Virchows Arch A (1987) 410:471–479

Virchows Archiv A
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Glycoconjugates in retinoblastoma

A lectin histochemical study of ten formalin-fixed and paraffin-embedded tumours*

Tero Kivelä

Department of Ophthalmology, Helsinki University Central Hospital, Helsinki, Finland

Summary. The binding of eleven biotin- or peroxidase-coupled lectins with different carbohydrate specificities to tumour tissue and remaining morphologically normal retina was studied in ten formalin-fixed and paraffin-embedded human eyes with retinoblastoma. In undetached retinas, outer and inner segments of photoreceptors bound concanavalin A (ConA) as well as *Lens culinaris* (LCA), wheat germ (WGA) *Ricinus communis* (RCAI) and peanut (PNA) agglutinins. Both nuclear and plexiform layers bound ConA, LCA and, in some specimens, WGA and RCAI. These results agree with those obtained with normal adult human retina, the main difference being that PNA labelled some rods in addition to cones in the retinoblastoma eyes. Flexner-Wintersteiner rosettes reacted with ConA and LCA, and often with WGA, PNA and RCAI. Undifferentiated retinoblastoma cells always bound ConA and LCA, and in some tumours WGA, PNA and RCAI. Pretreatment with neuraminidase increased the number of cells that bound PNA and RCAI, but diminished binding of WGA. Pokeweed mitogen and *Bandeiraea simplicifolia I, Dolichos biflorus*, soybean, *Ulex europaeus I* and *Lotus tetragonolobus* agglutinins labelled only vascular endothelial cells. Retinoblastoma cells most closely resembled photoreceptor cells in their lectin-binding patterns.

Key words: Retinoblastoma – Photoreceptor cells – Glycoconjugates – Lectin histochemistry – Neuraminidase

Retinoblastoma is the most common intraocular malignancy occurring during childhood. Although generally considered a neuroectodermal tumour of the retina, its precise origin continues to be a matter of dispute (Tso 1980). It has a remarkable tendency to differentiate into cell types that resemble normal photoreceptor cells both morphologically (Tso 1980) and immunologically (Bridges et al. 1985; Donoso et al. 1985, Donoso et al. 1986; Mirshahi et al. 1986). Although retinoblastoma cells may share neuronal and glial antigens in culture (Kyritsis et al. 1984; Jiang et al. 1984), glial differentiation is extremely rare in surgical human specimens (Tso 1980; Lane and Klintworth 1984; Terenghi et al. 1984; Molnar et al. 1984; Messmer et al. 1985; Kivelä et al. 1986). Evidence on differentiation into other neuronal cell types than photoreceptors is also very scarce (Tarkkanen et al. 1983).

Lectins, which are proteins found both in plants and animals, share the property of binding to specific saccharide moieties (Goldstein and Hayes 1978). They can be used to localize glycoconjugates in routinely processed histological specimens (Leathem and Atkins 1983; Virtanen et al. 1986). In particular, lectins have been used to detect changes in cell surface glycoproteins during malignant change (Raedler and Raedler 1985). In normal retina, many lectin conjugates label photoreceptor cells very strongly (Bridges and Fong 1980; Bee 1982; Blanks and Johnson 1983; Uehara et al. 1983, Uehara et al. 1985). It has also been reported that cultured retinoblastoma cells bind several lectins (Felberg et al. 1985). Prompted by these findings, the present study was undertaken to determine the binding patterns of eleven commonly used lectins to different retinoblastoma types in order to shed more light on the relationship between retinoblastoma cells and normal photoreceptors.

Material and methods

Histological specimens. During the years 1962–1985 a total of 66 retinoblastoma specimens from 57 patients have been examined in the Ophthalmic Pathology Laboratory, Department of

* This study was supported by grants from the Emil Aaltonen Foundation and Rasmussens Stiftelse

Offprint requests to: T. Kivelä, Melkonkatu 3B 29, SF-00210 Helsinki, Finland

Table 1. Clinicopathological characteristics of the retinoblastomata studied

Case	Type	Rosettes	Size	Necrosis	Patient	
					Blood group	Age
1.	Unilateral	Absent	Medium	Extensive	A/Rh+	1 y 10 mo
2.	Unilateral	Few	Large	Moderate	B/Rh+	3 y 6 mo
3.	Unilateral	Moderate	Small	Moderate	A/Rh+	1 y 7 mo
4.	Unilateral	Many	Large	Extensive	B/Rh+	1 y 1 mo
5.	Unilateral	Many[a]	Small	Minimal	A/Rh+	1 y 7 mo
6.	Bilateral	Absent	Medium	Extensive	A/Rh+	8 y 3 mo
7.	Bilateral	Absent	Small	Minimal	A/Rh+	8 mo
8.	Bilateral	Many	Medium	Extensive	O/Rh+	8 mo
9.	Bilateral	Many	Large	Moderate	AB/Rh+	7 mo
10.	Bilateral	Many	Medium	Moderate	A/Rh+	4 y 7 mo

[a] Contained many incomplete rosettes and fleurettes

Table 2. Characteristics of the eleven lectins studied

Plant of origin		Abbreviation	Lot	Sugar specificity[a]	
Botanical name	Common name			Nominal specificity[b]	Inhibitor[b]
Arachis hypogaea	Peanut	PNA	14F-8105-1	β-D-Gal(1→3)-D-GalNAc	D-Gal
Bandeiraea simplicifolia	–	BSAI	34F-9685-1	α-D-Gal > α-D-GalNAc	α-Met-D-Gal
Canavalia ensiformis	Jack bean	ConA	62F-3934	α-D-Man > α-D-Glc	α-Met-D-Man
Dolichos biflorus	Horse gram	DBA	103F-9615-1	α-D-GalNAc	D-GalNAc
Glycine max	Soybean	SBA	124F-9510	α-D-GalNAc > β-D-GalNAc	D-GalNAc
Lens culinaris	Lentil	LCA	103F-8105	α-D-Man > α-D-Glc	α-Met-D-Man
Lotus tetragonolobus	Asparagus pea	LTA	24F-9645-1	α-L-Fuc	α-L-Fuc
Phytolacca americana	Pokeweed	PWM	65F-9580	$(\beta$-D-GlcNAc$)_n$	D-GlcNAc
Ricinus communis	Castor bean	RCAI	34F-4028	β-D-Gal > α-D-Gal	α-lactose
Triticum vulgaris	Wheat germ	WGA	45F-9615	$(\beta$-D-GlcNAc$)_n$/NeuNAc	D-GlcNAc
Ulex europaeus	Gorse	UEAI	24F-9505-1	α-L-Fuc	α-L-Fuc

[a] Goldstein and Hayes (1978)
[b] Glc glucose, Man mannose, Gal galactose, Fuc fucose, GlcNAc N-acetylglucosamine, GalNAc N-acetylgalactosamine, NeuNAc N-acetylneuraminic acid, and Met methyl derivative of sugar

Ophthalmology, Helsinki University Central Hospital. Ten formalin-fixed and paraffin-embedded eyes containing an intraocular retinoblastoma were selected from the fifty paraffin blocks that remained for study (Table 1). Due to the great number of sections needed, this selection was necessary to conserve material for future studies. The selection was made so that all common retinoblastoma types were included among the tumours studied. The case histories were reviewed to determine whether preoperative retinal detachment had been present and to ascertain that any preoperative radiation therapy or other treatment had not been given. The blood groups of all patients could be retrieved from the case histories (Table 1). Sections (5 μm thick) were cut from the specimens and mounted on chromium-gelatin-treated glass slides to ensure adherence (0.05 g potassium chromium(III)sulphate dodecahydrate and 0.5 g gelatin in 100 ml distilled water).

Lectin histochemistry. The sections were routinely deparaffinized in xylene and hydrated in an ethanol series. In addition, duplicate series were treated either with pepsin to enhance the availability of lectin binding sites in formalin-fixed and paraffin-embedded material (Leathem and Atkins 1983; Virtanen et al. 1986), or with neuraminidase to expose penultimate carbohydrate residues blocked by sialic acid (Uehara et al. 1985).

For pepsin treatment, the sections were washed thrice in phosphate-buffered saline (PBS, pH 7.4), and then treated with 0.4% pepsin (Merck, Darmstadt, FRG) in 0.01 N hydrochloric acid at 37° C for 20 min. A new batch of pepsin was used, and the optimal treatment time was determined by preliminary stainings.

For neuraminidase treatment, other sections were washed in an acetate buffer (35.2 ml of 0.2 M sodium acetate, 14.8 ml of 0.2 M acetic acid and 150 ml of distilled water, final pH 5.0). Neuraminidase (EC 3.2.1.18) from *Clostridium perfringens* (Type V, Lot 63F-8172, Sigma, St. Louis, MO, USA) was used at a concentration of 0.5 U/ml diluted in the acetate buffer containing 2% bovine serum albumin (BSA; Merck, FRG). The specimens were briefly covered with the same diluent, which was then blotted off, and incubation with neuraminidase carried out in a moist chamber at 37° C for 30 min. After either enzymatic treatment, the sections were washed three times in PBS.

Endogenous peroxidase activity was destroyed by treatment for 30 min in 200 ml of methanol, containing 3.2 ml of

Table 3. Binding of the lectins to morphologically normal retina and tumour tissue in the retinoblastoma eyes studied

	Lectin[a]										
	BSAI	ConA	DBA	LCA	LTA[b]	PNA	PWM	RCAI	SBA	UEAI	WGA
Undetached retina											
Photoreceptor											
Outer segment	−	+ + +	−	+ + +	−	+ +[e, g]	−	+ +	−	−	+ + +[f]
Inner segment	−	+ +	−	+	−	+ +[e, g]	−	+	−	−	+ +[f]
Outer limiting membrane[c]	−	+ + +	−	+	−	+[d]	−	+[e]	−	−	+ + +[f]
Outer nuclear layer	−	+ +	−	+	−	−	−	+[e]/−	−	−	−/+[f]
Outer plexiform layer	−	+ +	−	+ +	−	+ +[d]	−	+ +[e]	−	−	+[f]
Inner nuclear layer	−	+ +	−	+	−	−	−	+[d]	−	−	−/+[f]
Inner plexiform layer	−	+ +	−	+ +	−	+[d]	−	+ +[e]	−	−	+[f]
Ganglion cell layer	−	+ + +	−	+ +	−	−	−	+[e]	−	−	+ +[f]
Nerve fiber layer	−	+ +	−	+	−	−	−	+[e]	−	−	+[f]
Pigment epithelium	−	+ + +	−	+ +	−	+[d]	−	+[e]	−	−	+ +[f]
Blood vessels	+ +[d]	+	+ +[e]	+ +	+ +[d]	+ + +[d]	+[e]	+ + +	+ +[d]	+ +	+ + +[f]
Retinoblastoma											
Differentiated											
Rosettes	−	+ + +	−	+ +		+ +[e]/−	−	+ + +[e]	−	−	+ +[f]/−
Diffuse areas	−	+ + +	−	+		−/+[e]	−	+[e]/−	−	−	+[f]/−
Undifferentiated	−	+ +	−	+		−/+[d]	−	+[e]/−	−	−	−/+ +[f]
Tumour blood vessels	+ +[d]/−	+	+[d]/−	+ +		+ +[d]	−/+ +	+ + +	−/+ +[d]	−/+	+ + +[f]
Macrophage-like cells	+ +[e]/−	+ + +	−	+ + +		+ + +[d]	−/+ +	+ + +	+ +[e]/−	−	+ + +[f]

[a] For abbreviations and sugar specificities see Table 2. − = no binding, + = weak binding, + + = moderate binding, and + + + = strong binding of lectin. +/− = majority/minority
[b] Unreliable results in retinoblastoma due to unspecific binding
[c] Mainly due to fibre baskets of Müller's cells
[d] Positive only after neuraminidase treatment
[e] Enhanced after neuraminidase treatment
[f] Diminished after neuraminidase treatment
[g] Preferential labelling of cones

30% hydrogen peroxide. The sections were then washed in PBS and, to reduce nonspecific binding of protein, incubated with 2% BSA in PBS in a moist chamber at room temperature for 30 min.

The biotinylated or peroxidase-coupled (RCAI) agglutinins used were commercially obtained (Sigma, St. Louis, MO, USA), diluted with PBS to a protein concentration of 500 µg/ml, and stored at −20° C until needed. Their names, abbreviations, sugar specificities and batches used are described in Table 2. These lectins were used at a concentration of 25 µg/ml (LCA, LTA, RCAI and SBA) and 50 µg/ml (all other lectins), diluted with PBS containing 2% BSA. Changing the buffer system to Tris (pH 7.6), supplemented with 1.0 mM Mg^{2+}-, Ca^{2+}- and Mn^{2+}-ions (Leathem and Atkins 1983) did not enhance the positive reaction. Parallel control sections were stained with lectins that had been preincubated for 30 min at room temperature with their corresponding hapten sugars (Table 2; Sigma, St. Louis, MO, USA), used at a concentration of 0.2 M. Incubation with the lectin or lectin-hapten complex was carried out in a moist chamber at 37° C for 90 min.

The avidin-biotinylated peroxidase complex (ABC) method of Hsu and Raine (1982) was used for visualization of biotin-coupled lectins. The ABC complex was prepared 30 min before use by mixing 32 µl of avidin DH (Vectastain ABC Standard Kit; Vector Laboratories, Burlingame, CA, USA) and 32 µl of biotinylated horseradish peroxidase (Vectastain ABC Kit) in 4.0 ml PBS-BSA. After three washes in PBS, the sections were incubated with the ABC complex in a moist chamber at 37° C for 30 min. This step was omitted when RCAI was used, as it was directly conjugated to horseradish peroxidase. Following final three washes in PBS, the specific colour reaction was developed with 40 mg of 3-amino-9-ethylcarbazole (Sigma, St. Louis, MO, USA; diluted in 12 ml of N, N-dimethylformamide, Merck, FRG) in 200 ml of the acetate buffer (pH 5.0) containing 200 µl of 30% hydrogen peroxide. Treatment time was 20 min. After a wash in running tap water, the coverslips were mounted with Aquamount (BDH Chemicals, Poole, UK).

Results

In three specimens morphologically normal retina remained that had not been detached. In these cases, the binding patterns obtained with all eleven lectins studied closely resembled those seen in normal adult human retina (Kivelä and Tarkkanen 1987) and these results are summarized in Table 3. However, when retinal detachment had been present, the binding intensities of all lectins were substantially reduced.

Mannosyl- and glucosyl-specific lectins

Concanavalin A (ConA) bound strongly to the membranes and cytoplasm of undifferentiated and

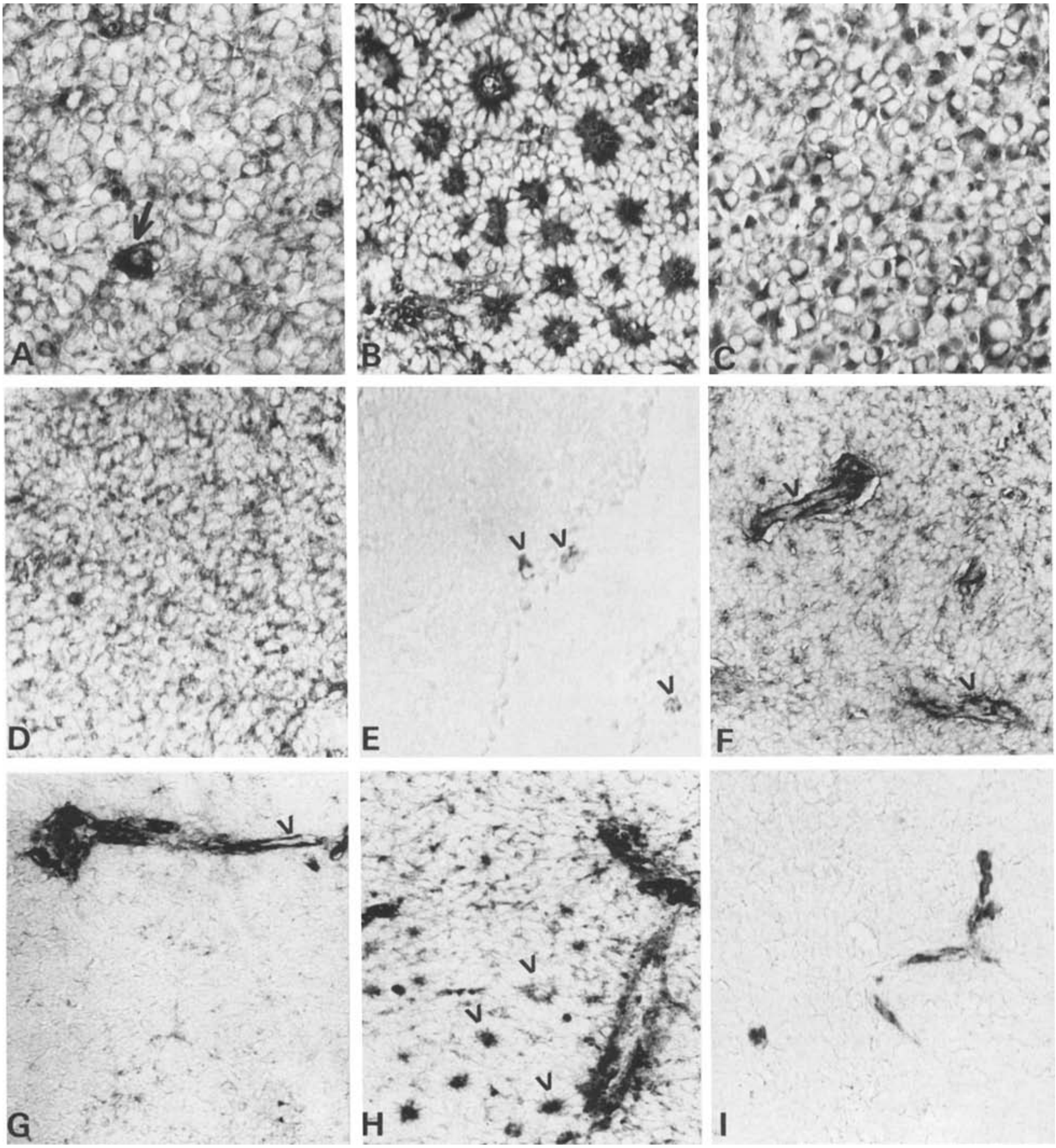

Fig. 1. Binding of mannosyl- and glucosyl-specific (**A–F**), as well as N-acetylglucosaminyl- and sialyl-specific (**G–I**) lectins to retinoblastoma (lectin histochemical staining). **A** Concanavalin A (ConA) labels an undifferentiated area of a differentiated retinoblastoma. An intensely labelled macrophage-like cell (*arrow*) is also seen (Case 9; ×490). **B** Rosettes react strongly with ConA (Case 10; ×285). **C** Cells with apical cytoplasm bind ConA in a tumour that was rich in fleurettes (Case 5; ×285). **D** Tumour cells of an undifferentiated retinoblastoma bind ConA (Case 1; ×285). **E** Section stained after preincubation of ConA with α-methyl-D-mannoside. No positive cells are seen, but lipofuscin-like granules (*arrowheads*) are visible in putative macrophages (Case 4; ×285). **F** *Lens culinaris* agglutinin binds strongly to stromal elements (*arrowheads*) and more weakly to tumour cells (Case 3; ×180); **G** Wheat germ agglutinin (WGA) labels stromal elements and blood vessels (*arrowhead*). Tumour cells are virtually negative (Case 4; ×180); **H** After treatment with pepsin, WGA binds to the apical parts of rosettes (*arrowheads*). Several undifferentiated tumour cells are labelled weakly (Case 4; ×285); **I** Pokeweed mitogen labels only vascular endothelial and red blood cells in retinoblastoma (Case 6; ×285)

differentiated retinoblastoma cells in all specimens (Fig. 1A–D). The overall reaction intensity was somewhat less intense in undifferentiated tumours. With *Lens culinaris* agglutinin (LCA), the binding pattern basically paralleled that of ConA, although the reaction intensity was substantially weaker (Fig. 1F). Blood vessels and stromal cells within the tumour were also positively labelled, especially with LCA. In addition, all tumours contained a few scattered cells of varying size and shape that were intensely positive. Many of these cells had lipofuscin-like granules in their cytoplasm and they were interpreted as macrophages (Fig. 1A and 1E). Treatment with pepsin slightly enhanced the binding of ConA and LCA, whereas neuraminidase pretreatment did not have any noticeable effect. Preincubation of either lectin with α-methyl-D-mannoside abolished the positive reaction in retinoblastoma cells and greatly reduced it in macrophage-like cells.

N-acetylglucosaminyl- and sialyl-specific lectins

Without any pretreatment, wheat germ agglutinin (WGA) bound strongly to blood vessels, stromal elements and macrophage-like cells within all retinoblastomas, but it did not label tumour cells (Fig. 1G). However, after pretreatment with pepsin, positive reaction was seen in the apical membrane and cytoplasm of retinoblastoma cells forming rosettes (Fig. 1H). In addition, areas of positively labelled undifferentiated retinoblastoma cells were seen in one undifferentiated and four differentiated tumours. Pretreatment with neuraminidase reduced the binding intensity of all positive elements. Preincubation of WGA with N-acetyl-D-glucosamine abolished the positive reaction in tumour cells, and reduced it in stromal elements and blood vessels. Pokeweed mitogen (PWM) reacted only with occasional macrophages and vascular endothelial cells in the retinoblastomas studied (Fig. 1I). This positive reaction was enhanced after pretreatment with pepsin, and it was reduced after preincubation of PWM with N-acetyl-D-glucosamine.

Galactosyl-specific lectins

In morphologically normal retinal parts, peanut agglutinin (PNA) labelled outer and inner segments of cones in untreated and pepsin-treated sections. It also reacted with occasional rods (Fig. 2A). After pretreatment with neuraminidase, the outer and inner segments of all photoreceptors as well as both plexiform layers were labelled, but the nuclear layers remained negative. In one tumour (Case 5), PNA bound strongly to the apical cytoplasm of a few cells situated near the transition of normal retina to retinoblastoma. They probably were normal photoreceptor cells incorporated into the tumour from the retina (Fig. 2B). In sections treated with pepsin, PNA labelled in five specimens the apical membranes of retinoblastoma cells that formed rosettes (Fig. 2C). In one specimen (Case 3), areas composed of undifferentiated tumour cells were weakly labelled (Fig. 2D). After pretreatment with neuraminidase, most rosettes were positively labelled in all specimens, and weak label was seen in areas of undifferentiated cells in one undifferentiated (Case 1) and five differentiated tumours. In addition, vascular endothelia, stromal elements and many macrophage-like cells became intensely labelled (Fig. 2E). In one particular tumour (Case 2), a few positive fibrillar elements were seen that may represent entrapped nerve fibres from the plexiform layers of the retina, since these were also positive after pretreatment with neuraminidase (Fig. 2F). Preincubation of PNA with D-galactose resulted in a negative reaction.

Ricinus communis agglutinin I (RCAI) reacted weakly with areas of undifferentiated cells in four specimens and the apical membranes of tumour cells that formed rosettes in three specimens before any pretreatment. In other cases, only stromal elements were positive (Fig. 2G). After pretreatment with pepsin or neuraminidase, all tumours but one (Case 4) contained undifferentiated areas that bound RCAI (Fig. 2H and 2I). The apical parts of differentiated cells in rosettes were labelled in all specimens (Fig. 2H). Regardless of pretreatment, stromal elements, macrophage-like cells and tumour blood vessels were intensely positive. Preincubation of RCAI with α-lactose entirely abolished the positive reaction in retinoblastoma cells.

Bandeiraea simplicifolia agglutinin I (BSAI) labelled only some vascular endothelial cells in the retinoblastoma specimens, and pretreatment with neuraminidase was necessary to demonstrate this reaction (Fig. 3A). The binding was abolished after preincubation of BSAI with α-methyl-D-galactoside.

N-acetylgalactosaminyl-specific lectins

Dolichos biflorus (DBA) and soybean (SBA) agglutinins bound to vascular endothelia, within the retinoblastomas after treatment with neuraminidase (Fig. 3B–E). However, the positive reaction was inconsistent and weak as opposed to the strong

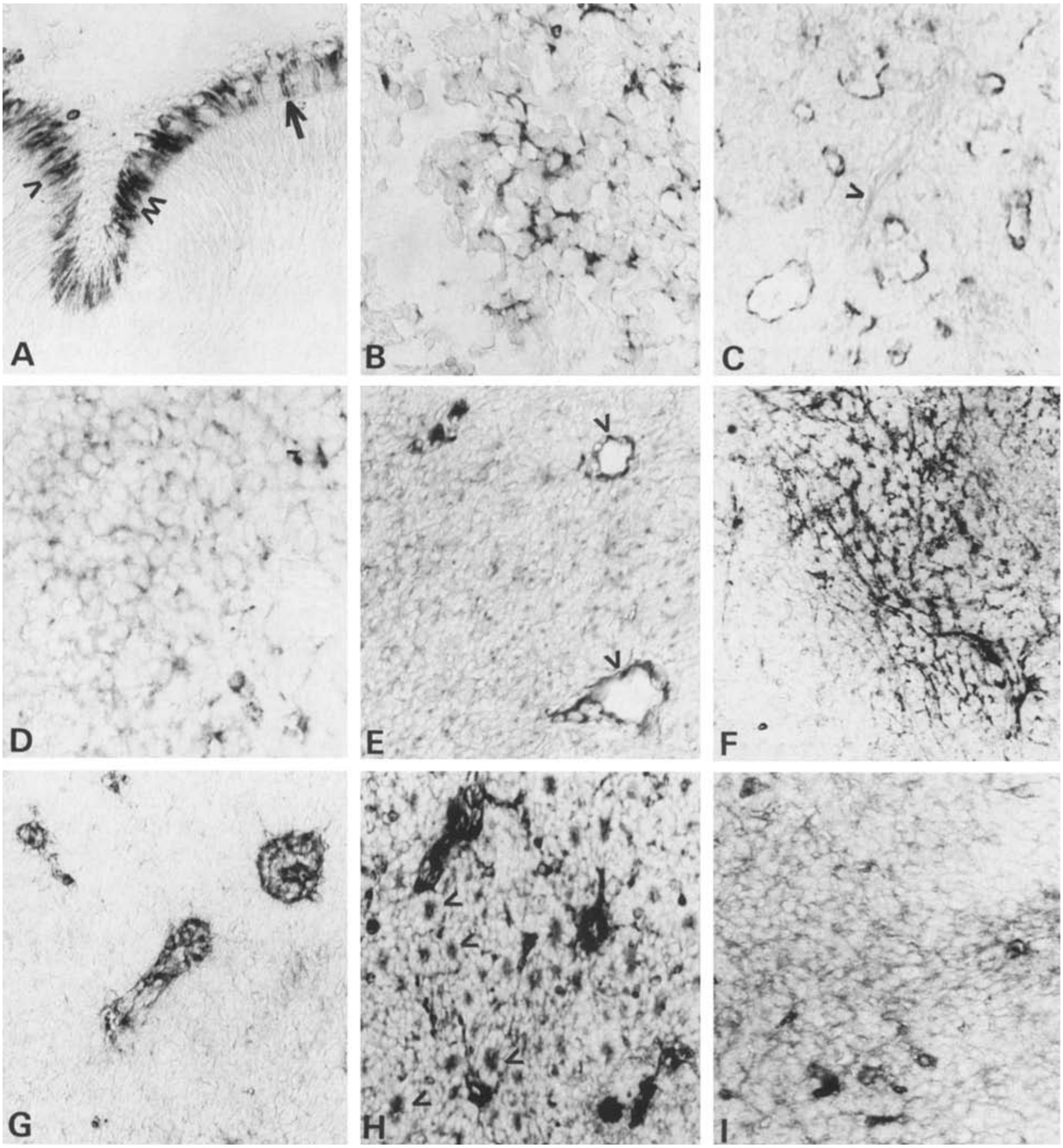

Fig. 2. Binding of β-galactosyl-N-acetyl-D-galactosaminyl-specific (**A–F**) and galactosyl-specific (**G–I**) lectins to retinoblastoma (lectin histochemical staining). **A** In uninvolved parts of the retina, peanut agglutinin (PNA) labels mainly cone outer and inner segments (*arrowheads*), but occasional rods (*arrow*) are also positively labelled (×265); **B** The apical cytoplasm of a few cells that probably have been incorporated into the tumour from adjacent infiltrated retina bind PNA (Case 5; ×415). **C** PNA labels apical membranes of tumour cells that form rosettes, whereas blood vessels (*arrowhead*) and undifferentiated tumour areas are negative (Case 10; ×265). **D** Undifferentiated areas of a differentiated tumour are weakly reactive with PNA (Case 3; ×410); **E** Most retinoblastoma cells are weakly reactive with PNA in an undifferentiated tumour after treatment with neuraminidase. Blood vessels (*arrowheads*) are also labelled (Case 7; ×265); **F** Positively labelled fibrillar structures revealed after treatment with neuraminidase that might derive from the plexiform layers of infiltrated retina (Case 2; ×180); **G** Without any pretreatment, only stromal elements of a differentiated retinoblastoma are labelled with *Ricinus communis* agglutinin (RCAI) (Case 5; ×265); **H** After treatment with pepsin, rosettes and diffuse areas of a differentiated tumour bind RCAI (Case 9; ×220). **I**. An undifferentiated retinoblastoma is labelled with RCAI after pretreatment with pepsin (Case 1; ×265)

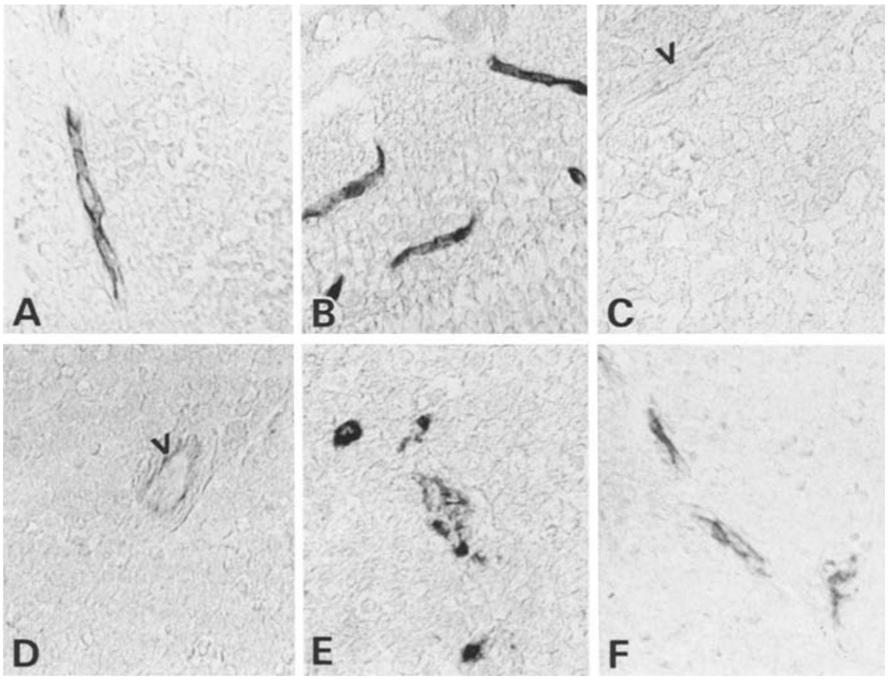

Fig. 3. Binding of α-galactosyl-specific (**A**), N-acetylgalactosaminyl-specific (**B–E**) and fucosyl-specific (**F**) lectins to retinoblastoma (lectin histochemical staining). Tumour cells are negative regardless of pretreatment. **A** *Bandeiraea simplicifolia* agglutinin I labels vascular endothelia after pretreatment with neuraminidase (Case 9; ×285); **B** Vascular endothelial cells bind *Dolichos biflorus* agglutinin (DBA) in the undetached retina of a patient from blood group A without pretreatment (×285); **C** In the same specimen, blood vessels of the tumour (*arrowhead*) do not bind DBA (×285); **D** Weak labelling of vascular endothelial cells is observed with DBA after pretreatment with neuraminidase (×285); **E** Soybean agglutinin labels macrophage-like cells (Case 9; ×285); **F** *Ulex europaeus* agglutinin I binds to vascular endothelia (Case 3; ×285)

and uniform labelling of vascular endothelial cells of undetached retinas. In addition, DBA bound to retinal vascular endothelia without any pretreatment if the patient belonged to the blood group A or AB (Fig. 3B), but blood vessels of the tumour remained negative (Fig. 3C). SBA bound strongly to the macrophage-like cells (Fig. 3E). Incubation with N-acetyl-D-galactosamine inhibited the binding of both lectins.

Fucosyl-specific lectins

Ulex europaeus agglutinin I (UEAI) bound only to vascular endothelial cells in undetached retina and within retinoblastomas. Treatment with pepsin enhanced the positive reaction, but even then the labelling of tumour blood vessels was rather weak and inconsistent (Fig. 3F). Neuraminidase did not alter the binding pattern. The positive reaction was abolished by preincubation of UEAI with α-L-fucose. *Lotus tetragonolobus* (LTA) agglutinin labelled retinal vascular endothelial cells after pretreatment with neuraminidase. Under the experimental conditions used, LTA bound unspecifically to the nuclei of retinoblastoma cells, and its binding to tumour cells could not be determined unequivocally.

Discussion

Very few differences were noted between lectin binding to morphologically normal parts of undetached retinas in eyes with retinoblastoma (Table 3) and to normal adult human retinas (Kivelä and Tarkkanen 1987). However, the nuclear layers bound wheat germ agglutinin (WGA) less intensely in some cases and *Ricinus communis* agglutinin I (RCAI) more intensely than was noted in normal adult human retina using identical methodology (Kivelä and Tarkkanen 1987). Also, peanut agglutinin (PNA) did not bind specifically to cones as has been reported for most normal retinas (Blanks and Johnson 1984; Uehara et al. 1983, Uehara et al. 1985; Kawano et al. 1984). WGA has a secondary affinity to sialic acid (Bhavanandan and Katlic 1983) which, on the other hand, can sterically hinder the binding of RCAI and PNA to glycoconjugates. These findings might thus be due to more extensive sialylation of glycoconjugates in the adult retina (McLaughlin et al. 1980).

Differentiated retinoblastoma cells that formed rosettes very closely resembled normal photoreceptor cells in their reaction patterns (Table 3). The entire cytoplasm of these cells was always labelled with concanavalin A (ConA) and *Lens culinaris* agglutinin (LCA). After pretreatment with pepsin, WGA and RCAI also labelled the luminal parts of most rosettes. Furthermore, when studied after pretreatment with pepsin, the luminal membranes of rosettes bound often PNA, which normally labels only photoreceptor outer and inner segments under these conditions. The other lectins studied did not react with retinoblastoma cells, but they bound weakly to some vascular endothelia within the tumours. The inconsistent binding may reflect their neovascular nature.

All lectins which labelled rosettes were also able to bind to undifferentiated cells in some undifferentiated and differentiated tumours, which might be considered as suggestive evidence of their relationship to photoreceptor cells. However, other possibilities must be considered. ConA, LCA, WGA and RCAI also react with other neuronal cell types in the inner nuclear and ganglion cell layers of the retina in retinoblastoma eyes (Table 3) or in adult human retina (Kivelä and Tarkkanen 1987). After treatment with neuraminidase, PNA labels both plexiform layers of the retina, whereas the nuclear layers remain negative. Although it is not known whether the positive reaction is associated with extracellular matrix of neuronal processes in the plexiform layers, it may be argued that the neurones sending their processes to these synaptic layers could express PNA-binding molecules in their cell bodies after malignant change. Also, major changes in glycoconjugates often occur during tumour development (Hakomori 1985), and the binding of PNA is particularly often affected (Walker 1985; Kahn and Baumal 1985; Böcker et al. 1984; Fisher et al. 1984).

The present observations agree with two previous reports. Felberg et al. (1985) studied cultured retinoblastoma cells using flow cytometry and fluorescein-conjugated lectins and noted that many Y79 cells bound ConA, WGA or RCAI. Bardenstein et al. (1986) briefly report on lectin binding to four differentiated retinoblastomas. The rosettes were labelled with ConA and LCA in all specimens, and with WGA and RCAI in three cases. Undifferentiated tumour areas bound LCA in two specimens and RCAI in three cases. Apparently, PNA did not bind to any of these tumours. As regards ConA, for obscure reasons Schwechheimer et al. (1983) were unable to detect any ConA-binding cells in their two retinoblastomas.

Felberg et al. (1985) were able to label Y79 retinoblastoma cells with *Ricinus communis* agglutinin II (RCAII), which is specific for N-acetylgalactosaminyl residues (Goldstein and Hayes 1978). In the present study and, apparently, in the study of Bardenstein et al. (1986), *Dolichos biflorus* and soybean agglutinins, which both share this same specificity, did not bind to any of the tumours studied. This apparent discrepancy is probably explained by the fact that while N-acetylgalactosamine is a potent monosaccharide inhibitor of these lectins, their binding to histological sections is differently affected by other carbohydrates on the same or adjacent glycoconjugates (Goldstein and Hayes 1978). As mentioned, cultured retinoblastoma cells can also differ in their antigenic structure as compared to intraocular tumours.

Fixation with formalin and embedding in paraffin can substantially alter binding intensities and patterns of some lectins (Leathem and Atkins 1983; Bell and Skerrow 1984; Virtanen et al. 1986). These alterations can often be counteracted effectively with slight proteolytic treatment (Leathem and Atkins 1983; Virtanen et al. 1986). It was necessary to use formalin-fixed and paraffin-embedded tissues in the present study to include all common types of this rare tumour, and the results should be controlled by staining frozen sections of future retinoblastomas. Further studies will also be needed to determine the identity of the glycoconjugates that bind lectins in retinoblastoma, and to clarify whether lectin histochemistry might be of use in tumour grading and classification. Finally, many tumours express endogenous lectins (Raz et al. 1984; Grabel et al. 1985; Gabius et al. 1985). As retinoblastoma cells have binding sites for several lectins with different sugar specificities, such tumour lectins might be important to cellular recognition and tumour cell adhesion in retinoblastoma.

Acknowledgements. I am very grateful to docent I. Virtanen for teaching me the basics of lectin histochemistry and for providing constructive criticism during my work. I also wish to thank Professor A. Tarkkanen for his enthusiasm and keen interest during all stages of this study. The expert technical assistance of M. Koikkalainen is greatly appreciated.

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Accepted November 27, 1986