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Lectin-binding sites in the anterior segment of the human eye

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Abstract. The anterior segment of the human eye was screened for differences in the lectin-binding patterns of Con A, PNA, GS-I, WGA, SBA, DBA, and UEA-I to enable cell typing for cell-culture purposes. An immunohistochemical technique combining an indirect antibody-lectin method with the avidin-biotin system was used. Con A and WGA were bound by all cells except the conjunctival goblet cells. UEA-I was exclusively bound by both vascular endothelial cells and some corneal and conjunctival epithelial cells. The binding of GS-I, PNA, SBA, and DBA showed an uneven pattern and differed among the cases investigated. The reasons for these differences are not clear. Our results indicate that the usefulness of lectins for cell-typing purposes is restricted and must be determined for every case.

Introduction

Cell-surface glycoconjugates are involved in cell-to-cell interactions such as differentiation, regulation, and tissue integrity. The lectins bind selectively to such carbohydrate moieties. The pattern of lectin-binding sites shows differences not only between the same organs of different species, but also within the organs of the same individual. For some of these differences, a correspondence to various stages of cellular differentiation, neoplastic alteration, and organ development could be shown [2, 3, 11, 12].

In the anterior part of the human eye, tissues of different embryologic origin are involved in a variety of functions. In this study we wanted to determine whether there is correspondence between the origin or function of cells and their ability to bind different lectins. Insights into the lectin-binding pattern could be of interest for cell typing of tissues and for tissue culture.

Lectins have previously been used in eye research. The number of studies using lectin binding to investigate the retinal pigment epithelium and photoreceptors in the posterior part of the eye has steadily increased [4, 7, 9, 13–15, 19]. In the anterior part of the eye, however, fewer studies focusing on special tissues such as cornea [5, 20], conjunctiva [8, 21, 24, 25], aqueous outflow pathways [23], and lens [22] have been done. The systematic investigation of Ahmed and Rahi [1], covering the whole eyeball with adjacent tissues, determined the distribution of only one lectin. We therefore decided to map the distribution pattern of a panel of lectins within the anterior part of the human eye, covering most of the main sugar-specific groups. For high sensitivity and good morphology, we combined an indirect antibody-lectin method with the avidin-biotin system on paraffin sections.

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Materials and methods

Materials

The studies were performed on five human autopsy eyes, as shown in Table 1. The enucleated eye bulbs were divided at their equator and fixed in the paraformaldehyde-lysine-periodate solution introduced by McLean and Nakane [16] for 4 h at 4° C. Next, the anterior parts of the bulbs were cut longitudinally into pieces 3–5 mm in width, containing the cornea and sclera with parts of the conjuctiva, the chamber angle, the iris, and the ciliary body up to the peripheral retina, and were then embedded in paraffin following common schedules.

Table 1. Human autopsy eyes used for the study

Donor number	Sex	Age	Enucleated within
H-505	Man	65 years	17 h post mortem
H-027	Man	59 years	8 h post mortem
H-119	Man	80 years	8 h post mortem
H-044	Woman	81 years	10 h post mortem
H-116	Woman	77 years	4 h post mortem

Table 2. Lectins used for the study

Plant source	Common name	Abbreviation
Canavalia ensiformis	Jackbean	Con A
Triticum vulgaris	Wheat germ	WGA
Griffonia simplicifolia	–	GS-I
Glycine max	Soybean	SBA
Arachis hypogaea	Peanut	PNA
Dolichos biflorus	Horse gram	DBA
Ulex europaeus I	Gorse	UEA-I

Immunohistochemistry

An indirect antibody-lectin method was used as suggested by Leathem and Atkins [10] for seven different lectins (Table 2). The immunohistochemical staining was carried out on 5-μm sagittal sections mounted on slides coated with 10% glue (Pritt, Henkel). The sections were deparaffinized in up to 0.15 *M* phosphate-buffered saline (PBS) pH 7.2. The sections were first pretreated for 30 min with 3% H_2O_2 freshly diluted from a 30% H_2O_2 stock solution (Merck) and then for 5 min with 1% pronase E from *Streptomyces griseus* (Sigma type XIV). Next, they were incubated with the lectins, diluted 1:100 (Con A/GS-I/DBA) or 1:200 (WGA/SBA/PNA/UEA-I), for 30 min. Afterwards, overnight incubation with antibodies against the respective lectins, raised in rabbits and diluted 1:200 (anti-Con A/GS-I/DBA) or 1:400 (anti-WGA/SBA/PNA/UEA-I), was performed. All lectins and anti-lectins were obtained from Dakopatts except GS-I and anti-GS-I, which were supplied by Medac. Thereafter, biotinylated antibodies against rabbit IgG, raised in mice and diluted 1:220, followed by a complex of avidin and biotinylated horseradish peroxidase, diluted 1:110 (both Vectastain ABC-Kit, Camon), were applied for 30 min each. The peroxidase was visualized using aminoethylcarbazole (Sigma) as described by Graham et al. [6]. All steps were carried out at room temperature using a moist chamber. Between the different steps the sections were thoroughly washed with PBS, which was also used for all dilutions mentioned. Finally, the sections were rinsed in distilled water, counterstained with Mayer's hemalum, and mounted in Kaiser's glycerine jelly (both from Merck).

Controls

The controls included (a) the use of lectins preabsorbed for 30 min with a 0.1 *M* solution of their respective major specific sugars (all from Sigma), as shown in Table 4; (b) the replacement of the specific anti-lectin antibodies by non-immunized horse serum (Vectastain ABC-Kit, Camon); and (c) staining of sections without lectin incubation for detection of the possible existence of endogenous lectins in the tissues.

Results

All lectins bound to conjunctival and corneal epithelial cells, with some differences in binding intensity and location within the cellular layers. Whereas Con A and WGA bound throughout all epithelial layers, binding of SBA, DBA, and UEA-I took place only at the superficial and intermediate cells and GS-I was bound by the superficial cells only. PNA was outstanding in that it bound only to a few scattered cells within the basal and intermediate epithelial layers. All goblet cells of the conjunctival epithelium reacted strongly with GS-I, SBA, and PNA; some of them reacted weakly with DBA as well, but none showed a response to Con A, WGA, or UEA-I (Table 3).

Con A and WGA were bound by the endothelial cells of the cornea, trabecular beams, and Schlemm's canal

Table 3. Lectin-binding sites in the anterior human eye

Anatomical site	Lectin						
	Con A	WGA	GS-I	SBA	PNA	UEA-I	DBA
Epithelial cells of conjunctiva and cornea:							
Goblet cells	–	–	+++	+++	+++	–	–
Superficial cells	+	+++	+++	++	–	+++	+
Wing cells	+	+++	–	+	+++[2]	+++	+
Basal cells	++	+	–	–	+++[2]	–	–
Endothelial cells of:							
Cornea	+++	+++	++(2)	–	++(1)	–	–
Trabecular beams and Schlemm's canal	+++	+++	–	–	++(1)	–	–
Nonpigmented ciliary epithelium	+++	+++	++	++	+++	–	++
Ciliary muscle	+++	+	+(2)	–	–	–	+(2)
Iris muscles	+++	+	+(2)	–	+	–	+(2)
Endothelial cells of the vessels	+++	+++	+++(3)	+++(2)	+++(2)	+++	–
Smooth muscle cells of the vessel media	+++	+	–	–	–	–	–
Fibroblasts	++	++	–	–	–	–	–
Free cells	++	++	+++	–	–	–	–

Binding of [2] few scattered cells only

Numbers in parentheses represent the number of cases among the total of 5; –, no binding; +, weak binding; ++, moderate binding; +++, strong binding

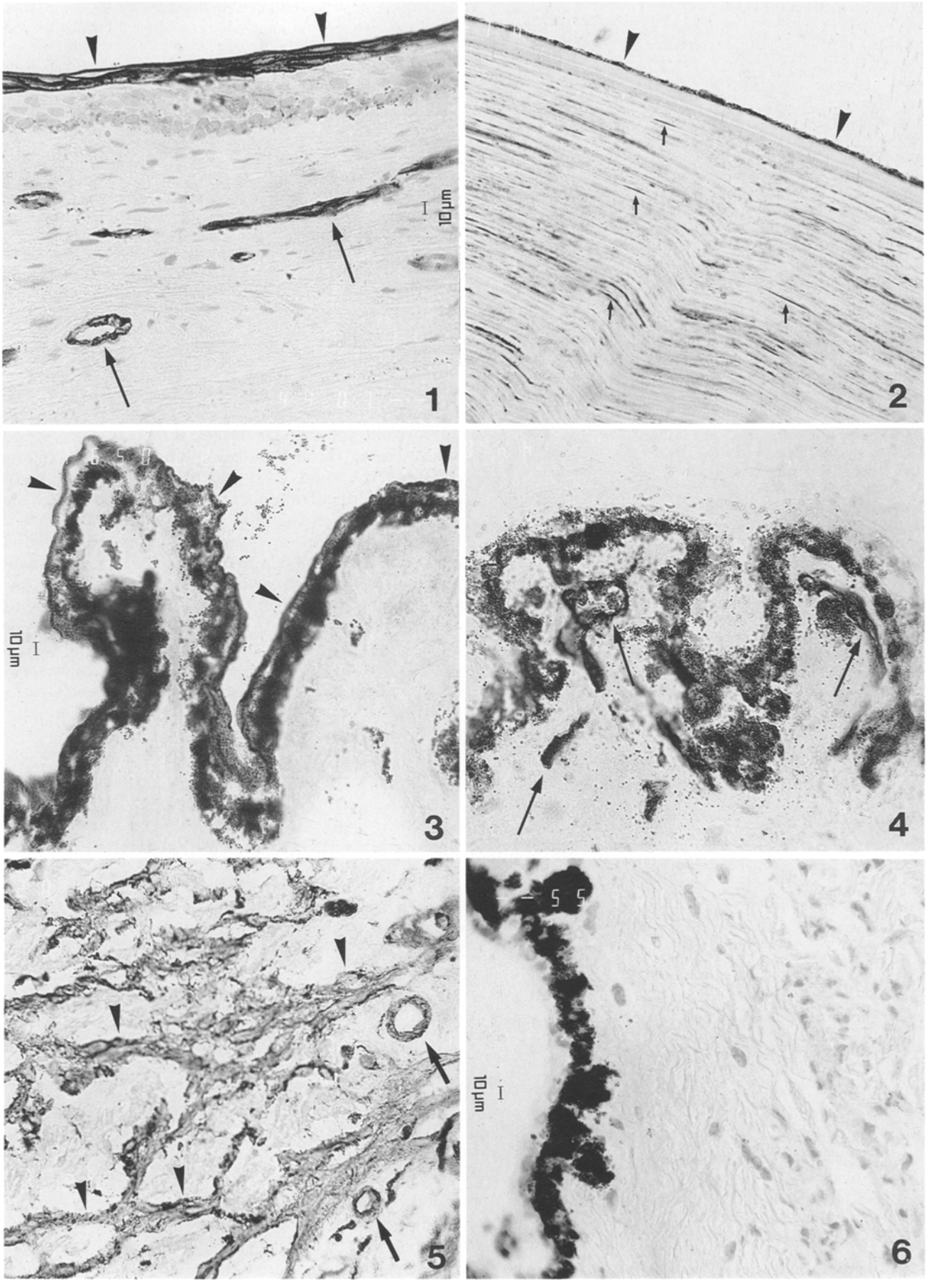
10µm
1
2
10µm
3
4
10µm
5
10µm
6

Table 4. The major and minor sugar[a] and blood-group specificities of the lectins used (adapted from [11])

Specificity	Con A	WGA	GS-I	SBA	PNA	UEA-I	DBA
Mannose	x						
Glucose	(x)						
N-Acetylglucosamine	(x)	x					
Galactose			x	(x)	x		
N-Acetylgalactosamine			(x)	x	(x)		x
L-Fucose						x	
N-Acetylneuraminic acid		(x)					
Blood groups			B		T	O/H	$A_{1/2}$

[a] All sugars are in the D-form unless otherwise noted
x, major sugar-binding affinity; (x), minor sugar-binding affinity

in all eyes, whereas PNA was bound only in case H-116. GS-I was bound by the corneal endothelium in cases H-116 and H-027. Binding of SBA, DBA, and UEA-I was not observed (Table 3). In all eyes, Con A, WGA, and UEA-I were bound strongly by the vascular endothelial cells. In addition, GS-I was bound in cases H-119 and H-116; SBA, in cases H-119 and H-027; and PNA, in case H-119. No binding of DBA to vascular endothelial cells could be observed. In case H-044 regional differences occurred: GS-I and PNA were bound only by the endothelium of the episcleral vessels (Table 3).

The muscle cells of the vessel media and all stromal cells bound to Con A and WGA, with the free cells also binding to GS-I. SBA, PNA, DBA, and UEA-I were bound neither by the muscle cells of the vessel media nor by the stromal cells (Table 3). The ciliary muscle and the iris muscles bound strongly to Con A and weakly to WGA. In addition, GS-I and DBA were bound in cases H-116 and H-027. PNA was bound only by the iris muscles. Neither SBA nor UEA-I were bound by ciliary or iritic muscle cells (Table 3). The nonpigmented layer of the ciliary epithelium bound to all lectins except UEA-I. Con A, WGA, and PNA were bound more strongly than GS-I, SBA, or DBA (Figs. 1–6).

Fig. 1. Light micrograph of GS-I binding in the conjunctiva bulbi (case H-044; ×250). In the conjunctival epithelium, the superficial cell layer (*arrowheads*) is stained, whereas the intermediate and basal-cell layers show no binding. The endothelial cells of the limbus vessels (*arrows*) stain in this case as well. **Fig. 2.** Light micrograph of Con A binding in the inner cornea (case H-044; ×250). The corneal endothelium (*arrowheads*) and the keratocytes (*arrows*) are stained. **Fig. 3.** Light micrograph of PNA binding in the ciliary processes (case H-027; ×250). Staining can be seen at the unpigmented ciliary epithelium (*arrowheads*). Note that no staining of ciliary capillaries is present. **Fig. 4.** Light micrograph of UEA-I binding in the ciliary processes (case H-044; ×250). The endothelium of the ciliary capillaries (*arrows*) is stained, whereas the unpigmented ciliary epithelium remains unstained. **Fig. 5.** Light micrograph of WGA binding in the ciliary muscle (case H-505; ×250). The ciliary muscle cells (*arrowheads*) and the ciliary vessels (*arrows*) are stained. **Fig. 6.** Light micrograph of the inner ciliary body (case H-116; ×250) of a control section. SBA preabsorbed with galactose and *N*-acetylgalactosamine was used. Note the absence of staining of all cells within the different tissues

Controls

All sections in which the lectins were blocked by their appropriate sugar before use, those in which the specific antibody against the lectin was replaced by nonimmunized horse serum, and those in which the lectin was omitted showed no staining indicating lectin-binding sites.

Discussion

With the seven lectins we covered five of the six main sugar-specific groups (Table 4). The group of lectins binding mainly or exclusively to *N*-acetylneuraminic acid (sialic acid) was not included, as we could not obtain respective anti-lectins and did not want to use directly conjugated lectins for the following reason: for UEA-I, Con A, DBA, and PNA we compared the sensitivity of methods using either directly biotinylated lectins (Medac) or unlabelled lectins followed by biotinylated anti-lectins. In agreement with the methodical statements of Leathem and Atkins [10], we found the labelled lectin method to be far less sensitive than the unlabelled lectin-antibody technique (data not shown). This should be kept in mind when results are compared that have been obtained by different methods.

Generally our results are in agreement with those of other study groups. Two groups [20, 25] found no binding of GS-I, SBA, UEA-I, or PNA in tissues in which we found binding sites [19, 22]; however, the results of these investigators were obtained using less sensitive methods. One group [1] reported binding of SBA by the corneal and trabecular endothelia; we can offer no obvious explanation for the negative results we obtained for such binding. Different findings for the binding patterns of WGA, UEA-I, DBA, and Con A were reported in conjunctival epithelium obtained at surgery [8, 21, 24]; this could indicate postmortem changes in our specimen. GS-I, PNA, UEA-I, and DBA are known to possess blood-group specificities (Table 4), which could explain some of these discrepancies.

In the present study we hoped to find either markers for different cell types or a correlation between the lectin-binding pattern and the embryological origin of the tissues. In general, our investigations of embryological

origin were disappointing. None of the lectins studied bound continuously to cells of either mesenchymal, ectodermal, or neuroectodermal origin (Table 3). On the other hand, our results could be useful for preliminary identification of different cell types under pathological or tissue-culture conditions.

We demonstrated an uneven distribution of lectin-binding sites within the cell layers of the conjunctival and corneal epithelia (Table 3). Similar differences have been found for the cell layers of the skin [17]. A recent investigation showed a change in lectin-binding patterns during wound repair in the skin [18]. It may therefore be of diagnostic interest if lectin-binding patterns correlate with different degrees of epithelial cell lesions and stages of epithelial wound repair in the conjunctiva and cornea as well. UEA-I is a known marker for endothelial cells [11]; in the inner eye, it was indeed bound only by endothelial cells during the present study (Table 3). As most organ cultures or tissue explants contain vessel components, UEA-I binding could be used for identification of growing endothelial cells among other cells in histological sections. GS-I, SBA, PNA, and DBA were bound differently by the endothelial cells, fibroblasts, and smooth-muscle cells (Table 3). These lectin-binding patterns, however, showed individual variations that require the determination of individual binding patterns in control sections before these lectins can be used for cell typing.

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