Glycans of the trabecular meshwork in primary open angle glaucoma

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Abstract

Aims—Glycan expression was compared in glaucomatous trabecular meshwork (TM) and normal TM in order to determine any differences which may reflect pathological changes underlying primary open angle glaucoma (POAG).

Methods—Resin embedded TM from trabeculectomy specimens from 15 eyes with POAG and from 12 eyes with normal anterior segments were probed with a panel of biotinylated lectins and an avidin-peroxidase revealing system at the light microscope level. Statistical analyses were performed on the comparative staining results.

Results—The lectins ConA and ePHA showed strong staining in all areas of both glaucomatous and normal TM; ePHA staining of Schlemm's canal (SC) from POAG TM was significantly less than that from normal TM (ePHA-SC $p=0.04$). The lectins PSA, LCA, and SNA bound moderately strongly to SC endothelium and weakly to the endothelium of the corneoscleral meshwork (CSM); glaucomatous SC endothelial binding was significantly less than that of normal SC endothelium for PSA and LCA (PSA-SC $p=0.002$, LCA-SC $p=0.002$). STA and DSA showed moderately strong binding while WGA, ECA, AHA, and MPA bound weakly throughout the TM; for DSA and MPA this staining was significantly greater in POAG than in normal TM (DSA-SC $p=0.001$, DSA-CSM $p=0.002$, MPA-SC $p=0.01$, MPA-CSM $p=0.02$). Jac stained strongly throughout the TM and showed no significant difference in POAG compared with normal TM (Jac-SC $p=0.6$, Jac-CSM $p=1$). 1PHA, SBA, DBA, CTA, UEA-1 and LTA did not bind to glaucomatous TM or normal TM. There were no age-related changes seen.

Conclusions—The expression of some complex and hybrid, bisected and non-bisected *N*-linked glycans is significantly diminished in glaucomatous TM compared with normal TM. Some glycans with multiple *N*-acetylglucosamine residues and *O*-linked glycans with terminal and subterminal galactosyl groups are significantly increased in POAG TM. Glycan expression does not change significantly with age in POAG or normal TM.

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Schlemm's canal (SC) in primary open angle glaucoma (POAG) are well documented.^{1–6} Despite considerable research, however, the pathogenesis of this condition remains unresolved.⁷ One of the major histological characteristics of TM from eyes with POAG is a lower cellularity compared with normal TM.^{8–10} Other features which are usually associated with cellular degeneration include pigment accumulation and increased basement membrane deposition.^{11 12} It is also well known that such alterations may be seen to a lesser extent as part of the normal aging process.^{8 13} Because of these observations, trabecular cell dysfunction has been implicated in the pathogenesis of POAG.^{8 11 13}

The glycans of cell surface glycoproteins are important in determining structural and functional properties such as cellular permeability,¹⁴ resistance of glycoproteins to degradation,¹⁵ and surface antigenic characteristics,¹⁶ so changes or aberrations in cell surface glycoconjugates may reflect pathological changes within the cell.^{17 18} Previous investigations of the glycoconjugates of the TM have included a comparative histochemical study¹⁹ which concluded that there is a greater concentration of sialic acid groups on the abluminal surface of SC endothelium in the TM of glaucomatous tissue compared with normal tissue. The lectins of *Canavalia ensiformis* (ConA), *Triticum vulgaris* (WGA), *Phaseolus vulgaris* erythroagglutinin (ePHA), and *Ulex europaeus* (UEA-1) have also been used in a comparative lectin binding study of TM from normal and glaucomatous eyes,²⁰ but this employed lectin binding to western blots of polyacrylamide gels prepared from TM extracts and so could not localise the lectin binding sites within the tissue.

In a previous report on the glycans of normal human TM,²¹ we used the biotinylated lectins of *Sambucus nigra* (SNA), *Maackia amurensis* (MAA), and *Limax flavus* (LFA) as probes at the ultrastructural level. We demonstrated that sialic acid is present mainly in $\alpha(2,6)$ linkage in the SC endothelium, and in $\alpha(2,3)$ linkage where it is associated with extracellular fibrillar material in the juxtacanalicular tissue (JCT). In the present study, we have selected 20 lectins on the basis of their preferential binding to the main groups of D-pyranose sugars (most commonly occurring in *N*- and *O*-linked glycoproteins) namely, glucose/mannose, galactose/

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Abbreviations:

α -D-man, α -D-mannose; α -D-Glc, α -D-glucose; GalNAc, *N*-acetylgalactosamine; fuc, fucose; GlcNAc, *N*-acetylglucosamine; Neu5Ac, 5*N*-acetylneuraminic acid (sialic acid).

The morphological changes observed in the human trabecular meshwork (TM) and

Table 1 Details of the age, presurgical antiglaucomatous medication, and last pretrabeculectomy intraocular pressure of patients with primary open angle glaucoma

Age (years)	Medication	IOP (mm Hg)
41	Pilocarpine 4%, Eppy 1%, Metipranolol 0.3%	20 (28)
50	Pilocarpine, Betoptic 0.5%	NA
51	Pilocarpine 2%, Timoptol 0.25%, Diamox	24 (27)
67	Pilocarpine 4%, Ganda, Diamox	15 (28)
68	Pilocarpine 2%, Timoptol 0.25%	24 (24)
68	Pilocarpine 2%, Timolol, Allopurinol 300 mg	18 (27)
69	Pilocarpine 3%, Ganda 0.2%, Timoptol 0.25%	27 (28)
70	Pilocarpine 2%, Eppy 5%, Glaucine 0.3%	40 (NA)
72	NA	NA
78	Pilocarpine 2%, Eppy 1%, Betoptic 0.5%, Diamox	24 (NA)
78	NA	NA
81	NA	NA
81	Pilocarpine 2%, Timoptol 0.25%, Diamox Predsol	16 (60)
81	Pilocarpine 4%, Timoptol 0.25%, Diamox	30 (NA)
83	Diamox, Timoptol 0.25%	34 (40)

IOP=intraocular pressure before operation. Figure in brackets is the highest recorded IOP.
NA=not available.

N-acetylgalactosamine, *N*-acetylglucosamine, L-fucose, and sialic acid.¹⁷ Using an avidin-biotin peroxidase revealing system, we have compared the lectin binding to TM in 15 specimens from eyes with POAG and 12 specimens from eyes with a normal anterior segment (and with a comparable age range) to ascertain whether there is any discernible difference in either the types of glycans expressed by the TM of the two groups or in the topographical pattern of their expression.

Materials and methods

TISSUE

The TM specimens examined in this study were from two groups: 15 trabeculectomy specimens (obtained from patients with POAG) and TM from 12 eyes with no pathology involving the anterior segment. To obtain the 15 trabeculectomy specimens for study, a total of 93 specimens were processed and examined, of which 24 included some TM tissue. Of these, 15 contained both TM and

SC. A description of the trabeculectomy group, with the presurgical antiglaucomatous medication of the subjects, is shown in Table 1; none had undergone laser trabeculectomy. The age range of the subjects providing the 15 trabeculectomy specimens was 41–83 years. The specimens (which were approximately 2×1×1 mm in size) were immediately fixed in 5% (v/v) formaldehyde containing 0.1% (w/v) cetylpyridinium chloride. Following a 24 hour wash in 0.01 M sodium cacodylate, pH 7.4, containing 3 mM calcium chloride, the tissue blocks were processed into Araldite resin. Briefly, the procedure was as follows: the tissue was dehydrated in graded alcohols (40%, 70%, and 100 v/v), immersed in propylene oxide (two changes of 10 minutes), and left overnight in equal parts (v/v) of Araldite epoxy resin (Araldite MY 753, Ciba Geigy, Cambridge) to propylene oxide. Infiltration was continued the next day with a 3/1 (v/v) mixture of resin to propylene oxide, before finally embedding in undiluted Araldite. Polymerisation was carried out at 60°C for 12 hours.

The normal trabecular meshworks were obtained from 12 human eyes (age range 32–84 years) and have been reported previously.²² These eyes comprised four specimens obtained at necropsy within 3 hours after death, and eight tissue blocks of whole globes with malignant melanoma arising in the posterior choroid and no other pathology (selected from the archives of the Pathology Department, Manchester Royal Eye Hospital). All eight melanoma eyes had normal intraocular pressure before enucleation.

The eyes from necropsy were fixed in 2.5% (v/v) glutaraldehyde in 0.01 M sodium cacodylate buffer pH 7.4. Part of the iridocorneal angle was then removed and cut into pieces 1 mm thick which were washed in 0.01 M sodium cacodylate buffer containing 3 mM

Table 2 Biotinylated lectins used in this study: those with similar specificities are grouped together

Source	Abbrev	Major specificity	Reference
<i>Canavalia ensiformis</i> (jack bean)	ConA	α-D-man, α-D-Glc, terminal or α1,2 N-linked sequences. Bisected or non-bisected	26 27
<i>Pisum sativum</i> (garden pea)	PSA	α-D-man in non-bisected bi/tri antennary complex N linked sequences	27 28
<i>Lens culinaris</i> (lentil)	LCA	α-D-man, similar to but not identical with PSA	27 29
<i>Phaseolus vulgaris</i> erythroagglutinin (kidney bean)	ePHA	Bisected bi/tri antennate complex N linked sequences	30 31
<i>Phaseolus vulgaris</i> leucoagglutinin (kidney bean)	1PHA	Tri/tetra antennate non-bisected complex N linked sequences	32
<i>Glycine max</i> (soybean)	SBA	GalNAcα1-	33 34
<i>Dolichos biflorus</i>	DBA	GalNAcα1,3(Fuca1,2)Galβ1,4GlcNAc-	35 36
<i>Bandeiraea simplicifolia</i> (Griffonia)	BSA-1B ₄	Galα1,3Galβ1,4GlcNAcβ1-	37
<i>Erythrina corallodendron</i> (coral tree)	CTA	Galβ1,4GlcNAc-(multiple)	38
<i>Erythrina crista-galli</i> (coral tree)	ECA	Galβ1,4GlcNAc-	39
<i>Triticum vulgaris</i> (wheat germ)	WGA	(-4GlcNAcβ1,4GlcNAcβ1-) _n , (-3Galβ1,4GlcNAcβ1-) _n	26 40
<i>Solanum tuberosum</i> (potato)	STA	-β1,4GlcNAc oligomers	27
<i>Datura stramonium</i> (Jimson weed)	DSA	-GlcNAcβ1,4GlcNAc-	27 41
<i>Arachis hypogaea</i> (peanut)	AHA	Galβ1,3GalNAcα1->Galβ1,4GlcNAcα1-	42
<i>Maclura pomifera</i> (Osage orange)	MPA	Galβ1,3GalNAcα1->GalNAcα1-	43
<i>Artocarpus integrifolia</i> (Jacalin)	Jac	Galβ1,3GalNAc-, Galα1,6-	44
<i>Ulex europaeus</i> (gorse)	UEA-1	Fuca1,2Galβ1,4GlcNAc-	45 46
<i>Tetralagonolobus purpureus</i> (lotus)	LTA	α-L-fucosyl terminals	45
<i>Sambucus nigra</i> (elderberry bark)	SNA	Neu5Acα2,6Gal/GalNAc-	47
<i>Maackia amurensis</i>	MAA	Neu5Acα2,3Galβ1,4Glc/GlcNAc-	48

Table 3 Inhibition of lectin binding by specific sugars

Lectin	Inhibitory sugar	Concentration
ConA	α MeMan	0.2 M
PSA	α MeMan	0.2 M
LCA	α MeMan	0.2 M
ePHA	None	
1PHA	None	
SBA	α -D-GalNAc	1 mM
DBA	α -D-GalNAc	1 mM
BSA1B ₄	α -D-Galactose	0.2 M
CTA	α -D-Galactose	0.2 M
ECA	α -D-Galactose	0.2 M
WGA	(GlcNAc) ₂	1 mM
STA	(GlcNAc) ₂	1 mM
DSA	(GlcNAc) ₂	1 mM
AHA	α -D-Galactose	1 mM
MPA	α -D-Galactose	0.2 M
Jac	α -D-Galactose	0.2 M
UEA-1	L-fucose	1 mM
LTA	L-fucose	1 mM
SNA	None	
MAA	None	

α MeMan= α -methyl mannopyranoside.
(GlcNAc)₂=d-N-acetylchitobiose.

calcium chloride. The tissue was then processed as detailed above for the trabeculectomy specimens. To process the melanoma specimens previously embedded in paraffin wax, part of the angle was cut from each block. The tissue was dewaxed in xylene at 60°C for 1 hour, passed through alcohols, and rehydrated into sodium cacodylate buffer pH 7.4. The tissue was then processed and re-embedded in undiluted Araldite as above.

LECTIN HISTOCHEMISTRY

A panel of 20 biotinylated lectins (obtained from Sigma, Poole, Dorset, EY Labs [Bradsure Biologicals Ltd, Leics] or Boehringer Mannheim, East Sussex) was used to identify specific carbohydrate sequences

Table 4 Median ranking scores of lectin binding to 15 glaucomatous trabecular meshwork specimens

Lectin	Schlemm's canal endothelium	Juxtacanalicular tissue	Corneoscleral meshwork	
			EC	C
ConA	3.5	3	3	2
PSA	3.5	3	3	2
LCA	2	2	2	1.5
ePHA	2.5	2	2	2
BSA-1B ₄	3	2	2	2
ECA	3	3	3	2
WGA	3	3	3	3
STA	0.5	0.5	0.5	0.5
DSA	0.5	0.5	0.5	0.5
AHA	1.5	1	1	1
MPA	2	2.3	2	2
Jac	2	2	2	1
SNA	1.5	2	1.5	1.5
MAA	3	2	2	1
	3	3	2	2
	3	3	2	2
	3	3	2	2
	0.5	0	0	0
	0.5	0.1	0.1	0.5
	1.5	1.5	1.5	1.5
	1.5	1.5	1.5	1.5
	4	4	3	3
	4	4	3	3
	3	3	2	2
	1	1.5	1.5	1
	1.5	1.5	1.5	1
	1.5	1	1	1

EC=endothelial cell, C=core of trabecular beam; figures in bold=pretreated with neuraminidase.
Staining: 4=intense, 3=moderately strong; 2=medium, 1=weak, 0=none.

Table 5 Median ranking scores of lectin binding to 12 normal trabecular meshwork specimens

Lectin	Schlemm's canal endothelium	Juxtacanalicular tissue	Corneoscleral meshwork	
			EC	C
ConA	4	4	3.5	3.5
PSA	3.5	3.5	3.5	3.5
LCA	2	2	2	2
ePHA	3.5	2.5	1.5	1
BSA-1B ₄	2.5	2.5	2	2
ECA	4.0	3.5	3.5	2
WGA	3.5	3	3.5	2.5
STA	0.5	0.5	0.5	0.5
DSA	1	1	1	1
AHA	1.5	2	1	1.5
MPA	1.0	1	1.5	1
Jac	1.5	1	1	1
SNA	3.5	3	2.5	2
MAA	3.5	3.5	3.5	2.5
	1.5	1.5	1.5	1
	1.5	1.5	1.5	1
	1.5	1.5	1.5	1
	0	0	0.5	0.5
	1.5	1.5	1.5	1
	0.5	0.5	0.5	0.5
	2	2	2	1
	4	4	3	2.5
	4	4	4	2.5
	3.5	3.5	2	2
	1.5	1.5	1.5	1.5
	1	1	1	1
	1.5	1.5	1.5	1.5

EC=endothelial cell, C=core of trabecular beam; figures in bold=pretreated with neuraminidase.
Staining: 4=intense, 3=moderately strong; 2=medium, 1=weak, 0=none.

(Table 2). The method (detailed in a previous study²²) was carried out as follows. The resin embedded sections (1 μ m thick) were put onto multispot slides (C A Hendley, Essex), precoated with aminoalkyl silane²³ (Sigma) and dried at 37°C overnight. Epoxy resin was removed from each section by etching for 15 minutes with 50% (w/v) saturated sodium ethoxide solution in absolute ethanol. The sections were rehydrated using 100%, 70%, and 40% (v/v) alcohol solutions and finally distilled water. Endogenous peroxidase activity was blocked with 10% (v/v) hydrogen peroxide (diluted from 30% v/v) for 8 minutes. Sections were then trypsinised with 0.1% (w/v) crude trypsin (type II, Sigma) in 0.05 M TRIS-buffered saline (TBS) pH 7.6, containing 0.1% (w/v) calcium chloride, for 5 minutes at 37°C. Biotinylated lectin (Table 2) was then applied at a concentration of 10 μ g/ml in 0.05 M TBS, pH 7.6, containing 1 mM calcium chloride, and the sections were incubated at 37°C for 60 minutes. The sections were given three 5 minute washes in TBS with 1 mM calcium chloride before being incubated in avidin peroxidase (Sigma) at a concentration of 5 μ g/ml in 0.125 M TBS for 60 minutes.²⁴ After three 5 minute washes in TBS, the lectin binding sites were visualised with a 0.05% (w/v) solution of diaminobenzidine hydrochloride (DAB) in TBS containing cobaltous ions (ml/100 ml of 1% (w/v) cobaltous chloride solution).²⁵ Sections were placed in this solution and, after 4 minutes, 0.015% (v/v) hydrogen peroxide was added and staining continued for a further 3 minutes. The sections were washed in running tap water for 2 minutes, dried, and mounted in XAM (Gurr, Speke, Liverpool).

Figure 1 ConA staining of trabecular meshwork (TM) from primary open angle glaucoma (POAG) (a,b) and normal (c) TM. (a) Moderately strong staining of trabecular cells (large arrowheads) and cores of beams of POAG corneoscleral meshwork (CSM). Schlemm's canal (SC) endothelium is present with some apparent disruption (arrowheads). Melanin is visible as dense, particulate deposits (arrows) and can be clearly distinguished from diaminobenzidine hydrochloride deposit which is more diffuse (magnification $\times 536$). (b) ConA binding to a different glaucomatous TM. Staining of trabecular cells in the CSM and juxtacanalicular tissue (JCT) and both inner and outer SC endothelium as well as adjacent sclera is stronger than in (a) (staining intensity is graded 4 compared with 3 in (a)). Discontinuity with possibly some endothelial deficit is visible in SC endothelium (arrowheads) (magnification $\times 536$). (c) SC endothelium and trabecular cells of the normal CSM stain very strongly (large arrowheads) (magnification $\times 536$).

CONTROLS

A negative control, where the lectin was replaced with 0.05 M TBBS, pH 7.6, containing 1 mM calcium chloride, was included with each experimental run. The specificity of lectin binding was confirmed by inhibitory sugars at appropriate concentrations where possible (Table 3). The effect of neuraminidase (see below) was taken as confirmation of lectin specificity for sialyl residues.

Table 6 Group 1 versus group 2 Mann-Whitney U test

Lectin	Median 1	Median 2	p Value
ConA-SC	3.5	4.0	0.18
ConA-CSM	3.0	3.0	0.38
PSA-SC	2.0	3.5	0.002*
PSA-CSM	2.0	2.0	0.87
LCA-SC	2.5	3.5	0.002*
LCA-CSM	2.0	2.0	0.61
ePHA-SC	3.0	3.5	0.04*
ePHA-CSM	3.0	3.5	0.25
ECA-SC	1.0	1.0	0.33
ECA-CSM	1.0	1.5	0.14
WGA-SC	2.0	1.5	0.27
WGA-CSM	2.0	2.0	0.14
1PHA-SC	1.0	0	0.28
1PHA-CSM	1.0	0	0.09
STA-SC	3.0	3.5	0.28
STA-CSM	2.0	2.5	0.71
DSA-SC	3.0	1.5	0.001*
DSA-CSM	3.0	1.5	0.002*
AHA-SC	0	0	0.31
AHA-CSM	0	0	0.96
MPA-SC	1.5	1.0	0.01*
MPA-CSM	1.5	1.0	0.02*
Jac-SC	4	4	0.6
Jac-CSM	3	3	1.0
SNA-SC	3.0	3.5	0.45
SNA-CSM	2	2	0.67
MAA-SC	1.5	1.0	0.04*
MAA-CSM	1.5	1.0	0.01*

*p Value < 0.05 is significant.

NEURAMINIDASE DIGESTION

After etching, blocking of endogenous peroxidase and trypsinisation, some of the sections were incubated in 0.1 units/ml neuraminidase (from *Clostridium perfringens*, type VI, Sigma) in 0.2 M sodium acetate buffer, pH 5.5, containing 1% (w/v) calcium chloride for 1 hour at 37°C. After washing, sections were treated with the selected biotinylated lectins (10 μ g/ml) and avidin peroxidase (5 μ g/ml) and visualised with DAB/cobalt as described above.

STATISTICAL ANALYSIS

Four groups were analysed: group 1 (glaucomatous TM), group 2 (normal TM), group 3 (neuraminidase treated glaucomatous TM), and group 4 (neuraminidase treated normal TM). For each group the staining results with each lectin for SC endothelium (lectin-SC) and for the endothelium of corneoscleral meshwork (lectin-CSM) were included in the analysis. The results for the JCT were excluded as they were very similar to the results for the CSM endothelium. Lectin staining was evaluated by a ranked procedure for a representative section of each specimen, based on a scale of 0–4 where 0=no staining, 1=weak staining, 2=moderate staining, 3=moderately strong staining, and 4=intense staining. Intermediate results were classified as 1.5, 2.5, and 3.5. Staining with some lectins was very weak or possibly zero. For these, only ranks 0 and 1 were used. Grading of staining intensity was evaluated by consensus between two of the authors (SAC and REB). It was not possible to perform the evaluations masked because the source of the tissue was apparent from its morphology.

Groups 1 and 2 and groups 3 and 4 were compared by the Mann-Whitney U test for independent samples where the ranking was spread over the range 0–4. A cross tabulation method for the χ^2 test was used when staining was rated 0 or 1.

Figure 2 PSA staining of trabecular meshwork (TM) from primary open angle glaucoma (a) and normal (b) TM. (a) Variable and intermittent staining of Schlemm's canal (SC) endothelium can be seen (small arrowheads) with weaker staining of the TM (magnification $\times 536$). (b) Continuous strong staining of collector channel (CC) and SC endothelium is evident (arrowheads) (magnification $\times 536$).

Groups 1 and 3 and groups 2 and 4 were compared by the Wilcoxon matched pairs signed ranks test. The McNemara test for related variables, with two values only, was used for analysis where lectin staining was ranked 0 or 1.

To test for correlation between patient age and intensity of lectin staining, Spearman correlation coefficients were determined for groups 1 and 2.

All analyses were performed using the SPSS (SPSS Inc) software package.

Results

LECTIN HISTOCHEMISTRY

The specimens of TM from the POAG patients (group 1) showed a broadly consistent pattern of lectin staining with no obvious differences corresponding to the different earlier drug regimes of the subjects. There were variations in the morphology of SC and TM in the POAG specimens and in five cases SC appeared to be compressed or blocked so that the canal was not patent and the canal

Table 7 Group 1 versus group 3 Wilcoxon matched pairs

Lectin	Median 1	Median 3	p Value
ConA-SC	3.5	3.5	0.75
ConA-CSM	3.0	3.25	0.80
PSA-SC	2.0	3.0	0.30
PSA-CSM	2.0	2.0	0.47
LCA-SC	2.5	3.0	0.90
LCA-CSM	2.0	2.0	0.17
ePHA-SC	3.0	3.0	0.18
ePHA-CSM	3.0	3.0	0.14
ECA-SC	1.5	2.25	0.03*
ECA-CSM	1.0	1.75	0.01*
WGA-SC	1.75	1.5	0.91
WGA-CSM	1.5	1.25	0.39
BSA-SC	0.5	0.5	0.39
BSA-CSM	0.5	0.5	0.39
STA-SC	3.0	3.0	0.04*
STA-CSM	2.5	3.0	0.23
DSA-SC	3.0	3.0	0.23
DSA-CSM	2.75	3.0	0.31
AHA-SC	0.5	0.5	0.75
AHA-CSM	0.5	0.5	0.75
MPA-SC	1.5	1.5	0.50
MPA-CSM	1.5	1.5	0.68
Jac-SC	4.0	4.0	0.36
Jac-CSM	3.5	3.75	0.40
SNA-SC	3.0	1.0	0.01*
SNA-CSM	2.5	1.25	0.01*
MAA-SC	1.5	1.5	0.90
MAA-CSM	1.5	1.5	0.90

*p Value < 0.05 is significant.

endothelium was less clearly defined. In three cases the canal endothelium was incomplete or was apparently absent.

Table 4 gives the median scores of lectin staining of the glaucomatous TM and SC and Table 5 gives the median results of lectin binding to the TM from the normal eyes (discussed in more detail in a previous report²²). The re-embedding of the eight normal eyes from wax into Araldite and the different fixative used did not result in any inconsistencies in the staining pattern compared with that of tissue which was embedded straight into Araldite after glutaraldehyde fixation. The determination of Spearman correlation coefficients showed that there was no significant correlation between lectin staining and age in either group 1 or group 2 with any of the lectins despite the wide age ranges (41–83 years and 32–84 years respectively).

Table 8 Group 2 versus group 4 Wilcoxon matched pairs

Lectin	Median 2	Median 4	p Value
ConA-SC	4.0	3.5	0.28
ConA-CSM	3.25	3.5	1.0
PSA-SC	3.5	2.0	0.02*
PSA-CSM	2.0	1.5	0.04*
LCA-SC	3.75	2.25	0.09
LCA-CSM	2.25	2.25	0.59
ePHA-SC	4.0	3.5	0.46
ePHA-CSM	3.5	3.5	0.75
ECA-SC	1.0	1.5	0.09
ECA-CSM	1.5	1.5	0.04*
WGA-SC	1.0	1.5	0.46
WGA-CSM	1.0	1.0	0.40
STA-SC	3.0	3.0	0.79
STA-CSM	2.0	3.0	0.13
DSA-SC	1.5	1.5	0.18
DSA-CSM	1.5	1.5	0.75
AHA-SC	0	1.5	0.01*
AHA-CSM	0.5	1.5	0.01*
MPA-SC	1.0	1.5	0.03*
MPA-CSM	1.0	1.5	0.03*
Jac-SC	4.0	4.0	0.59
Jac-CSM	3.0	4.0	0.61
SNA-SC	3.5	2.0	0.01*
SNA-CSM	3.0	1.5	0.01*
MAA-SC	1.0	1.0	0.04*
MAA-CSM	1.25	1.25	0.04*

*p Value < 0.05 is significant.

Figure 3 ePHA staining of trabecular meshwork (TM) from primary open angle glaucoma (a) and normal (b) TM. (a) Moderately weak binding to TM and Schlemm's canal (SC) endothelium (magnification $\times 618$). (b) Strong staining of SC endothelium and slightly weaker staining of juxtacanalicular tissue (JCT) and trabecular cells of corneoscleral meshwork (magnification $\times 618$).

ConA binding

ConA bound moderately strongly to SC endothelium, JCT, and CSM (except the cores of beams) of the glaucomatous tissue (group 1), with some variation between cases (Figs 1a and b). Staining of SC endothelium was variable and in some cases there were discontinuities in the pattern of surface staining (although the endothelium was still intact). In normal TM (group 2), ConA staining was strong throughout, particularly in the SC endothelium where staining was continuous (Fig 1c). Statistically there was no difference in staining intensity between glaucomatous (group 1) and normal (group 2) TM (ConA-SC, $p=0.18$, ConA-CSM, $p=0.38$ Table 6), but there was greater variation of staining within group 2.

PSA and LCA binding

The binding of PSA and LCA to the JCT and CSM was less intense than that of ConA in both glaucomatous (group 1) and normal tissue (group 2) and there was variation between the two groups in the staining of SC endothelium. In the glaucomatous tissue

(group 1), some sections showed moderately weak binding to both SC endothelium and the CSM, but in others the staining was moderately strong. There were occasional discontinuities in the staining, either because the endothelium was present but the lectin binding was irregularly weak, or because of deficits in the endothelium (Fig 2a). The SC endothelium of normal tissue (group 2), on the other hand, showed continuous and moderately strong binding of PSA (Fig 2b) and LCA. This stronger staining of SC endothelium in normal tissues was shown to be statistically significant (PSA-SC, $p=0.002$, LCA-SC, $p=0.002$ Table 6). PSA staining of glaucomatous TM (group 1) was not affected by pretreatment with neuraminidase (PSA-SC, $p=0.30$, PSA-CSM, $p=0.47$ Table 7) whereas normal SC endothelium and CSM endothelium (group 2) showed a significant reduction in PSA staining following treatment with this enzyme (PSA-SC, $p=0.02$, PSA-CSM, $p=0.04$ Table 8).

ePHA and 1PHA binding

The pattern and intensity of ePHA staining of glaucomatous TM was similar to that of ConA and again there was more variation in intensity of staining between the group 1 specimens compared with the normal group. Normal TM bound ePHA consistently strongly with more intense staining of the SC endothelium and JCT than the CSM (Figs 3a, 3b) and group 2 ePHA binding to the SC endothelium was found to be significantly greater than that in glaucomatous TM (ePHA-SC, $p=0.04$ Table 6). 1PHA did not bind to the TM of either group.

WGA, STA, and DSA binding

The staining patterns of WGA and STA (which have overlapping binding requirements) showed differences in affinity and localisation from each other but not between groups 1 and 2. In both groups WGA bound weakly to all parts of the TM whereas STA stained with greater intensity on the SC endothelium than the JCT and CSM. DSA staining, on the other hand, was statistically greater in glaucomatous (group 1) than in normal TM (DSA-SC, $p=0.001$, DSA-CSM, $p=0.002$ Table 6).

ECA binding

ECA bound weakly throughout the TM in both groups. Pretreatment with neuraminidase significantly increased the staining with ECA in both series (Figs 4a, 4b) (ECA-SC, $p=0.03$, ECA-CSM, $p=0.01$ Table 7; ECA-CSM, $p=0.04$ Table 8).

SNA and MAA binding

The sialic acid specific lectins SNA and MAA showed different binding patterns from each other. SNA bound strongly to the inner and outer walls of the SC, the JCT, and to a much

Figure 4 The effect of pretreatment with neuraminidase on ECA staining (a,b) and SNA staining (c,d) of primary open angle glaucoma trabecular meshwork (TM). (a) Despite damage to the tissue, there is weak staining throughout the TM. Narrowed lumen of Schlemm's canal (SC) is visible (magnification $\times 588$). (b) Pretreatment with neuraminidase increases ECA staining throughout the section which includes TM and sclera (S) (magnification $\times 588$). (c) Strong staining of SC endothelium with some discontinuity where a cell body can be seen (arrowheads). Other deficits in the SC endothelium are more likely to be a result of trauma (large arrowheads) (magnification $\times 588$). (d) Pretreatment with neuraminidase reduces SNA staining (magnification $\times 588$).

Figure 5 Jac staining of trabecular meshwork (TM) from primary open angle glaucoma (a) and normal (b) TM. (a) Schlemm's canal (SC) endothelium shows quite strong but intermittent staining (small arrowheads). Trabecular cells show variable staining with weaker binding to the cores of beams (large arrowheads) (magnification $\times 522$). (b) Strong but intermittent staining of SC endothelium (small arrowheads) and continuous staining of cells of juxtacanalicular tissue and corneoscleral meshwork (large arrowheads) (magnification $\times 522$).

lesser extent, the trabecular cells of the CSM in both groups. There were some discontinuities in the trabeculectomy tissue and the staining of the SC appeared slightly weaker than in normal meshwork but this was not statistically significant. Staining with SNA in both groups (Figs 4c, 4d) was reduced significantly, though not completely, after pretreatment with neuraminidase (glaucomatous TM SNA-SC, $p=0.01$, SNA-CSM, $p=0.01$ Table 7; normal TM SNA-SC, $p=0.01$, SNA-CSM, $p=0.01$ Table 8). MAA, on the other hand, bound weakly throughout the TM of both groups of specimens. Binding was significantly increased in normal TM following treatment with neuraminidase (MAA-SC, $p=0.04$, MAA-CSM, $p=0.04$ Table 8) but was unaffected in glaucomatous TM.

Jac, MPA, AHA, and BSA-1B₄ binding

Jac bound strongly throughout the outflow pathway in both groups and with greatest intensity in the JCT and SC endothelium where, in both the glaucomatous (group 1) and normal tissue (group 2), staining was sometimes irregular (Figs 5a, 5b). MPA bound weakly in both groups but statistically more avidly in Group 1 (MPA-SC, $p=0.01$, MPA-CSM, $p=0.02$ Table 6). However, although MPA and AHA have a similar specificity, there was negligible binding with AHA. Pretreatment with neuraminidase did not affect MPA binding to glaucomatous TM but there was a significant increase in binding to normal TM (MPA-SC, $p=0.03$, MPA-CSM, $p=0.03$ Table 8). AHA binding to normal TM also significantly increased after neuraminidase

Table 9 Group 3 versus group 4 Mann-Whitney U test

Lectin	Median 3	Median 4	p Value
ConA-SC	3.5	3.5	0.93
ConA-CSM	3.25	3.5	0.54
PSA-SC	3.0	2.0	0.07
SA-CSM	2.0	1.5	0.05
LCA-SC	3.0	2.25	0.90
LCA-CSM	2.5	2.25	0.96
ePHA-SC	3.0	3.5	0.46
ePHA-CSM	3.0	3.0	0.52
ECA-SC	2.25	1.5	0.06
ECA-CSM	1.75	1.5	0.35
WGA-SC	1.5	1.5	0.33
WGA-CSM	1.25	1.0	0.73
BSA-SC	0.5	1.0	0.01*
BSA-CSM	0.5	1.0	0.02*
STA-SC	3.0	3.0	0.82
STA-CSM	3.0	3.0	0.72
1PHA-SC	0.25	0	0.63
1PHA-CSM	0.25	0	0.63
AHA-SC	0.5	1.5	0.01*
AHA-CSM	0.5	1.5	0.01*
MPA-SC	1.5	1.5	0.79
MPA-CSM	1.5	1.5	0.82
Jac-SC	4.0	4.0	0.64
Jac-CSM	3.75	4.0	0.93
SNA-SC	1.0	2.0	0.09
SNA-CSM	1.25	1.5	0.5
MAA-SC	1.5	1.25	0.61
MAA-CSM	1.5	1.25	0.61

*p Value<0.05 is significant.

(AHA-SC, $p=0.01$, AHA-CSM, $p=0.01$ Table 8) while binding to glaucomatous TM was unaffected. The resultant AHA binding to normal TM, following this enzyme digestion, was significantly greater than for glaucomatous TM (AHA-SC, $p=0.01$, AHA-CSM, $p=0.01$ Table 9). Staining with BSA-1B₄ was very weak throughout the meshwork in both groups following pretreatment with neuraminidase.

Other lectins

SBA, DBA, CTA, UEA-1, and LTA showed negligible staining in all parts of the TM in both groups. The McNemar test for related groups where results have only two values showed no significant test results for lectins SBA, DBA, CTA, UEA-1, and LTA.

Discussion

Trabeculectomy tissue is one of the few sources of material readily available for observing the pathological effects of POAG and has been used for morphological^{5,6,49} and quantitative studies of meshwork cellularity⁸ and collagen content.⁵⁰ Limitations of trabeculectomy specimens include the small size of the tissue blocks and the potential for distortion of the specimen during the operative procedure. These considerations were taken into account in evaluating this comparative lectin histochemical study of glaucomatous and normal TM and only cases where there was an adequate amount of TM and SC were selected. In some of the trabeculectomy specimens the SC endothelium could not be distinguished clearly because the canal was blocked and, occasionally, the canal endothelium appeared to be missing. This feature of POAG has been reported in earlier studies of trabeculectomy tissue.^{49,51}

The moderately strong binding of ConA and ePHA to SC endothelium and JCT in both groups indicates that high mannose *N*-linked

glycans (both complex and intermediate) are expressed by the TM of both glaucomatous and normal tissue. The specificities of ConA and ePHA also show that these glycans may be of the bisected or non-bisected subsets and are probably biantennate. More highly branched non-bisected glycans, such as tri/tetra antennate, are not present as there is no reaction with 1PHA. The diminished binding of ePHA to glaucomatous SC endothelium compared with normal TM suggests a reduction in the expression of bisected glycans in the SC endothelium. PSA and LCA also show reduced binding to the SC endothelium of the glaucomatous TM compared with normal tissue which suggests a reduction in some *N*-linked glycans in this area.

A preliminary report of lectin binding to human TM, using SDS/polyacrylamide gel electrophoresis and lectin binding to western blots,²⁰ concluded that ConA, 1PHA, and UEA-1 bound more strongly to normal TM than that from POAG subjects. Our findings with respect to ConA show that there is a greater variation in binding in glaucomatous eyes compared with normals but there was no statistically significant difference in median ranking for intensity of staining and hence glycan expression. We found negligible or no binding with both 1PHA and UEA-1. UEA-1 binds to L-fucose, a relatively hydrophobic residue, and the discrepancy (compared with the previous report²⁰) may reflect the presence of glycolipid. This glycoconjugate is not affected by the biochemical method which uses unfixed, unprocessed tissue but is removed during processing for lectin histochemistry. However, a lectin histochemical study of cultured TM cells (from cynomolgus monkey and bovine eyes) also found no visible staining with UEA-1⁵² but positive staining with ConA, AHA, WGA, and PHA (erythroagglutinin and leucoagglutinin combined). The electrophoretic separation of TM components would have used much larger quantities of material than were analysed here, possibly rendering low levels of glycan associated with a particular glycoprotein detectable on lectin blotting.

The binding of STA to both trabeculectomy and normal tissue suggests that trabecular cells express β 1,4GlcNAc oligomers, or glycan residues in the form of repeated *N*-acetylglucosamine groups (β 1,4GlcNAc-). WGA, DSA, and STA possess similar, but not identical, specificities and the variation in binding between the lectins, in both groups, suggests that different glycans may be involved, particularly as DSA shows more intense staining with glaucomatous than normal TM. DSA has a similar specificity to STA but a greater affinity for multiple *N*-acetylglucosamine groups and can bind to *O*-linked glycans. The weak staining with ECA, which was increased by neuraminidase pretreatment, indicates the presence of subterminal *N*-acetylglucosamine groups with some terminal sialylation. This particular grouping, with α (2,6) linked sialic acid, is known to occur in fibronectin⁵³ which has been shown to be present on SC endothelium in aged human eyes⁵⁴ and in the

subendothelial region of the trabecular beams in normal and trabeculectomy tissue.⁵⁵

Glycans which are O-linked to serine or threonine of the protein chain are also present throughout the TM of trabeculectomy and normal tissue as shown by the strong binding of Jac (specific for Gal β (1,3)GalNAc- and Gal α (1,6)). MPA (which has similar but not identical specificities) shows statistically stronger staining in glaucomatous TM compared with normal TM. This lesser intensity of staining in normal TM may result from masking by sialic acid groups as neuraminidase pretreatment causes increased MPA binding, whereas there is no statistical difference following desialylation of glaucomatous TM. A biochemical study of trabecular cell surface glycoconjugates in the cynomolgus monkey eye concluded that all accessible terminal galactose/acetyl galactosamine groups were sialylated.⁵⁶

The trabeculectomy specimens used in this study were from subjects where various combinations of drugs including pilocarpine, Timoptol (timolol), Ganda (guanethidine monosulphate), and Diamox (acetazolamide) prescribed over varying periods of time, had failed to normalise the intraocular pressure. The effect of medication on the morphology and biochemistry of the TM is an important question to be addressed as it is not known what, if any, influence drug therapy might have on the expression of glycan residues present in the TM. The number of patients in this study is insufficient to draw any conclusions about the effect of medication, but a previous comparative study of trabeculectomy specimens for treated and untreated subjects with POAG concluded, following qualitative and quantitative analysis, that medication produces no visible effect on SC and JCT morphology.³

We have demonstrated that, from the overall similarity in their lectin binding patterns, there are no major differences in the types of glycans expressed in glaucomatous and normal TM. However, differences were observed in the intensity of staining of certain lectins, suggesting alterations in concentration of some of the glycans, which could reflect either an alteration in glycoprotein levels or glycan content. Thus high mannose, intermediate and complex, non-bisected and bisected N-linked glycans (which are not highly branched, but probably biantennary) are expressed in glaucomatous TM, particularly on SC endothelium, to a lesser degree than in normal tissue. Glycans showing an increased expression in glaucomatous TM compared with normal TM are (a) O-linked glycans with terminal and subterminal galactose, with concomitantly less sialylation, and/or (b) groups with multiple N-acetylglucosamine residues which may be N- or O-linked to the polypeptide backbone.

It has been suggested that the pathology of POAG is very similar to exaggerated aging changes in normal TM.⁵⁷ However, we found no correlation between age and glycan expression in either normal or glaucomatous TM. We conclude that the observed changes in glycan expression are relevant to the pathogenesis

of POAG. Identification of the glycoproteins to which these glycans are linked should provide some insight into the pathological processes which constitute POAG.

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