watkins WM. – Biochemical genetics of blood group antigens: retrospect and prospect. *Biochem Soc Trans,* 1987; 15: 620–624.
59. Lhermitte M, Lamblin G, Lafitte JJ, Degand P, Roussel P, Mazzuca M. – Human, bronchial-mucus glycoproteins: a comparison between chemical properties and affinity for lectins. *Carbohydr Res,* 1981; 92: 333–342.
60. Lafitte JJ, Lamblin G, Lhermitte M, Humbert P, Degand P, Roussel P. – Etude des glycoprotéines bronchiques humaines de type mucique obtenues par lavage de bronches macroscopiquement saines. *Carbohydr Res,* 1977; 13: 383–389.
61. Lamblin G, Lafitte JJ, Lhermitte M, Degand P, Roussel P. – Mucins from cystic fibrosis sputum. *Modern Problems in Paediatrics,* 1978; 19: 153–164.
62. Degand P, Roussel P, Lamblin G, Havez R. – Purification des mucines de kystes bronchogèniques. *Biochim Biophys Acta,* 1973; 320: 318–330.
63. Rahmoune H, Rounding HP, McDonald-Gibson, Lamblin G, Hall RL, Roussel P. – Sulfated O-glycoproteins secreted by guinea-pig trachea in organ culture. *Am J Respir Cell Mol Biol,* 1991; 4: 156–165.
64. Vishwanath S, Ramphal R. – Tracheobronchial mucin receptor for *Pseudomonas aeruginosa:* predominance of amino sugars in binding sites. *Infect Immun,* 1985; 48: 331–335.
65. Brennan MJ, Joralmon RA, Cisar JO, Sandberg AL. – Binding of *Actinomyces naeslundii* to glycosphingolipids. *Infect Immun,* 1987; 55: 487–489.
66. Murray PA, Levine MJ, Tabak LA, Reddy MS. – Specificity of salivary-bacterial interactions: II. Evidence for a lectin on *Streptococcus sanguis* with specificity for a NeuAcα2,3Galβ1,3GalNAc sequence. *Biochem Biophys Res Commun,* 1982; 106: 390–396.
67. Parkkinen J, Rogers GN, Korhonen T, Dahr W, Finne J. – Identification of the O-linked sialyloligosaccharides of glycophorin A as the erythrocyte receptors for S-fimbriated *Escherichia Coli. Infect Immun,* 1986; 54: 37–42.
68. Andersson B, Dahmen J, Frejd T, Leffler H, Magnusson G, Noori G, Svandborg Eden C. – Identification of an active disaccharide unit of a glycoconjugate receptor for pneumococci attaching to human pharyngeal epithelial cells. *J Exp Med,* 1983; 158: 559–570.
69. Ramphal R, Carnoy C, Fievre S, Michalski JC, Houdret N, Lamblin G, Strecker G, Roussel P. – *Pseudomona aeruginosa* recognizes carbohydrate chains containing Type 1 (Galβ1-3GlcNAc) or Type 2 (Galβ1-4GlcNAc) disaccharide units. *Infect Immun,* 1991; 59: 700–704.
70. Roberts DD, Olson LD, Barile MF, Ginsburg V, Krivan HC. – Sialic acid-dependent adhesion of *Mycoplasma pneumoniae* to purified glycoproteins. *J Biol Chem,* 1989; 264: 9289–9293.
71. Loveless RW, Feizi T. – Sialo-oligosaccharide receptors for *Mycoplasma pneumoniae* and related oligosaccharides of poly-N-acetyllactosamine series are polarized at the cilia and apicalmicrovillar domains of the ciliated cells in human bronchial epithelium. *Infect Immun,* 1989; 57: 1285–1289.
72. Krivan HC, Olson LD, Barile MF, Ginsburg V, Roberts DD. – Adhesion of *Mycoplasma pneumoniae* to sulfated glycolipids and inhibition by dextran sulfate. *J Biol Chem,* 1989; 264: 9283–9288.
73. Susuki Y, Nagao Y, Kato H, Matsumoto M, Nerome K, Nakajima K, Nobusawa E. – Human influenza A virus hemagglutinin distinguishes sialyloligosaccharides in membrane-associated gangliosides as its receptor which mediates the adsorption and fusion processes of virus infection. *J Biol Chem,* 1986; 261: 17057–17061.
74. Ramphal R, Houdret N, Koo L, Lamblin G, Roussel P. – Differences in adhesion of *Pseudomonas aeruginosa* to mucin glycopeptides from sputa of patients with cystic fibrosis and chronic bronchitis. *Infect Immun,* 1989; 57: 3066–3071.
75. Creeth JM, Bridge JL, Horton JR. – An interaction between lysozyme and mucus glycoproteins. *Biochem J,* 1979; 181: 717–724.
76. Van-Seuningen I, Davril M, Hayem A. – Evidence for the tight binding of human mucus proteinase inhibitor to highly glycosylated macromolecules in sputum. *Biol Chem Hoppe-Seyler,* 1989; 370: 749–755.
77. Ramphal R, Lhermitte M, Filliat M, Roussel P. – The binding of anti-pseudomonal antibiotics to macromolecules from cystic fibrosis sputum. *J Antimicrob Chemother,* 1988; 22: 483–490.