

Respiratory pathophysiologic responses

Effect of antigen on the glycoconjugate profile of tracheal secretions and the epithelial glycocalyx in allergic sheep

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*To characterize the glycoconjugate composition of tracheal secretions and the apical glycocalyx of the tracheal epithelium under baseline conditions and after antigen challenge, sheep allergic to *Ascaris suum* were intubated with a double-balloon nasotracheal tube to create a tracheal chamber. After an initial tracheal lavage, the animals were either exposed to intratracheally nebulized phosphate-buffered saline (PBS) (3 ml, n = 6) or *A. suum* antigen (251,000 protein nitrogen units in 3 ml of PBS, n = 6). Tracheal lavage was repeated 2 hours later, and the animals were killed. An enzyme-linked lectin assay and lectin histochemical analysis were used to characterize carbohydrate residues in lyophilized, resuspended tracheal secretions and the apical glycocalyx of the tracheal epithelium, respectively. Eight lectins were used to detect GalNAc, α -Gal, β -Gal, α -Fuc, (1-3)Man, α -Man/Glu, α -Man, and α -(2-3)sialyl residues. The amounts of total nondialyzable solids, proteins, and lipids in tracheal secretions were approximately twice as high after exposure to *A. suum* than after exposure to PBS. All carbohydrate residues were present in tracheal secretions after exposure to PBS and *A. suum*, but the reactivity was higher after exposure to *A. suum* for β -Gal (+125%), α -Man/Glu (+150%), α -(1-3)Man (+287%), α -(2-3)sialyl (+353%), and α -Man (+448%) ($p < 0.05$). Likewise, the apical glycocalyx contained all carbohydrate residues after exposure to PBS and *A. suum*; after exposure to *A. suum*, the reactivity was greater for α -GalNAc (+18%), α -(2-3)sialyl (+90%), β -Gal(1-3)GalNAc (+433%), and α -(1-3)Man (+482%) ($p < 0.05$). There were no differences in other carbohydrate residues in either tracheal secretions or the apical glycocalyx. These antigen-induced changes in the respective glycoconjugate profiles of tracheal secretions and the apical glycocalyx of the tracheal epithelium could have an effect on airway defenses, including bacterial adhesion to the epithelium. (J ALLERGY CLIN IMMUNOL 1994;93:585-93.)*

Key words: Antigen, lectin, glycosyl, glycoconjugate, tracheal secretions, lavage, histochemistry, epithelial glycocalyx, allergic sheep

The terminal carbohydrate residues of glycoconjugates contained in airway secretions serve several biologic functions, including host defense. For example, the secreted glycoconjugates can attach to surface proteins on bacteria colonizing the tracheobronchial tree, thereby preventing the attachment of the bacteria to the epithelium, a

Abbreviations used

OD: Optical density (absorbance)
PBS: Phosphate-buffered saline

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prerequisite for infection. Specific proteins bind to specific oligosaccharides,¹ and the terminal carbohydrate residues of the epithelial glycocalyx and secreted glycoconjugates all are believed to represent alternative binding sites for bacterial adhesion proteins. The respective carbohydrate profiles might therefore influence bacterial attachment to the epithelium. We have previously used lectin histochemistry to characterize glycoconjugates contained in sheep airway mucus cells.² However, there may be qualitative and quantitative differences between the glycoconju-

gates present in airway mucus cell granules and those present in airway secretions or the epithelial glycocalyx. Therefore a direct assessment of the glycoconjugates in the apical glycocalyx of the airway epithelium and airway secretion would be necessary to delineate their potential in antibacterial defenses.

Excessive lower airway secretions are a characteristic feature of bronchial asthma, and they seem to result from increased production and decreased clearance of airway liquids.³ It is possible that these quantitative changes are associated with changes in the glycoconjugate profiles of the secretions and of the apical glycocalyx of the epithelium. The purpose of the present investigation was to test this possibility in an animal model of airway anaphylaxis. We therefore determined in allergic sheep whether antigen challenge *in vivo* causes changes in the glycoconjugates contained in the tracheal glycocalyx as detected by lectin histochemistry, and the comparative alterations in the tracheal secretions as assessed with an enzyme-linked lectin assay.

METHODS

Animals and protocol

Clinically healthy, conscious adult ewes (20 to 32 kg) with *A. suum* hypersensitivity documented by cutaneous testing were loosely restrained and intubated while under topical anesthesia (2% lidocaine) with a specially designed double-balloon nasotracheal tube (diameter = 8 mm). The distance between the balloons was 15 cm. This created a tracheal chamber into which two extramural catheters were incorporated for lavage.⁴ Tracheal lavage was carried out by injecting phosphate-buffered saline (PBS) through the proximal catheter and collecting the effluent from the distal (dependent) catheter. After intubation and an initial lavage with 200 ml PBS, the sheep underwent a tracheal aerosol challenge with either PBS (control, $n = 6$) or purified *A. suum* antigen (Greer Diagnostic, Lenoir, N.C.) (251,000 protein nitrogen units or 500 μ g of *A. suum* extract, $n = 6$). As an irrelevant antigen control, three additional sheep were challenged with ovalbumin (500 μ g). Three milliliters of PBS or *A. suum* in 3 ml of PBS or ovalbumin in 3 ml of PBS were ultrasonically nebulized (model 670, Monahan, Plattsburgh, N.Y.) and introduced into the tracheal chamber through one of the extramural catheters, with the other catheter serving as a vent.⁴ The mass median aerodynamic diameter of the aerosol was 3.0 μ m (geometric standard deviation = 1.4). Approximately 5% of the aerosol was deposited in the trachea.

Two hours later, the trachea was lavaged with 50 ml of PBS. The returned fluid was centrifuged at 1600 rpm for 10 minutes, and the supernatant was dialyzed (molecular weight cutoff, 6 to 8 $\times 10^3$), lyophilized, and

weighed. Total protein contents were determined with Kodak Ektachem (Eastman Kodak Co., Rochester, N.Y.), total lipid content with the Bligh-Dyer method,⁵ and sheep serum albumin with an ELISA method. In the lavaged samples after *A. suum* and ovalbumin challenges, a correction was made for the nebulized proteins by subtracting from the measured total protein content 5% of the nebulized preteins (fraction deposited in the trachea). This amounted to 0.01% of the average total protein content in tracheal lavage. After the second tracheal lavage, the sheep were injected with an overdose of pentobarbital sodium (50 to 80 mg/kg). The trachea and lungs were removed en bloc, and the trachea was cannulated and insufflated with buffered HgCl₂ (B-5 fixative) at a pressure of 30 cm H₂O. Tracheal cross-sections were taken from the proximal, mid, and distal segments, all within the tracheal segment between the two cuffs of the endotracheal tube. The tissues were washed and embedded in paraffin for lectin histochemistry. To determine whether tracheal intubation and lavage per se had an effect on the glycoconjugate profile of the epithelial glycocalyx, lectin histochemistry was performed as described above in five additional control sheep in which no airway manipulations were carried out before they were killed.

Eight lectins that bind to specific glycosides were used to characterize the glycoconjugate profile in the tracheal lavage and epithelial glycocalyx (Table I).

Enzyme-linked lectin assay

Microtiter plate wells were coated with lectins (0.2 ml, 5 μ g/ml) by incubating them at 37° C for 3 hours. After three washes with PBS and Tween-20 treatment, the lyophilized tracheal lavage samples were serially diluted twofold in seven steps from 250 μ g/ml PBS and incubated for 45 minutes at 37° C in triplicate to allow specific lectin binding. After another wash with PBS, the plates were reacted with biotinylated UEA I (0.2 μ g/ml), a lectin to detect the captured glycoconjugate. After unbound lectin was washed off, avidin-horse-radish peroxidase was added and reacted for 15 minutes; then the plates were washed again with PBS. The OD (absorbance) of the substrate (ABTS, Boehringer Mannheim, Corp., Indianapolis, Ind.) reaction product was determined at 405 nm in an MR600 microplate reader (Dynatech Laboratories, Inc., Chantilly, Va.) after 15 minutes. The results were expressed as OD per 10 μ g nondialyzable solids. The reactivity was expressed as OD per 10 μ g solids because acceptable standards for sheep mucus are lacking. On a semilogarithmic plot of glycoconjugate concentration, the standard curve was a straight line. The sensitivity of the assay was 2 μ g/ml. Nonbiotinylated lectin substitutions, lectin deletion, and specific glycoside preincubations were used as controls to confirm the specificity of the assay. *A. suum* antigen at a concentration of 1 μ g/ml (exceeding the calculated *A. suum* antigen concentration after *A. suum* challenge) failed to react or reacted

TABLE I. Lectins used to characterize carbohydrate residues of glycoconjugates

Lectin/agglutinin (common name)	Acronym	Carbohydrate residue sequences recognized by lectin	Carbohydrates that competitively inhibit lectin binding
<i>Arachis hypogea</i> (peanut)	PNA	β -Gal(1-3)GalNAc	β -D-Galactose(1-3)-N-acetyl-Galactosamine-Galactose
<i>Dolichos biflorus</i> (horsegram)	DBA	α -GalNAc	α -D-Galactose-N-acetyl-Galactosamine
<i>Bandeiraea simplicifolia</i> I or <i>griffonia</i> I Subunits B ₄ Subunits A ₄	BSA-I _{B4}	α -Gal α -D-Galactosamine	α -Methyl-Galactose
<i>Ulex europeus</i> (gorse)	UEA-I	α -Fuc	α -L-Fucose
<i>Galanthus nivalis</i> (snow drop)	GNL	α -(1-3)Man	α -Methyl-Mannose
<i>Lens culinaris</i> (lentil)	LCA	α -Man/Glc	α -Methyl-Mannose/Glucose
<i>Pisum sativum</i> (pea)	PSA	α -Man	α -Methyl-Mannose
<i>Maackia amurensis</i> II	MAL II	α -(2-3)sialyl	Sialic acid

only minimally within the noise level with any of the eight lectins used in the experiments (Table I).

Lectin histochemical analysis

The histochemical method used has been previously described.² Briefly, from each trachea, three complete rings were serially sectioned (24 sections 4 to 6 μ m thick), and epithelial glycoconjugate residues were visualized with the biotinylated lectins, as used for the enzyme-linked lectin assay. Two controls were run with each lectin: deletion and lectin preincubation with the appropriate carbohydrate (Table I). The specificity of the binding was ascertained by competitive binding inhibition with a 0.1 mol/L solution of the appropriate specific carbohydrates. The preincubation was carried out for 45 minutes. The site of the lectin binding was detected with a Vector ABC kit (Vector Laboratories, Inc., Burlingame, Calif.) and visualized without counterstain with Differential Interference Contrast optics (Olympus of America, Inc., Lake Success, N.Y.).

The abundance of specific carbohydrate residues, as detected by the lectin binding of the tracheal epithelial glycocalyx on the apical epithelial cell membrane (including cilia) over cartilaginous areas of the tracheal rings, was quantitated by stereologic volumetric estimation. The number of intersections of the test-grid lines (8.6 μ m apart, positioned perpendicularly to the epithelial surface) with the positive reaction product on the glycocalyx per 1500 total test lines was counted at oil immersion magnification. A percentage was determined by point counting randomly started 1500 hits around a complete tracheal section (500 per section $\times$ 3 tracheal sections) per animal, for each of the eight examined lectins. A weighted reactivity was calculated by multiplying the determined percent hits (number of intersections on the reaction product di-

vided by 1500 test-grid lines) by the following factors: 5 for densely positive, 4 for strongly positive, 3 for moderately positive, 2 for reactive, and 1 for negative (no reaction product). The difference in the values obtained by two morphologists unaware of the source of the sections was within 1 standard deviation.

Statistical analysis

A paired *t* test was used to evaluate the morphometric data. A nonparametric analysis of variance (Kruskal-Wallis test), followed by nonparametric pairwise comparisons, was used to compare the results of the tracheal lavage analyses. Differences were considered significant when *p* < 0.05 with a two-tailed test. Values in the text are expressed as means $\pm$ SD.

RESULTS

The volume of the tracheal lavage fluid approximated the introduced PBS volume (Table II). After PBS control exposure, the mean total proteins and lipids comprised 25% and 52% of the total nondialyzable solids, respectively. Similar values were seen after ovalbumin challenge. After *A. suum* antigen nebulization, the total nondialyzable solid content was about two times greater than that after PBS control, and total proteins and lipids comprised 17% and 43%, respectively. Albumin comprised 4.6%, 4.2%, and 11% of total solids after PBS, ovalbumin, and *A. suum* antigen challenges, respectively.

After PBS control nebulization, the lavage fluid reacted with all eight lectins used, and the mean reactivities ranged from 0.106 OD per 10 μ g for MAL II to 0.470 OD per 10 μ g for UEA I lectin

TABLE II. Tracheal lavage analysis

Protocol	Total volume (ml)	Total nondialyzable solids (μg/ml)	Total protein (μg/ml)	Total albumin (μg/ml)	Total lipids (μg/ml)
Control (PBS) (n = 6)	48.8 ± 1.97	1114.5 ± 330.9	231.5 ± 42.9	51.5 ± 37.7	580.0 ± 265.1
<i>A. suum</i> antigen (n = 6)	50.8 ± 1.17	2355.7 ± 371.92	402.5* ± 32.60	254.8* ± 68.7	1008.1* ± 240.2
Ovalbumin antigen (n = 3)	48.3 ± 1.53	1321.4 ± 323.0	223.0 ± 34.7	56.4 ± 12.8	516.8 ± 110.8

Values are expressed as means ± SD.

**p* < 0.05 versus control.

(Fig. 1). This indicated the presence of β-Gal(1-3)GalNAc, α-GalNAc, α-Gal, α-L-Fuc, α-(1-3)Man, α-Man/α-Glu, α-Man, and α-(2-3)sialyl residues, with the lowest reactivity for sialyl residues and the highest reactivity for α-L-Fuc. The carbohydrate residues in the glycocalyx also reacted with all lectins, but there was a considerable variability in the level of reactivity with high levels for α-GalNAc, α-Fuc, α-Man, and possibly α-Glu and low levels for sialyl and very low levels for β-Gal(1-3)GalNAc and α-(1-3)Man residues (Fig. 2).

After *A. suum* exposure, there was a greater (*p* < 0.05) reactivity in the lavage fluid than after PBS exposure for all carbohydrate residues but α-GalNAc. The mean OD was higher by 106% for α-Gal and α-Fuc and 125% for β-Gal(1-3)GalNAc. Even larger differences were found for α-Man/Glc (+150%), α-(1-3)-Man (+287%), α-(2-3)sialyl residues (+353%), and α-Man (+448%) (Fig. 1). Lectin histochemical analysis of the glycocalyx showed a greater (*p* < 0.05) reactivity after *A. suum* exposure than after PBX exposure mainly for β-Gal(1-3)GalNAc (+433%) and α-(1-3)-Man (+482%) and to a lesser degree for α-GalNAc (+18%) and sialyl residues (+90%). There were no significant differences in the other carbohydrate residues (Fig. 2).

There were no significant differences in the lectin reactivity profiles in the lavage and the glycocalyx between ovalbumin and PBS nebulizations (Figs. 3 and 4). Likewise, there was no significant difference in the lectin reactivity profile of the tracheal glycocalyx between animals after PBS nebulization and animals that were not intubated and received no tracheal lavage (Fig. 5).

DISCUSSION

Baseline condition (PBS control)

The total solids in the tracheal lavage had a higher content of lipid than protein (Table II).

With respect to glycoconjugates, it is possible that glycoproteins and glycolipids both accounted for them. The exact source of the lipids is not known, but they must be derived from the trachea because of the mechanical seal provided by the two cuffs of the endotracheal tube. Because the tracheal lavage fluid was centrifuged and only the supernatant was used for analysis, lipids derived from cell membranes probably contributed little to the total lipid content. At least some of the lipids may be secreted by the airway epithelium, as suggested by tracheal epithelial cell culture experiments.^{6,7} In one of those studies, only 6% of total solids secreted by human bronchial explants was comprised of lipids.⁷ This is considerably less than the lipid content of the tracheal lavage in our experiment (52% of total solids). In vivo, nonepithelial sources of lipids (e.g., serum transudates) must also be considered. In dogs and human subjects with tracheostomy, the nondialyzable material of lower airway secretions has also been shown to contain lipids in addition to proteins, glycoproteins, and proteoglycans; and some of the lipids were identified as glycolipids.^{8,9} In one study the nondialyzable solids in lower airway secretions obtained from patients who had undergone laryngectomy contained approximately equal amounts of carbohydrate, protein, and lipid.¹⁰ The somewhat higher lipid content in the present study could have resulted from the methodology of the lipid assay used. The method is based on chloroform extraction. Because of cross-linking between lipids and glycoproteins,⁹ some proteins may have been extracted in the chloroform layer.

Although the tracheal sections did not reveal tissue damage, the inflated balloons of the endotracheal tube and the physical effect of lavage could have stimulated epithelial secretion. The sheep that underwent PBS challenge, although used as a reflection of tracheal glycoconjugate profiles under baseline conditions, may therefore

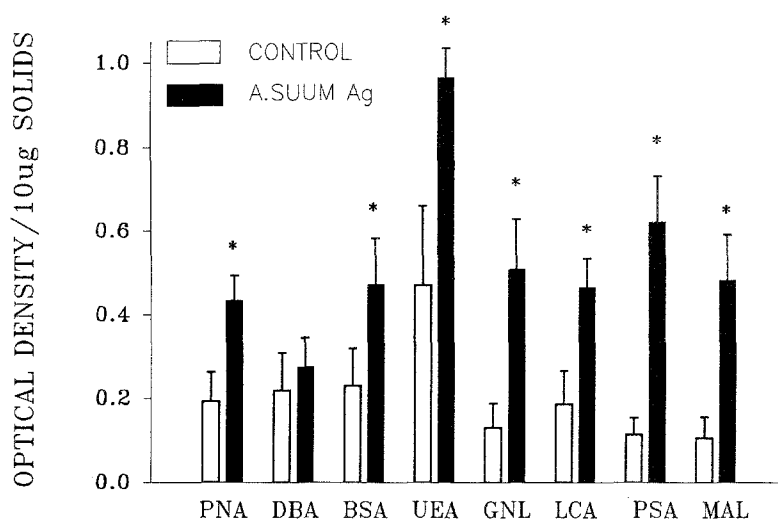


FIG. 1. Lectin reactivity in tracheal lavage as determined by enzyme-linked lectin assay. Mean value $\pm$ SD for PBS control ($n = 6$) and *A. suum* antigen ($n = 6$) challenges. * $p < 0.05$ versus control. See Table I for explanation of lectin acronyms.

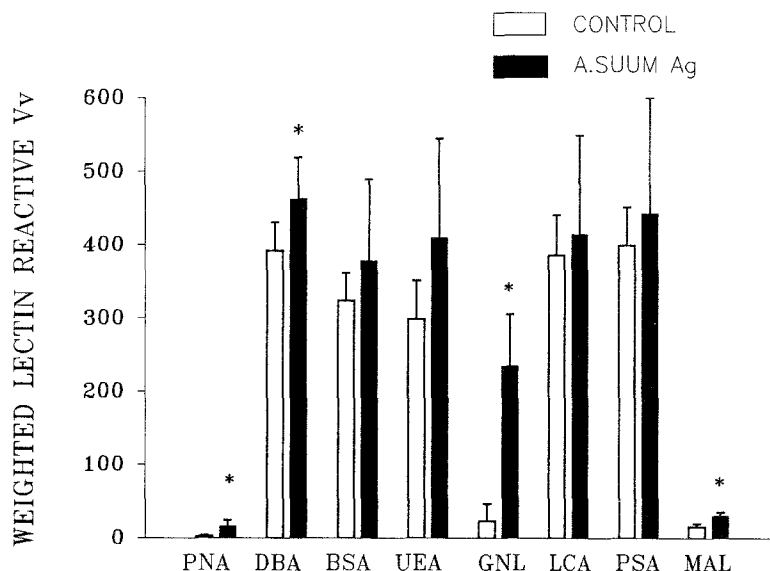


FIG. 2. Lectin reactivity of apical epithelial glycocalyx in the trachea as determined by lectin histochemistry and stereologic volumetric estimation. The weighted lectin-reactive volume fraction (V_v) was obtained by multiplying the percent hits by the staining intensity. Mean values $\pm$ SD of PBS control ($n = 6$) and *A. suum* antigen ($n = 6$) challenges. * $p < 0.05$ versus control. See Table I for explanation of lectin acronyms.

not have truly represented the secreted glycoconjugate profiles in normal sheep that have not undergone instrumentation of the airway. The lectin-binding profile of the epithelial glycocalyx was apparently not altered by the instrumentation because we found no differences between intubated, PBS-lavaged sheep and nonintubated sheep.

There were marked differences in the glycoconjugate profiles of the tracheal secretions and the glycocalyx of the apical membrane of surface epithelial cells (Table III). All eight lectins reacted with the resuspended lyophilized material derived from the supernatant of tracheal lavage and with the epithelial glycocalyx. Thus α - and β -galactose residues and α -GalNAc, α -Fuc,

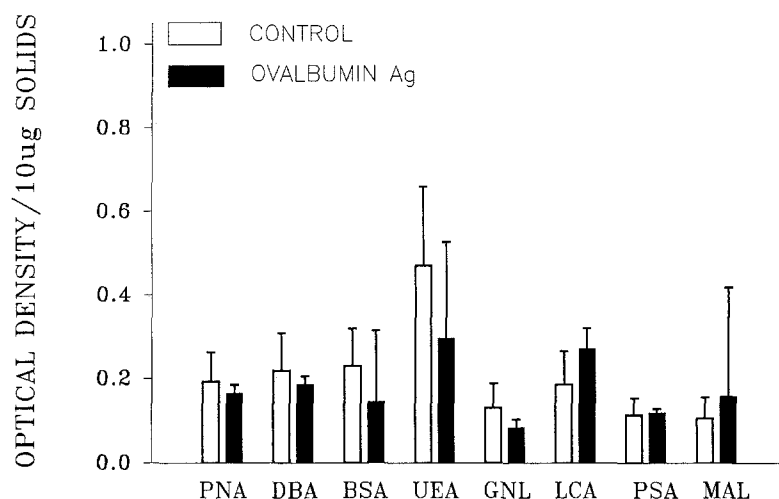


FIG. 3. Lectin reactivity in tracheal lavage as determined by enzyme-linked lectin assay. Mean value $\pm$ SD for PBS (control, $n = 6$) and ovalbumin ($n = 3$) challenges. See Table I for explanation of lectin acronyms.

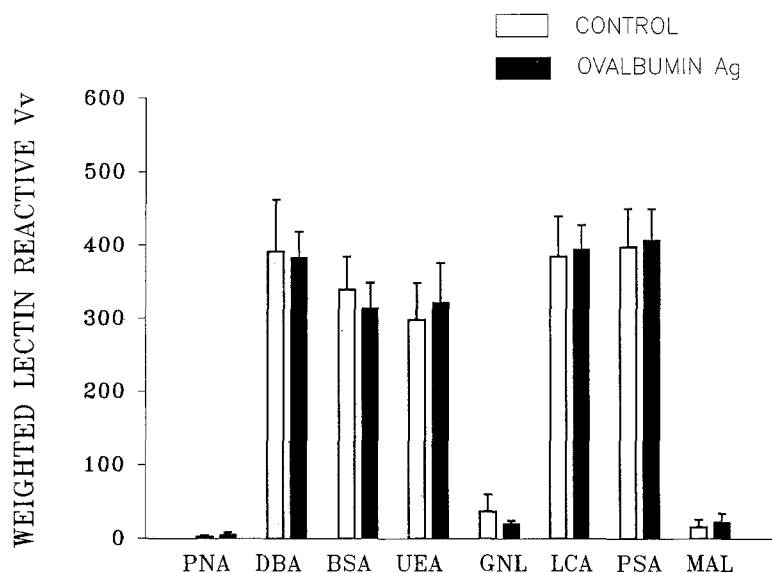


FIG. 4. Lectin reactivity of apical epithelial glycocalyx in the trachea was determined by lectin histochemistry and stereologic volumetric estimation. The weighted lectin-reactive volume fraction (V_v) was obtained by multiplying the percent hits by the staining intensity. Mean values $\pm$ SD of PBS (control, $n = 6$) and ovalbumin ($n = 3$). See Table I for explanation of acronyms.

α -Man, α -(1-3)Man, and α -(2-3)sialyl were all detected as lectin-accessible carbohydrate residues. The variability of reactivity among the eight lectins was greater in the glycocalyx than in the tracheal secretions.

A semiquantitative technique was used in the present study to characterize the glycoconjugate profile of the apical glycocalyx of the tracheal epithelium. Because the distribution of lectin

staining and its intensity were inhomogeneous, the intensity was weighted by determining what percentage of the total number of "hits" fell on each of five intensity levels and by multiplying the percentage by the respective intensity level. To further improve the objectivity of the technique, two morphologists, who were unaware as to the source of the sections, were employed. Given the large differences between some of the lectins

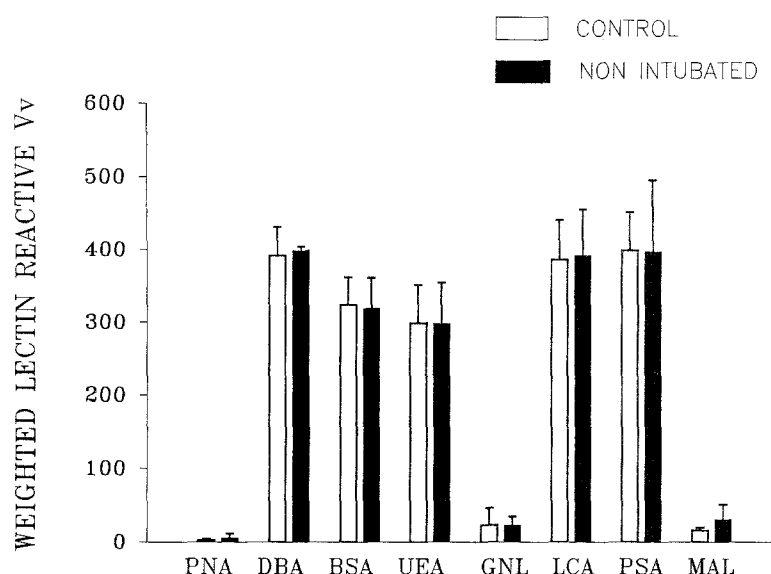


FIG. 5. Lectin reactivity of apical epithelial glycocalyx in the trachea as determined by lectin histochemistry and stereologic volumetric estimations. The weighted lectin-reactive volume fraction (V_v) was obtained by multiplying the percent hits by the staining intensity. Mean values $\pm$ SD of PBS lavage (control) in intubated animals ($n = 6$) and nonintubated animals that were not lavaged (nonintubated, $n = 5$). See Table I for explanation of acronyms.

TABLE III. Relative carbohydrate residue reactivities in tracheal secretions and apical epithelial glycocalyx

Carbohydrate residue	Lectin	Secretions		Glycocalyx	
		Control	<i>A. suum</i> Ag	Control	<i>A. suum</i> Ag
β -Gal(1-3)GalNAc	PNA	+	++	$\pm$	++
α -GalNAc	DBA	+	+	+++	++++
α -Gal	BSA I _{B4}	+	++	++	+++
α -L-Fuc	UEA I.	+	++	+++	++++
α -(1-3)Man	GNL	+	+++	—	+++
α -Man/Glc	LCA	+	+++	++++	++++
α -Man	PSA	+	++++	++++	++++
α -(2-3)sialyl	MAL II.	+	+++	+	++

Ag, Antigen; —, negative; +, lightly reactive; ++, positive; +++, moderately reactive; +++++, densely reactive.

used, the technique was considered adequate for the purpose of this study, and we believe that our findings reflect a true variability in the carbohydrate residues contained in the glycocalyx.

Although we used a large number of lectins to recognize a wide variety of carbohydrate moieties, a complete spectrum of terminal sugars was not obtained. For example, we did not specifically test for glucose, N-acetyl-glucosamine, and N-acetyl-neuraminic acid. These oligosaccharides can also be located on the periphery of the carbohydrate chains of respiratory glycoconjugates.¹¹ *Lens culi-*

naris antigen recognizes both mannose and glucose, whereas *Pisum sativum* antigen recognizes only mannose. In the present study, *Lens culinaris* antigen and *Pisum sativum* antigen activities were similar in tracheal lavage and glycocalyx after PBS control challenge. Whether glucose residues were present under baseline conditions therefore cannot be determined.

Effect of *A. suum* antigen

We have previously shown that intratracheal antigen challenge with the same technique as in

the present study causes tracheal constriction in allergic sheep.⁴ This suggests that the antigen challenge elicited an anaphylactic rather than a nonspecific epithelial response in the trachea. This was further supported by the observation that the irrelevant antigen, ovalbumin, failed to change the glycoconjugate profiles of tracheal secretions and the epithelial glycocalyx when compared with PBS. The immediate response to *A. suum* in allergic sheep, as determined by the assessment of respiratory and bronchovascular function, typically ends within 2 to 3 hours of challenge, and the late-phase response starts 4 to 5 hours after challenge.^{12, 13} Consequently, the 2-hour observation period must have reflected changes associated with the immediate allergic response. After *A. suum* exposure, there was an increase of more than twofold in total nondialyzable solids in the tracheal lavage. Because the increases in total protein and lipids were proportionally less, other constituents including carbohydrates must have comprised a significant fraction of the observed increase in total solids. Plasma extravasation could also have contributed to the changing glycoconjugate pattern after antigen challenge. This was supported by the finding that albumin concentration increased approximately fivefold after *A. suum* antigen challenge. Regardless of their sources, *A. suum* antigen increased the total solid and presumably glycoconjugate content in tracheal secretions and led to profound changes in their lectin-detectable carbohydrate profile. There were marked increases in the reactivity of all glycoconjugates except α -GalNAc.

In the glycocalyx the carbohydrate residues, which were strongly expressed after PBS control challenge (α -GalNAc, α -Gal, α -Fuc, and α -Man), remained unchanged after *A. suum* nebulization. Of the carbohydrates that had very low activities after PBS challenge, β -Gal(1-3)GalNAc and α -(2-3)sialyl increased moderately and α -(1-3)Man increased markedly after *A. suum* exposure.

It cannot be determined whether the changes in the lectin-detectable carbohydrate residues resulted from altered synthesis and expression of membrane glycoconjugates by the epithelial cell, the incorporation into the glycocalyx of glycoconjugates contained in the tracheal surface liquids, or a modified accessibility of lectins to the glycocalyx. The observed effects and the mode of *A. suum* antigen action on the tracheal epithelial cells and their surface glycocalyx is not entirely understood and requires further study.

Potential for bacterial adhesion

Bacteria colonizing the lower airways have been shown to bind to the apical membrane of epithelial cells by two major mechanisms.¹⁴ One possibility is the adhesion of bacteria to proteins expressed in the plasma membrane, the other is adhesion to specific glycoconjugates of the glycocalyx. By using lectins, we addressed the latter possibility. Both glycoproteins and glycolipids seem to participate in this process, and a given bacterium may have different adhesions that bind selectively to more than one terminal carbohydrate residue. The diversity and number of carbohydrate residues in respiratory secretions should determine how effectively colonizing bacteria are prevented from adhering to the epithelium. *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Mycoplasma pneumoniae* recognize carbohydrate residues that were detected in the tracheal secretions and the epithelial glycocalyx in our study. *P. aeruginosa* and *K. pneumoniae* bind to α -Man; *S. pneumoniae*, *S. aureus*, and *P. aeruginosa* bind to β -Gal(1-3)NAc; and *P. aeruginosa* and *M. pneumoniae* bind to sialyl.¹⁴⁻¹⁶ Several strains of *P. aeruginosa* recognize β -Gal and sialyl.^{17, 18}

Antigen had different effects on the glycoconjugate profile of tracheal secretions and the glycocalyx. In addition, the overall increase in total solids, and presumably glycoconjugates in the tracheal secretions could alter the potential for bacterial adhesion to mucus. Because the relative changes in the lectin reactivity of the tracheal secretions and epithelial glycocalyx after *A. suum* antigen challenge are not directly comparable, their effects on the adhesion of the various bacteria to the epithelium cannot be determined.

Our findings demonstrate that airway challenge with antigen in allergic sheep alters the respective glycoconjugate profiles of airway secretions and the airway epithelial glycocalyx. Whether similar changes can be observed in patients with asthma and what effect the changes may have on bacterial adhesion to the airway epithelium remains to be demonstrated.

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