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Sialyl-Le^x and sulfo-sialyl-Le^x determinants are receptors for *P. aeruginosa*

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***Pseudomonas aeruginosa*, the main pathogen in the airways of patients suffering from cystic fibrosis (CF), binds to carbohydrate chains of respiratory mucins. Using flow cytometry and polyacrylamide based fluorescent glycoconjugates, it was previously demonstrated that several strains of *P. aeruginosa* recognize a set of neutral and acidic carbohydrate epitopes found at the periphery of respiratory mucins, especially sialyl-Le^x. This structure, overexpressed in mucins from CF patients, could be responsible in part for the persistence of lung infection in CF patients. The aim of the present work was to determine whether a glycoconjugate bearing the 6-sulfo-sialyl-Le^x epitope, also found in abundance in CF airway mucins, is also preferentially recognised by different strains of *P. aeruginosa*. The study was conducted with a non-piliated strain 1244-NP and four mucoid strains isolated from CF patients. For four strains out of five, the affinity for 6-sulfo-sialyl-Le^x was as high as for sialyl-Le^x derivative. These results were confirmed for strain 1244-NP by a microtiter plate assay.**

***Keywords: Pseudomonas aeruginosa*, neoglycoconjugates, sialyl-Lewis x, sulfo-sialyl-Lewis x**

Introduction

Cystic fibrosis, a general exocrinopathy, is the most common genetic disease among Caucasians [1]. CF is due to mutations of the CF gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR), a cAMP-regulated chloride channel [2] which probably has other functions [3,4].

CF affects most of the exocrine glands but the severity of the disease is due essentially to bronchial mucus hypersecretion and chronic lung infection by *Pseudomonas aeruginosa*, a pathogen responsible for most of the morbidity and the mortality of the disease [5]. Previous works [6–8] have shown that *P. aeruginosa* binds to carbohydrate and especially to salivary and respiratory mucins and that its binding to CF mucins is higher than to non-CF mucins [9,10]. These data suggested that quantitative and/or qualitative modifications of the carbohydrate chains of CF mucins could be involved in this binding and lead to the bacterial colonisation of the airways of CF patients [11].

Respiratory mucins secreted by patients suffering from cystic fibrosis [11], by CF cells [12] or by CF human bronchial xenografts [13] are oversulfated. The airway mucosa of most CF patients is colonized by *Pseudomonas aeruginosa* and one might argue that oversulfation of mucins secreted by these patients is related to bacterial infection. In the xenograft model, there is no bacterial colonization and, still, there is an oversulfation of the mucins secreted by these xenografts. Therefore, the lack of infection in this model suggests a link between hypersulfation of CF mucins and the primary defect. Moreover, it has been proposed that the concentration of PAPS, the sulfate donor, in the Golgi lumen is directly regulated by CFTR, and therefore may influence the sulfation process [14]. Recent data indicate that, in addition to oversulfation, mucins from patients severely infected by *P. aeruginosa* undergo increased sialylation as well as an increased content of sialyl-Le^x epitopes associated with the strong inflammatory response observed in this disease [15].

In a previous work, polyacrylamide based fluorescent glycoconjugates and flow cytometry were used to show that several strains of *P. aeruginosa* recognized a set of neutral and acidic carbohydrate epitopes found at the periphery of mucins, especially the sialyl-Le^x epitope [16]. These results suggested that this epitope, overexpressed in CF mucins, could be responsible in part for the initiation and persistence of CF lung infection. Since the 6-sulfo-sialyl-Le^x determinant is also found in abundance in respiratory mucins of CF patients [17],

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one may raise the question of its implication in lung colonization by *P. aeruginosa*.

In the present work, we compared the affinity of sialyl-Le^x and 6-sulfo-sialyl-Le^x determinants for several strains of *P. aeruginosa*, using polyacrylamide based neoglycoconjugates. The binding of fluorescent polyacrylamide based glycoconjugates bearing Le^x and 3′-sulfo-Le^x was studied as a control. The study was conducted with a non-piliated strain of *P. aeruginosa*, 1244-NP, and four mucoid strains isolated from CF patients.

Materials and methods

Neoglycoconjugates

In the neoglycoconjugates (Syntesome, Munich, Germany) used in the study, carbohydrate determinants (Table 1) are linked via three-carbon spacers to a polyacrylamide type matrix (Gly-PAA). In these compounds approximately every fifth amide group of the polymer chains is *N*-substituted by the carbohydrate on a spacer arm (-$(CH_2)_3$-). The carbohydrate content of the neoglycoconjugates is 20% per mol and their molecular weight was about 40 000 [18]. Neoglycoconjugates labeled with a fluorescent probe (Gly-PAA-flu) were used for flow cytometry analysis.

Bacterial strains and culture conditions

All the strains were used in our previous study [16]. Strain 1244-NP (provided by S. Lory, from the University of Washington) is a non-isogenic non-piliated non-mucoid strain [19]; the other strains were isolated from CF patients as mucoid isolates. They were grown for 18 h at 37°C in tryptic soy broth (TSB medium, Difco, Detroit, Mich.). After centrifugation of the cultures at 4000 $\times g$ for 30 min, the cell pellet was washed twice by filtered physiologic saline containing 5% (v/v) TSB and then resuspended in the same solution. Optical density measurement was used to obtain a bacterial suspension of approximately 10^7 CFU/ml. The exact number of bacteria was determined by dilution and plating of the suspension.

Table 1. Synthetic glycoconjugates used in the study

Carbohydrate epitope	*Gly-PAA-(flu)*
Le^x	Galβ1-4[Fucα1-3]GlcNAcβ-PAA-(flu)
sialyl-Le^x	NeuAcα2-3Galβ1-4[Fucα1–3]GlcNAcβ-PAA-(flu)
6-sulfo-sialyl-Le^x	NeuAcα2-3Galβ1-4[HSO_3-6][Fuca1-3]-GlcNAcβ-PAA-(flu)
3-sulfo-Le^x	HSO_3-3Galβ1-4[Fucα1-3]GlcNAcβ-PAA-(flu)

PAA-flu-polyacrylamide carrier labelled with fluorescein.

Flow cytometry binding analysis

Before each experiment, fluorescent neoglycoconjugates were dissolved in filtered bidistilled water (250 µg/ml). Bacteria were suspended at a concentration of 2×10^6 CFU/ml in Phosphate Buffered Saline (PBS) containing 1% (v/v) of Bovine Serum Albumin (BSA), and 0.5 ml of aliquots were incubated with increasing concentrations (6.25 to 125 nM) of fluorescent glycoconjugates. Since fluorescent polyacrylate without any carbohydrate epitopes were not available, controls were obtained by omitting glycoconjugates in the incubation mixture. The mixtures were analyzed by flow cytometry as described previously [16], but using a FACScalibur cytometer (Beckton-Dickinson), and Q cell and Softwares for acquisition and analysis respectively. The green fluorescence was set on a logarithm scale and the mean fluorescence was converted in equivalent bound particles using fluorescent calibrated beads (Immuno-Britt, Coulter Counter). Results were expressed as equivalent bound particles after subtraction of the control values. Experimental data were expressed as mean value ± standard deviations. Binding capacities and dissociation constants (Kd) for the different Gly-PAA-flu were calculated according to Scatchard using the non-linear progression data analysis program Enzfitter (Cambridge).

As previously described [16,20], the dissociation constants were compared using the Student's test.

Inhibition experiments

Competition experiments were performed for the binding of 6-sulfo-sialyl-Le^x- and sialyl-Le^x-PAA-flu to *P. aeruginosa* 1244-NP. Bacterial suspension (0.5 ml) was incubated for 30 min with fluorescent conjugates at a concentration corresponding to 50% of saturation of the labeled glycoconjugate in presence of 100 fold molar excess of unlabeled polyacrylate derivatives. Bacteria incubated under the same experimental conditions, but without any potential inhibitors, were used as controls. After flow cytometry analysis, the values of mean fluorescence intensity (expressed as equivalent bound particles) were compared. The Kolmogorov-Smirnov two-sample test was used to calculate the probability that two histograms were different.

Incubation with sulfo-sialyl-Le^x-PAA-flu was performed in presence or in absence of the corresponding unlabeled glycoconjugate. Competition experiments of the binding of sialyl-Le^x-PAA-flu were performed in presence of unlabeled polyacrylate derivative bearing sulfo-sialyl-Le^x epitope.

Microtiter plate adherence assay

Adherence of *P. aeruginosa* to neoglycoconjugates was quantified using a microtiter plate assay [19]. Glycoconjugates were identical to those used in flow cytometry analysis but without any label [18]. They were dissolved (5 µg/ml) in 0.1 M sodium carbonate/sodium hydrogenocarbonate, pH 9 [21]. One hundred microliters of this solution was used to coat

the wells of a microtiter plate. After one night at 37°C, the wells were rinsed with phosphate buffered saline (PBS), then the bacterial suspension was added at a concentration between 5×10^6 and 5×10^7 CFU/ml. After incubation at 37°C for 30 min, the unbound bacteria were removed by washing the wells with PBS. The bacteria adhering to neoglycoconjugates were desorbed by adding a 0.5% solution of Triton X 100. After dilution and plating, the bacteria were quantified. A set of uncoated wells was used as a negative control. Only the experiments with little or no background binding were considered valid. Wells coated with purified respiratory mucins [19] were used as positive controls. All experiments were performed at least 4 times with 3 wells per experiment. Results were expressed in CFU $\times 10^2$ per well. They corresponded to the average number of bacteria of each well.

Results

Comparative binding of 6-sulfo-sialyl-Le^x- and sialyl-Le^x-PAA-flu derivatives to *P. aeruginosa* 1244-NP

The binding of sialyl-Le^x- and sulfo-sialyl- Le^x-PAA-flu to *P. aeruginosa* 1244-NP was analyzed by flow cytometry using a FACscalibur cytometer. The results show that these two fluorescent glycoconjugates have similar binding characteristics. The binding is saturable (Figure 1), and fitting the data by a non-linear regression reveals an interaction indicative of two classes of binding sites. This can be more easily visualized on the Scatchard plot (Figure 2). From a two-site ligand binding equation, using a molecular weight of 40 000 for the Gly-PAA-flu, apparent Kd values for the high affinity binding sites were calculated at 15 ± 9 nM for sialyl-Le^x-PAA-flu and at 14 ± 3 nM for 6-sulfo-sialyl-Le^x-PAA-flu. The numbers of apparent binding sites calculated for the two fluorescent glycoconjugates were also in the same order of magnitude (Table 2). Altogether, these results show that sialyl-Le^x- and 6-sulfo-sialyl-Le^x-PAA-flu have the same affinity for *P. aeruginosa* 1244-NP.

The binding of Le^x- and 3′-sulfo-Le^x-PAA-flu to *P. aeruginosa* 1244-NP was also analysed by flow cytometry, using the same experimental conditions. Analysis of the data showed that, unlike the binding of sialyl- and 6-sulfo-sialyl-Le^x-PAA-flu, the binding of Le^x- and 3′-sulfo-Le^x-PAA-flu was monophasic, with only one class of receptor of low affinity. The Kd values calculated at 62 ± l2 nM for Le^x-PAA-flu

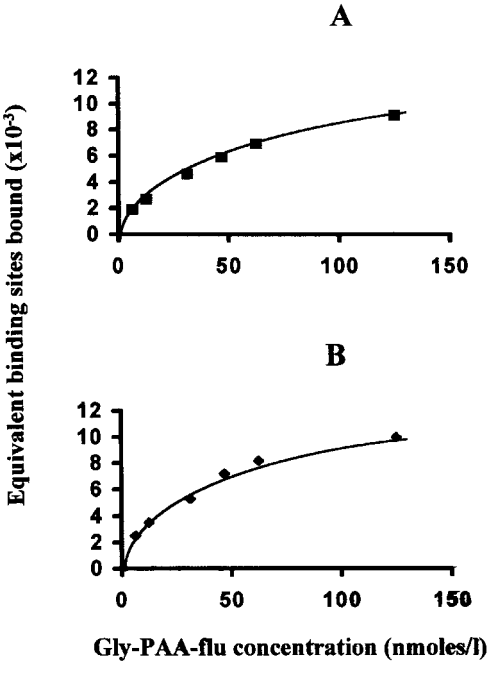

Figure 1. Influence of the concentration of sialyl-Le^x **(A)** and 6-sulfo-sialyl-Le^x **(B)**-PAA-flu on the binding to *P. aeruginosa* 1244-NP. Bacterial suspension was incubated with increasing concentrations (from 6.25 to 125 mM) of fluorescent glycoconjugates. After 30 min in the dark, a flow cytometry analysis was performed. Kinetics values were determined by non-linear regression using a two-site binding equation. Data points represent the mean values of 4 or 5 experiments. Error bars were too small to be recorded.

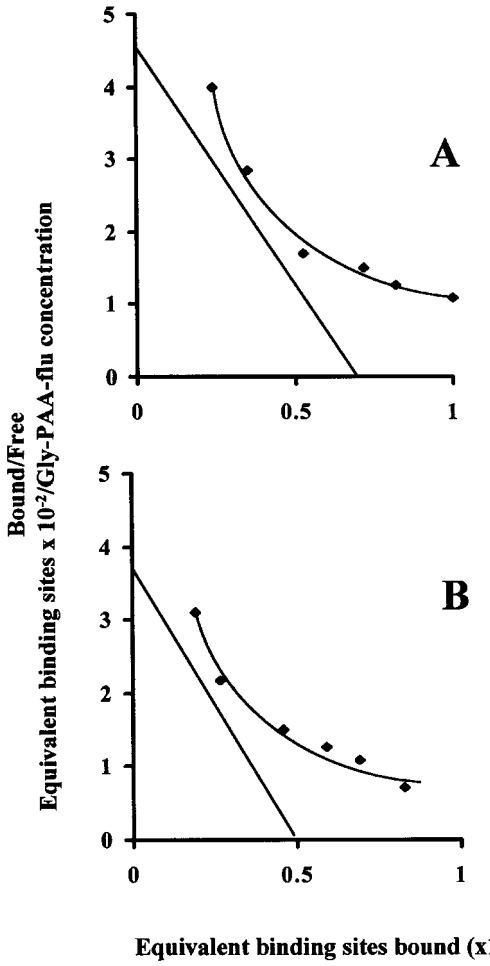

Figure 2. Scatchard plot representation of the binding of sialyl-Le^x **(A)** and 6-sulfo-Le^x **(B)** fluorescent derivatives to *P. aeruginosa* 1244-NP.

Table 2. Binding of Le^x-, sialyl-Le^x-, 6-sulfo-sialyl-Le^x- and 3′-sulfo-Le^x-PAA-flu to *P. aeruginosa* 1244 NP

Carbohydrate epitope	*Dissociation constant (Kd nM)*	*Number of binding sites*
Le^x [a]	62 ± 12	15 400 ± 1 800
sialyl-Le^x [b]	15 ± 9	7 000 ± 2 600
6-sulfo-sialyl-Le^x [c]	14 ± 3	5 000 ± 810
3′-sulfo-Le^x [d]	47 ± 12	13 100 ± 1 300

Results are expressed as mean ± standard deviations.
[a]number of data points used for Scatchard analysis = 24.
[b]number of data points used for Scatchard analysis = 21.
[c]number of data points used for Scatchard analysis = 29.
[d]number of data points used for Scatchard analysis = 18.

and at 47 ± 12 nM for 3′-sulfo-Le^x-PAA-flu were found to be significantly different ($p < 0.005$) from the values calculated for the high affinity binding sites of sialyl- and 6-sulfo-sialyl-Le^x-PAA-flu (Table 2). The number of apparent binding sites calculated at 15 400 ± 1800 and at 13 100 ± 1300 for Le^x- and 3′-sulfo-Le^x-PAA-flu was higher than the number of high affinity binding sites calculated for sialyl- and 6-sulfo-sialyl-Le^x-PAA-flu (Table 2). This difference can be explained by the presence of low affinity binding sites for these two neoglycoconjugates (data not shown). Altogether, these results suggest that substitution of the Le^x epitopes by 3-sialyl- or by 6-sulfo- and 3-sialyl- residues modifies its binding to *P. aeruginosa* 1244-NP.

It should be noticed that the Kd values obtained for the binding of Le^x-, sialyl-Le^x- and 3′-sulfo-Le^x-PAA-flu are identical to those obtained in our preceding study [16]. The number of apparent binding sites depends on the characteristics of the apparatus which is used. This number was higher with a cytometry analysis performed with a FACScalibur cytometer using a 1052 resolution channel than with a FACScann cytometer using a 250 resolution channel.

Specificity of the binding of 6-sulfo-sialyl-Le^x-PAA-flu to *P. aeruginosa* 1244-NP

Competition experiments were performed in the presence of a 62.5 nM solution of the fluorescent glycoconjugates; this concentration corresponded to 50–60% of the saturating concentration (Figure 1).

To check the specificity of the binding of sulfo-sialyl-Le^x-PAA-flu to *P. aeruginosa* 1244 NP, competition binding was performed in the presence of the unlabeled corresponding glycoconjugate. The results obtained after flow cytometry analysis demonstrated a decrease in the logarithm of fluorescence intensity when compared to control (3500 bound particles but 6000 in absence of unlabeled 6-sulfo-sialyl-Le^x derivative). Reduction of intensity was evidenced by the Kolmorogov-Smirnov two-sample test.

The value of mean fluorescence obtained for the binding of sialyl-Le^x-PAA-flu was not changed when incubation was performed in presence of unlabeled sulfo-sialyl-Le^x polyacrylate derivative. These results suggested that there was no cross reactivity in the binding of sulfo-sialyl-Le^x- and sialyl-Le^x-PAA-flu to *P. aeruginosa* 1244-NP.

Comparative adherence of *P. aeruginosa* 1244-NP to sialyl-Le^x and 6-sulfo-sialyl-Le^x-PAA

To compare the involvement of sialyl-Le^x and 6-sulfo-sialyl-Le^x epitopes in the adhesion process, we compared the adherence of *P. aeruginosa* 1244-NP to unlabeled polyacrylate derivatives coated on a microtiter plate assay. The numbers of adhering bacteria (10^2 CFU per well) obtained for the wells coated with sialyl-Lex-PAA and 6-sulfo-sialyl-Le-PAA were 374 ± 150 and 368 ± 156 respectively.

Comparative binding of sialyl-Le^x and 6-sulfo-sialyl-Le^x-PAA-flu to pathological strains of *P. aeruginosa*

In order to determine whether the pathological strains also preferentially recognize glycoconjugates bearing sialyl- and sulfo-sialyl-Le^x, the binding of sialyl-Le^x, 3-sulfo-Le^x- and sulfo-sialyl-Le^x-PAA-flu to four mucoid strains was compared using flow cytometry and Scatchard analysis. The calculated Kd values (Table 3) indicated that strain 690 bound better to the sialyl-Le^x fluorescent glycoconjugate than to the sulfo-sialyl-Le^x derivative. In contrast, for the three other strains (6190, 6118 and 130308), there were no differences between the Kd values obtained for sialyl-Le^x- and sulfo-sialyl-Le^x-PAA-flu. The Kd values obtained for the binding of 3′-sulfo-Le^x-PAA-flu indicated that all the strains, except 6118, bound better to sialyl-Le^x and/or to 6-sulfo-sialyl-Le^x-PAA-flu than to 3′-sulfo-Le^x-PAA-flu.

Discussion

The aim of the present data was to determine whether the 6-sulfo-sialyl-Le^x epitope is involved in the attachment of *P. aeruginosa* to respiratory mucins. The study was conducted with a non-piliated strain, strain 1244-NP, and four strains isolated from CF patients.

In order to determine carbohydrate receptors for *P. aeruginosa*, we have previously developed a flow cytometry assay using polyacrylamide-based glycoconjugates labeled with fluorescein. The results obtained with this technique have shown that several strains of *P. aeruginosa* recognized specifically the Le^x determinant and its sialylated or sulfated derivatives found at the periphery of respiratory mucins [16]. The glycoconjugate bearing the sialyl-Le^x epitope was found to be a better receptor than the other glycoconjugates. This result suggested that this structure might be a site of attachment for *P. aeruginosa*.

However, the broad diversity of carbohydrate chains of these glycoproteins, suggested that other receptors might be involved in this binding. The 6-sulfo-sialyl-Le^x epitope, which is also well represented in CF mucins [17], could be a good

Table 3. Binding of sialyl-Le^x-, 3′-sulfo-Le^x- and 6-sulfo-sialyl-Le^x-PAA-flu to four *P. aeruginosa* strains isolated from CF patients

	Kd (nm ± sd)			*p*		
	sialyl-LPF[a]	6-sulfo-sialyl-LPF	3′-LPF	sialyl-LPF vs. 6-sulfo-sialyl-LPF	sialyl-LPF vs. 3′-sulfo-LPF	3′-sulfo-LPF vs. 6-sulfo-sialyl-LPF
Strains						
6190	40 ± 15[b]	26 ± 8	79 ± 15	N.S.[c]	$p < 0.05$	$p < 0.05$
690	28 ± 4	43 ± 6	47 ± 1	$p < 0.05$	$p < 0.05$	N.S.
6118	46 ± 14	39 ± 13	48 ± 10	N.S.	N.S.	N.S.
130308	36 ± 2	43 ± 8	89 ± 21	N.S.	$p < 0.05$	$p < 0.05$

[a]LPF: Le^x-PAA-flu.
[b]Results are expresed as mean ± standard deviations. The number of data filled for Scatchard analysis ranged between 16 and 20.
[c]NS: not significant.

candidate for binding. The polyacrylamide-based glycoconjugate bearing this structure became available recently. Using this fluorescent derivative and flow cytometry, the present study demonstrates that all the strains of *P. aeruginosa* studied bound to the glycoconjugate bearing the 6-sulfo-sialyl-Le^x-PAA-flu. The specificity of the binding was studied by a competition experiment: the binding of 6-sulfo-sialyl-Le^x-PAA-flu to *P. aeruginosa* 1244-NP showed a decrease in the presence of an excess of the unlabeled corresponding glycoconjugate.

In our preceding study [16], competition assays were also performed. The binding of Le^x-PAA-flu showed a 50% inhibition in the presence of an excess of the corresponding unlabeled polyacrylate derivative. This partial inhibition may be due to a non-specific fixation of the fluorescent groups on the bacteria [22]. For similar reasons, it was also shown that competitive inhibition by respiratory mucins and mucin glycopeptides which bear more than a single epitope could not reach more than a 50% inhibition. A partial inhibition has also been observed for the binding of sialyl-Le^x-PAA-flu to CHO cells [23].

The Kd measurement indicates that, for all strains studied but one, the affinity for the 6-sulfo-sialyl-Le^x determinant was as high as the affinity for the sialyl-Le^x determinant. We also measured the adherence of strain 1244-NP to glycoconjugates bearing sialyl-Le^x and 6-sulfo-sialyl-Le^x coated on microtiter-plates. The results showed that the number of adherent bacteria was the same for the two glycoconjugates. Altogether, these results indicate that the 6-sulfo-sialyl-Le^x and the sialyl-Le^x epitopes are among the best receptors for *P. aeruginosa*. Moreover, competition experiments revealed the absence of cross-binding between polyacrylate derivatives bearing sialyl-Le^x derivatives and 6-sulfo-sialyl-Le^x determinants, suggesting that these two epitopes are recognized by different bacterial adhesins.

The occurrence of several adhesins with different specificity is quite possible. A variety of adhesins has been observed with *H. pylori* [24,25] and, in the case of *P. aeruginosa*, the binding of radiolabeled mucins to outer membranes revealed a number of bands [8].

Sialyl-Le^x and 6-sulfo-sialyl-Le^x determinants have been shown to play a role in cell adhesion via binding to selectins [26], and the 6-sulfo-sialyl-Le^x determinant is the best ligand of L-selectin [27] which is constitutively expressed on leukocytes. Human respiratory mucins containing such epitopes bind to L-selectin [28] and thus may interact with leukocytes which are abundant in the airways of CF patients. Human low-molecular weight salivary mucins (MG2) are also recognised by L-selectin and by different bacteria, and their binding is mediated by separate oligosaccharide determinants [29]. On the contrary, the results of the present data indicate that the binding of CF respiratory mucins to *P. aeruginosa* and to L-selectin may involve the same carbohydrate determinants. Altogether the results of the present work suggest that both the primary defect leading to oversulfation and the strong inflammatory reaction, generating an increase in mucin sialylation and an hyperexpression of sialyl-Le^x, may be responsible for the specific lung colonization by *P. aeruginosa* of CF patients. By binding with both leukocytes and bacteria, mucins with sialyl-Le^x and sulfo-sialyl-Le^x determinants might limit the defense properties of leukocytes against *P. aeruginosa*.

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References

1 Welsh J, Tsui LC, Boat TF, Beaudet AL, In *The metabolic and molecular basis of inherited diseases*, edited by Scriver CR, Beaudet AL, Sly WS, Valle D, (McGraw-Hill, 1995) pp. 3799–886.
2 Riordan JR, *Annu Rev Physiol* **55**, 609–30 (1993).
3 Boucher RC, *Am J Respir Crit Care Med* **150**, 581–93 (1994).

4 Devidas S, Guggino WB, *J Bioenerg Biomembr* **29**, 443–51 (1997).
5 Høiby N, *Acta Paediatr Scand (suppl.)* **301**, 33–54 (1982).
6 Ramphal RC, Carnoy C, Fièvre S, Michalski JC, Houdret N, Lamblin G, Strecker G, Roussel P, *Infect Immun* **9**, 700–4 (1991).
7 Rosenstein JJ, Chun Y, Stoll MS, Feizi T, *Infect Immun* **60**, 5078–84 (1992).
8 Carnoy C, Scharfman A, Van Brussel E, Ramphal R, Lamblin G, Roussel P, *Infect Immun* **62**, 1896–900 (1994).
9 Carnoy C, Ramphal R, Scharfman A, Lo-Guidice JM, Houdret N, Klein A, Galabert C, Lamblin G, Roussel P, *Am J Respir Cell Mol Biol* **9**, 323–34 (1993).
10 Devaraj N, Sheykhanazari M, Warren WS, Bhavanandan VP, *Glycobiology* **4**, 191–208 (1994).
11 Lamblin G, Degroote S, Perini JM, Delmotte P, Scharfman A, Davril M, Lo-Guidice JM, Houdret N, Klein A, Roussel, *BioLexis* **1**, http://www.biolexis.org/uk/index.html (2000).
12 Cheng PW, Boat TF, Cranfill K, Yankaskas JR, Boucher RC, *J Clin Invest* **82**, 68–79 (1989).
13 Zhang Y, Doranz B, Yankaskas JR., Engelhardt JF, *J Clin Invest* **96**, 2997–3104 (1995).
14 Pasky EA, Foskett JK, *J Biol Chem* **272**, 7746-7751.
15 Davril M, Degroote S, Humbert P, Galabert C, Dumur V, Lafitte JJ, Lamblin G, Roussel P (1999) *Glycobiology* **9**, 311–21 (1997).
16 Scharfman A, Degroote S, Beau J, Lamblin G, Roussel P, Mazurier J, *Glycobiology* **8**, 754–67 (1999).
17 Lo-Guidice JM, Wieruszeski JM, Lemoine J, Verbert A, Roussel P, Lamblin G, *J Biol Chem* **269**, 18794–813 (1994).
18 Bovin NV, *Glycoconjugate J* **15**, 431–46 (1998).
19 Ramphal R, Koo K S. Ishimoto, Toten AP, Lara JC, Lory S, *Infect Immun* **59**, 307–1311 (1991).
20 Partridge SR, Baker MS, Walker MR, Wilson M, *Infect Immun* **64**, 596–605 (1996).
21 Veerman EC, Bank VM, Namavar F, Appelmelk BJ, Bolscher JG, Nieuw Amerongen AV, *Glycobiology* **7**, 734–43 (1997).
22 Babiuk LA, Paul EA, *Can J Microbiol* **16**, 57–62, (1970).
23 Galinina OE, Tuzikov AB, Rapoport E, Le Pendu J, Bovin NV, *Anal Biochem* **265**, 282–9 (1998).
24 Ilver D, Arnqvist A, Ogren J, Frick IM, Kersulyte D, Incecik ET, Berg DE, Covacci A, Engstrand L, Boren T, *Science* **279**, 373–77 (1998).
25 Namavar F, Sparrius M, Veerman EC, Appelmelk BJ, Vandenbroucke-Grauls CM, *Infect Immun* **66**, 444–7 (1998).
26 McEver RP, Moore KL, Cummings RD, *J Biol Chem* **270**, 11025–28 (1995).
27 Galustian C, Lawson AM, Komba S, Ishida H, Kiso M, Feizi T, *Biochem Biophys Res Com* **240**, 748–51 (1997).
28 Crottet PY, Kim YJ, Varki A, *Glycobiology* **6**, 191–201, (1996).
29 Prakobhol A, Tangman K, Rosen SD, Hoover CI, Leffler H, Fisher SJ, *Biochemistry* **38**, 6817–25 (1999).

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