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PREFACE
Airway glycoconjugates and cystic fibrosis

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Cystic fibrosis (CF) is the most common severe genetic disease among Caucasians. This recessive disorder affects the exocrine glands and, in its most typical form, the main manifestations are chronic broncho-pulmonary disease, pancreatic insufficiency and elevated sweat chloride and sodium ions. In cystic fibrosis, there is bronchial mucus hypersecretion, as in chronic bronchitis, and severe lung infection. However, unlike most cases of chronic bronchitis, airway infection in CF is characterized by the predominance of *Staphylococcus aureus* in early life and, rapidly if not directly, of *Pseudomonas aeruginosa,* which is almost impossible to eradicate and which is responsible for most of the morbidity and mortality of the disease.

Cystic fibrosis is due to mutations of a gene localized on chromosome 7, *cftr*, encoding for a membrane glycoprotein called Cystic Fibrosis Transmembrane conductance Regulator (CFTR). CFTR is a PKA-activated chloride channel, which also influences the functioning of other epithelial ion channels. It may also have other undiscovered functions in cellular physiology, which influence the susceptibility to infection. In the USA and northern Europe, 70% of the CF chromosomes have a deletion of phenylalanine 508 (ΔF508), which is responsible for interrupting the traffic of CFTR in the RER, therefore preventing most of these molecules to reach the plasma membrane.

In spite of the discovery of the CF gene, in 1989, the pathophysiology of the lung infection is still mysterious and the treatment of the disease, although improving, is still largely empirical. The airway mucosa is normally protected by the mucociliary system, which is made of a layer of mucus mobilized by cilia beating in the fluid film covering the surface of the airway epithelium. This system acts as an escalator trapping inhaled particles and microorganisms, which are moved up to the pharynx where they are normally swallowed.

In CF, the abnormalities of the fluid phase are still a matter of debate. A decrease in the volume of the fluid phase is the most favored hypothesis. The question of water and solute modifications as the predominant factor in the genesis of the lung colonization is also a matter of controversy and, so far, the pharmacological clinical trials aimed at the correction of these modifications have not been very successful.

In the typical forms of CF, the airways are inflamed and infected. They have a tendency to be filled with mucus plugs (*mucoviscidosis)* that have entrapped bacteria and leukocytes and which are difficult to eliminate by normal mucociliary activity or coughing. Long before the discovery of the CF gene, investigators have been searching for glycosylation abnormalities of the airways that might pave the way of lung colonization but no unique alteration could be found.

The main objective of the present issue of the *Glycoconjugate Journal* is to review and discuss some current trends of research concerning glycoconjugates in the airways from CF patients, which might help in understanding some aspects of the pathophysiology of the disease, especially with regard to interactions with bacteria and mucus hypersecretion. This might lead ultimately to new therapeutic approaches of the hypersecretion, inflammation and infection of the airways.

As far as the *modifications of glycosylation in CF* are concerned, Rhim et al. [1] report that the membrane glycoproteins from airway cells expressing a mutated *cftr* are less sialylated and more fucosylated than those from cells expressing a wild type cftr. Similarly, it had been previously shown that glycolipids were also less sialylated [2].

Conversely, Lamblin et al. report that airway mucins prepared from patients suffering from CF, or from severe chronic bronchitis, infected by *Pseudomonas aeruginosa* are more sulfated and even more sialylated than mucins secreted by less infected patients suffering from chronic bronchitis [3]. These mucins from severely infected patients also contain more acidic derivatives of the Lewis x epitope [3] and, as shown by Morelle et al., the sialyl Lewis x epitope can be expressed on polylactosamine chains [4].

Moreover, using CHO or BHK cells transfected with ΔF508 or a wild type *cftr* gene, Brockhausen et al. [5] show that several glycosyl- or sulfo-transferases involved in the biosynthesis of mucin type-O glycans by these cells are not modified by transfection with ΔF508 or *cftr* gene.

These different data which concern the glycosylation alterations of airway mucins and of membrane glycoproteins of CF airway cells may appear to be conflicting, but

several elements have to be taken into consideration in their interpretation:

(i) The airway mucosa is made of different cells: goblet cells, ciliated and non-ciliated cells, at the surface, and serous and mucous cells in the tubular glands of the submucosa. The glycosylation machineries of these different cells do different things. The goblet cells of the surface and the mucous cells of the glands secrete mostly mucins, which are heavily O-glycosylated and are the main components of the bronchial mucus. The other surface epithelial cells synthesize membrane glycoconjugates, and serous cells synthesize a few glycoproteins, such as the bronchotransferrin, which are mostly N-glycosylated. Moreover, the glycosylation processes may vary according to development.

(ii) Not all the airway cells do not normally express CFTR. It is expressed in the epithelial cells of the surface, in some cells of the gland ducts and in the serous glands [6,7], but it is not expressed, or at a very low levels, in the goblet cells and in the mucous glands. The glycosylation abnormalities observed in glycoconjugates synthesized by these different types of cells might therefore be under different mechanisms of control, directly related to CFTR when cells express CFTR, but due to other mechanisms, such as inflammation, in mucin synthesizing cells, which do not express CFTR.

(iii) Cell lines are invaluable tools in deciphering pathophysiological mechanisms but their phenotype (expression of various genes such as mucin genes, glycosyltransferases and receptor genes) may be different from that of the airway cells in their *in vivo* environment.

The data of Rhim et al. [1] and those of Lamblin et al. [2] suggest that two different types of glycosylation alterations may be observed in the CF airway mucosa.

The alterations of membrane glycoproteins from airway cells expressing a mutated CFTR (increased fucosylation and decreased sialylation) do correspond to a direct effect of CFTR on the glycosyltransferases of these airway epithelial cells, by a mechanism which is still under discussion (modification of the pH in the Golgi, mislocalization of glycosylransferases?). The exact phenotype of these airway cells is unknown but, since they express CFTR, they should probably correspond to cells, which do not synthesize secreted mucins, such as MUC2, MUC5AC or MUC5B. The previous data concerning the sialylation defect of gangliosides [2] might have a similar interpretation.

The modifications observed in the secreted airway mucins from patients suffering from CF or from severe chronic bronchitis, infected by *Pseudomonas aeruginosa* (increased sialylation and sulfation as well as increased expression of the sialyl Lewis x determinants) are most probably related to inflammation acting on goblet cells and mucous cells, which have no, or weak, expression of *cftr*. Its mechanism may be secondary to bacterial infection, or primary as suggested by the hyperreactivity of the airways of the CF mice, or by several clinical studies indicating that inflammation in CF airways precedes bacterial infection. Different mediators might be involved in such an effect. As a matter of fact, TNFα applied to airway explants has been shown to increase the expression of enzymes responsible for the biosynthesis of sialylated and/or sulfated Lewis x epitopes of airway mucins (reviewed in [2]).

Infection of the CF mucosa by P. aeruginosa and its chronic colonization are most probably multifactorial phenomenona where different carbohydrate determinants may play a role. The glycosylation alterations of the surface of the mucosa, or of the secreted mucins, may be important for increasing the interactions between *P. aeruginosa* and airway cells and/or secreted mucus. In the airways, *P. aeruginosa* can interact with mucins using a variety of carbohydrate epitopes [8]. It also interacts with cells using glycolipid receptors [9]. The relative importance of these different alterations in the airway colonization will have to be determined, however, one should notice that in the CF airways, most of the bacteria reside in the mucus. In the future, drugs able to interfere in these interactions may represent an interesting approach in the treatment of the disease.

Mucus hypersecretion, especially *mucin hypersecretion*, is another common feature of cystic fibrosis. Understanding of the signaling networks controlling mucin production in response to Gram-Positive and Gram-Negative bacteria represents also an important area of research that might open new avenues in the treatment of the disease [10]. Similarly the role of inflammatory mediators on mucus hypersecretion will also have to be investigated.

Finally, as a disease of the airway epithelium, cystic fibrosis has been rapidly considered as a good model for *gene therapy*. Airway cells, normal and CF, express membrane lectins recognizing various ligands, such as αD-mannosyl, lactosyl and αD-glucsosyl residues. Two different groups [11,12] report on the preparation and use of neoglycoconjuagtes bearing such ligands to target the normal *cftr* gene to the airway cells. Complexes made with cDNA and *lactosylated polylysine* are demonstrated to give nuclear localization in CF airway cells [12] and to allow high expression of reporter genes.

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