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Immunohistochemical evaluation of factor VIII related antigen, filament proteins and lectin binding in haemangiomas

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Summary. Immunohistochemical identification of factor VIII related antigen (F VIII RAG) filament proteins (actin, myosin, filamin, vimentin and desmin) and lectin binding patterns of Con A, PNA, SBA, WGA, RCA-1, UEA-1 and DBA in the endothelial cells and the musclar layers of haemangiomas and normal blood vessels are reported, using paraffin sections with the HRP method.

The endothelial cells of haemangiomas were usually strongly positive to F VIII RAG as were those from capillary vessels and other small vessels. Some of the endothelium from haemangiomas and angiokeratomas was negative for factor VIII. The vessel walls of hemangiomas showed staining slightly positive for microfilaments (actin, myosin, filamin). The smooth muscle layer in small vessels showed a more marked staining with actin. Vimentin and desmin reactions in the vessel walls of haemangioma and in normal vessels were slight or moderate. UEA-1 lectin binding was constantly positive in endothelial cells from hemangiomas and in blood vessels. SBA and WGA binding appeared in the border layer of endothelium in haemangiomas and normal vessels.

Key words: Haemangioma – Factor VIII rAG – Filament protein – Lectin

Introduction

The haemangioma is a hamartomatous benign neoplasm usually occurring in the skin and mucosa of head and neck region, at birth and in childhood. Histologically, it is composed of proliferating vascular vessels in irregular arrangement and is classified as capillary, cavernous and racemose types, and types with scanty stromal connective tissue fibers. The vascular components of haemangioma consist of endothelial cells, a thin smooth muscle layer and connective tissue fiber as an outer zone.

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Recent studies have shown that histochemical markers are found in the vascular wall – vascular endothelial cells synthesized factor VIII related antigen (F VIII RAG) (Jaffe 1977; Sodetz et al. 1979; Mukai et al. 1980; Jones et al. 1981; Jeanneau et al. 1982; McComb et al. 1982) and showed marked UEA-1 binding (Pereira et al. 1978; Holthöfer et al. 1982, 1983; Kariniemi et al. 1983; Soda et al. 1983; Möller and Lennert 1984). Vascular smooth muscle characteristically has vimentin intermediate sized filament proteins (Frank and Warren 1981; Gabbiani et al. 1982; Quinlan and Franke 1982; Schmid et al. 1982; Travo et al. 1982; Kocher et al. 1984) and the connective tissue fibers in the outer zones displayed desmin (Quinlan and Franke 1982; Schmid et al. 1982; Travo et al. 1982). It has been reported that F VIII RAG appears in a variety of vascular endothelial cells in reactive lesions (Burgdorf et al. 1981; Sehested and Hou-Jensen 1981), and in benign and malignant vascular tumours (Guarda et al. 1981; Sehested and Jou-Jensen 1981; Barwick and Madri 1982; Bell and Flotte 1982; Guarda et al. 1982; Jurco et al. 1982; Said et al. 1982; Pfaltz et al. 1983; Crocker and Smith 1984). The present report describes F VIII RAG histochemically and different filament proteins with the use of immunoperoxidase method. Sugar residues are demonstrated by a lectin binding technique which specifically interacted with corresponding sugar residues; the report also compares these reactions in normal vessels and haemangiomas.

Materials and methods

Specimens were selected following diagnosis. Surgically removed haemangiomas and angiokeratomas (3) were fixed in 10% formalin and routinely made into blocks. Twenty serial sections at 4 μm were made for all the specimens.

Immunohistochemical methods (F VIII RAG, Actin, Myosin, Filamin, Vimentin, and Desmin.)

Deparaffinized sections were immersed in a 0.3% H_2O_2 methanol solution for 20 min to inactivate endogenous peroxidase and then rinsed well in phosphate-buffer saline (PBS). the sections were treated in the following manner.

1) Reaction with normal swine serum (1:20 Dekopatts, Copenhagen, Denmark).

2) Rinsed in PBS 3 times.

3) Reaction with rabbit antiserum (F VIII RAG 1:100, Dakopatts, Copenhagen, Denmark. Actin, 1:300., Myosin, 1:300., Filamin, 1:100., Vimentin, 1:100., Desmin, 1:100, Transformation Research Inc. Framingham, MA, USA) for 1–2 h.

4) Rinsed in PBS for 15 min

5) Immersed in swine anti-rabbit IgG (1:50 Dakopatts, Copenhagen, Denmark) for 30 min.

6) Rinsed in PBS for 15 min.

7) Reaction with peroxidase antiperoxidase complexes (PAP; 1:100 Dakopatts, Copenhagen, Denmark.) for 30 min.

8) Rinsed in PBS for 15 min.

9) Immersed in 0.05 M Tris buffer containing 0.0003% DAB with 0.03% H_2O_2 solution for 10 min.

10) rinsed in water, dehydrated and mounted in balsam.

According to the documentation of Transformation Research Inc., the source of vimentin was cultured human fibroblasts, the purified vimentin did not stain microfilaments or microtubules. The antigen source of desmin was chicken gizzard and anti-desmin serum showed no reaction to fibroblast and epithelial cells. Microfilaments, actin, filamin, and myosin were purified from chicken gizzard and breast muscle (myosin), and anti-sera were cross-reacted with skeletal and cardiac muscle of mammals and birds.

Control method. After inactivation of endogenous peroxidase, control tissue sections were made to react with normal rabbit serum in place of the rabbit serum (anti-FVIII RAG, actin, myosin, filamin). The other steps were the same as those of above (4–10), and we got negative reaction.

Lectin binding method. Peroxidase conjugated lectins of PNA, RCA-1, DBA, SBA, WGA, and UEA-1 and non-conjugated lectin of Con A were used in this study. Galactose-binding lectins are PNA and RCA-1, N-acetyl-galactosamine-binding lectins are DBA and SBA, fucose-binding lectin is UEA-1, N-acetyl-glucosamine-binding is WGA and mannose or glucose-binding lectin is Con A.

The sections were deparaffinized and then immersed in methanol solution containing 0.3% H_2O_2 for inactivation of endogenous peroxidase for 30 min. Then reaction with conjugated lectin solutions (100 μg/ml) was done for 40 min, and in the case of Con A staining, the sections were made to react with horse radish peroxidase/PBS solution (0.0005%) after Con A solution (150 μg/ml).

Competitive sugar inhibition test for lectin staining. 0.1 M and 0.5 M α-methyl-mannoside, D-galactose, GalNAc, GlcNAc, α-L-fucose (Sigma Chemical St. Louis, MO USA) were employed as competitive inhibitors of their binding reaction. Incubation media of Con A was added with α -methyl-mannoside, that of RCA-1 was galactose, that of PNA was galactose and GalNAc, that of SBA was galactose and GalNAc, that of DBA was GalNAc, that of WGA was GlcNAc and NANA, and that of UEA-1 was α-L-fucose, and we got weak reaction with 0.1 M sugar concentration, and negative reaction with 0.5 M.

Results

Factor VIII related antigen (F VIII RAG)

Endothelial cells from the blood vessels showed varying positive staining to F VIII RAG. Endothelial cells of capillary vessels were characterized by the strong staining of F VIII RAG, staining in the vein was comparatively stronger than that in the artery. Large vessels, either vein or artery, showed lack of F VIII RAG in their endothelial cells. Endothelial cells in almost all haemangioma displayed strong staining to F VIII RAG, it was particularly abundant in their surface lining, in generally irrespective of histological variant of haemangioma examined (Fig. 2a–d). On the contrary, in angiokeratoma and some cases of haemangioma, nor or little staining to F VIII RAG was found in endothelial cells of dilated or wide spaced lumens (Fig. 1b). Proliferating endothelial cells in the peripheral parts of the haemangiomas showed positive F VIII RAG ractions in whole cytoplasm, but not so great a staining intensity. Homogenous coaglating material in the lumens in normal vessels and haemangiomas occasionally showed strong staining for F VIII RAG.

Actin, myosin and filamin

These three microfilaments have a similar profiles in their distribution with little difference in staining in either normal or haemangiomatous blood vessels. Normal vessels of small size displayed moderate or strong staining with actin in smooth muscles and endothelial cells (Fig. 1c), slight-to-moderate staining with myosin and filamin in the smooth muscle layer and no staining in endothelial cells (Fig. 1d). The muscle layers in large vessels,

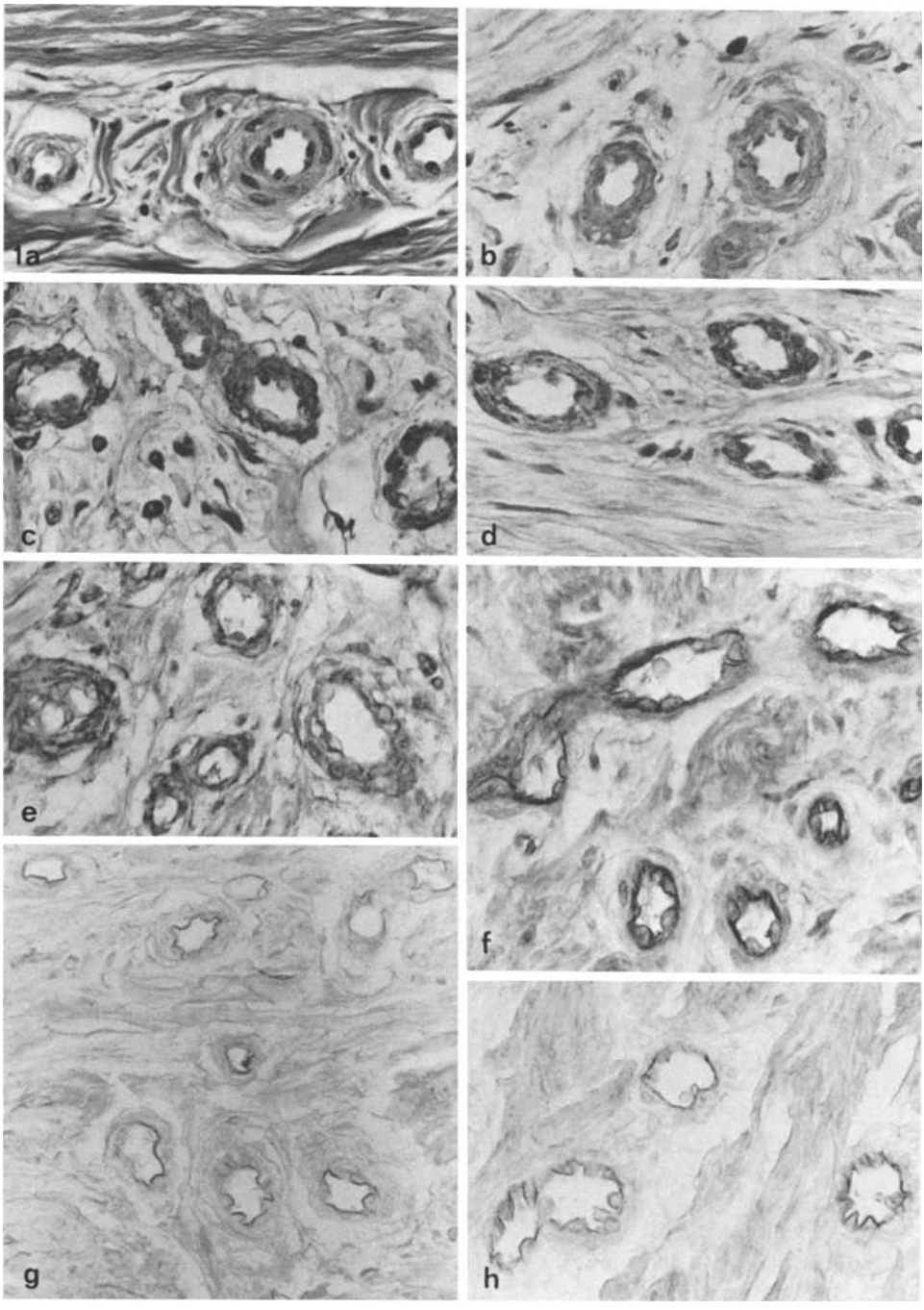

Fig. 1a–h. Small sized blood vessels regenerating tissue. ×200. **a** There are 3 to 5 endothelial cells and a smooth muscle layer. (HE). **b** Factor VIII related antigen (F VIII RAG). Note slight staining of the factor in the blood vessels wall. **c** Actin. Note marked actin staining in smooth muscle layer. **d** Myosin. Moderate myosin staining in blood vessels wall. **e** Vimentin. Slight vimentin staining in smooth muscle layer. **f** UEA-1 lectin. Comparatively strong staining to UEA-1 lectin in endothelial cells. **g** WGA lectin. Positive WGA staining in luminal surface of endothelial cells. **h** SBA lectin. Positive SBA staining in surface border of endothelial cells

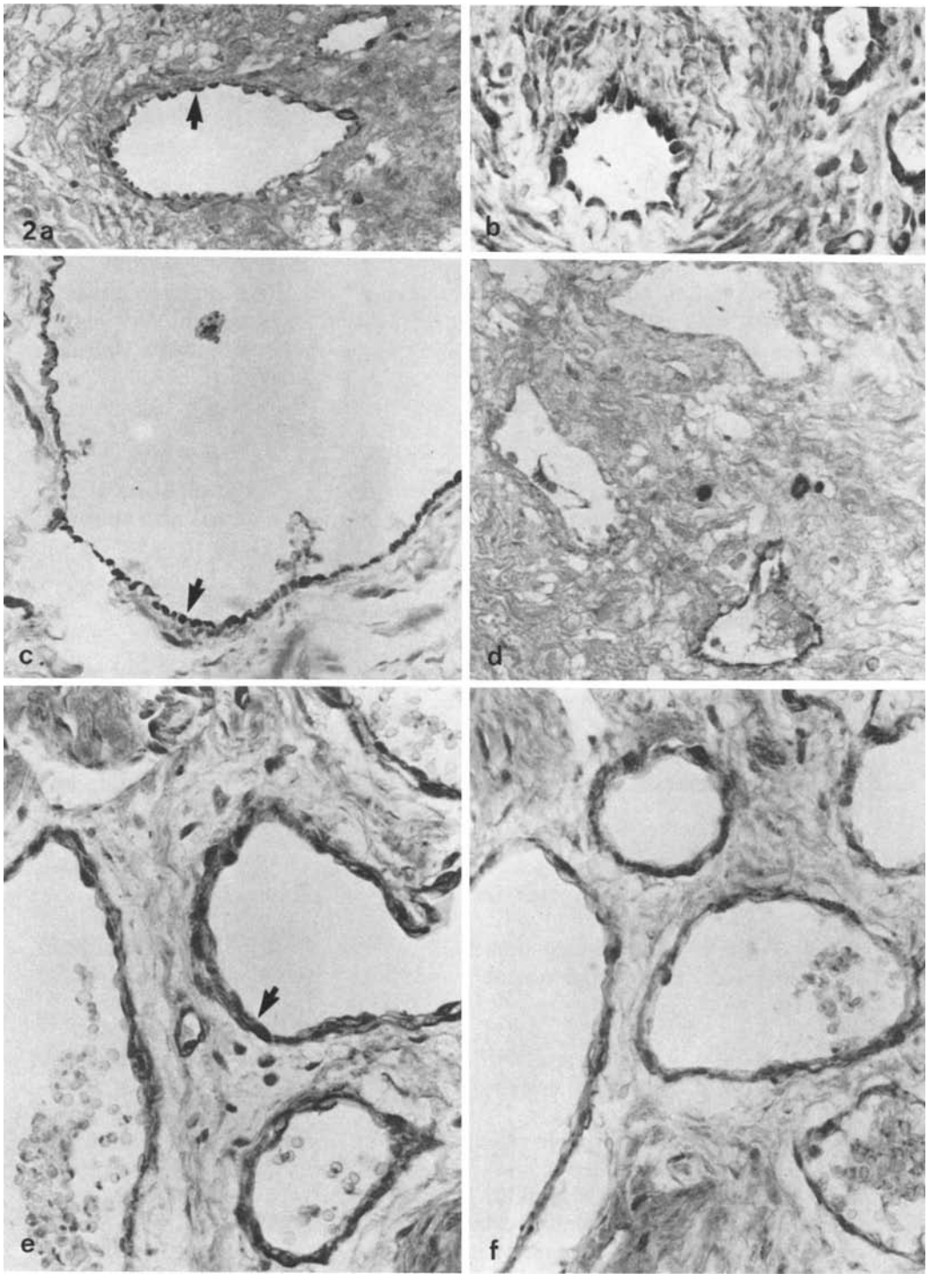

Fig. 2a–f. Haemangioma. ×200. **a, b.** Endothelial cells are positive or negative **c** (arrow) (FVIII). **d** F VIII RAG. Endothelial cells are positive or negative. **e** Actin. Thin wall of vessel shows positive actin staining. **f** Myosin. Thin capillary wall shows positive myosin staining as well as actin distribution

both artery and vein, showed relatively slight reactions to microfilament staining. Endothelial cells were generally reactive with weak evidence of microfilaments in the normal and haemangiomatous vessels. They were strongly positive in the proliferating endothelial cells of haemangioma (Fig. 2e, f).

Vimentin

The smooth muscle layers in normal blood vessels displayed slight-to-moderate staining with vimentin, whereas those in haemangiomas had very slight reaction. Endothelial cells in proliferating areas of haemangiomas showed positive vimentin staining (Fig. 1e).

Desmin

The normal blood vessels and haemangioma walls exhibited slight stainsing for desmin and the connective tissue stroma in haemangiomas also showed slightly positive staining.

Lectin binding

Con A: Con A binding was slightly positive in the endothelial cell and muscle layer of the normal vessels as well as in haemangiomas.

PNA: PNA binding in endothelial cells from normal vessles and haemangiomas was moderately positive. In smooth muscle slight levels of staining were found.

SBA: Moderate SBA staining was confined to the surface border of endothelial cells of the normal vessels and haemangiomas (Fig. 1h, 3 c).

WGA: WGA binding reacted slightly or moderately at the luminal surface in endothelial cells of the normal vessels as well as in haemangiomas (Fig. 1g, 3d).

RCA-1: RCA-1 binding in endothelial cells and the muscle zone was moderately positive in the normal vessels and haemangiomas.

UEA-1: Endothelial cells were characterized by existence of strong staining for UEA-1 lectin, and UEA-1 binding expression was strong in blood capillaries and haemangiomas. Endothelial cells from vein and artery showed similar staining to UEA-1 lectin binding pattern (Fig. 1f, 3a, 3b). Some of the endothelium in haemangiomas were devoid of UEA-1 staining. Smooth muscle and connective tissue stroma showed no UEA-1 lectin staining.

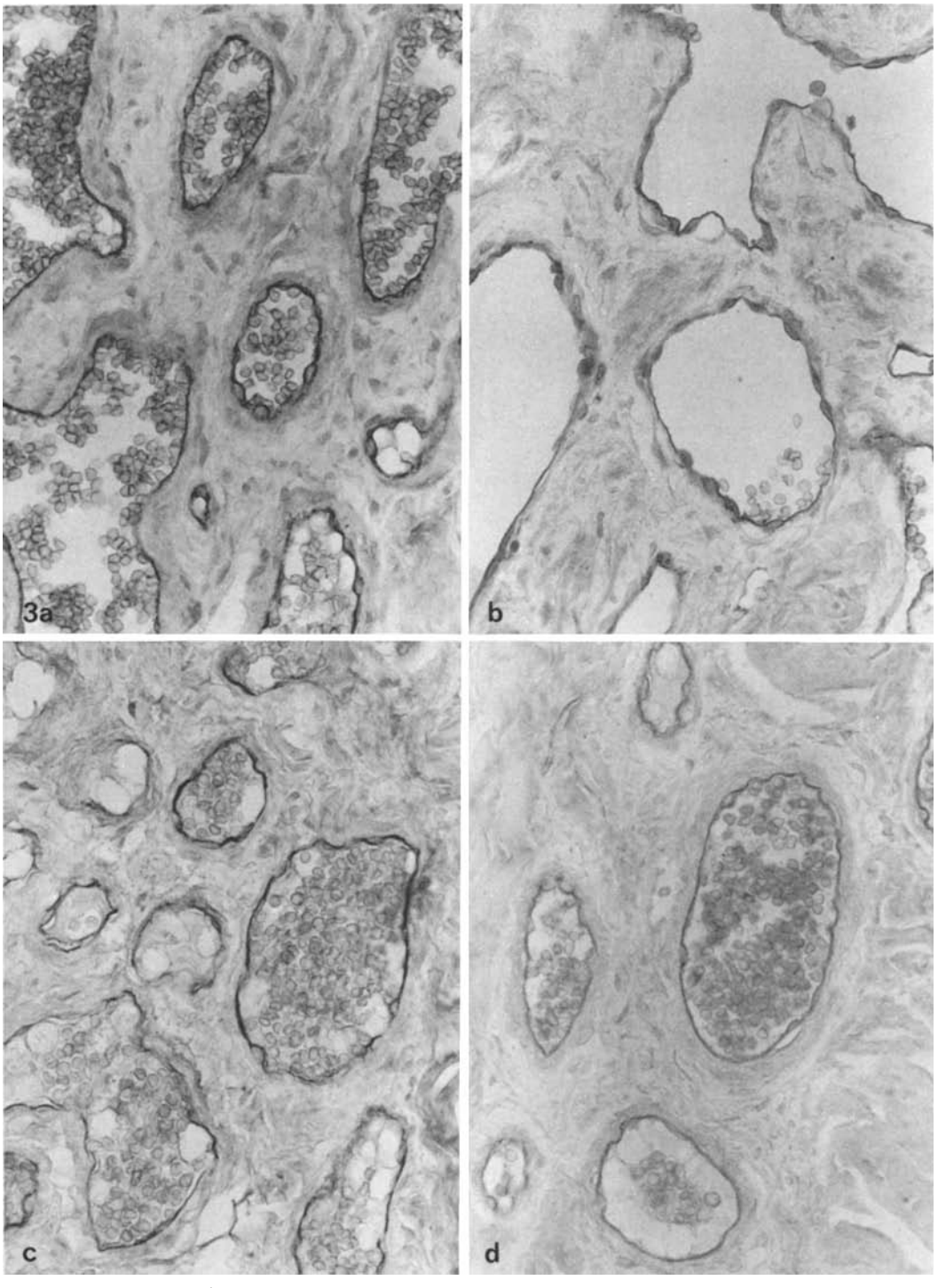

Fig. 3a–d. Lectin binding in hemangioma ×200. **a** UEA-1 lectin. Endothelial cells show strong positive binding to UEA-1 lectin. **b** UEA-1 lectin. Some area of haemangioma, endothelial cells show slight or very weak staining to UEA-1 lectin. **c** SBA lectin. Endothelial lining in haemangioma show positive SBA staining as well as UEA-1 staining. **d** WGA lectin. Note thin layer staining to WGA in haemangioma wall

Table 1. Stainability in blood vessels

	Normal Capillary	Large vessels		Small vessels		Haem-angioma
	E	E	S	E	S	E
FVIII	2	1–2	0	2–3	0	1–3
Actin	1	1–2	2–3	2	2–3	1–2
Myosin	1	1–2	2–3	2	2–3	1
Filamin	0	0	2–3	0	3	0–1
Vimentin	1–2	2	2	2	2	1
Desmin	1	0	1	1	2	1
UEA-1	3	2–3	0	3	0	3
WGA	2	1–3	0	2–3	1	2
SBA	2	1–2	1	2–3	0–1	2

E: Endothelial Cell; S: Smooth Muscle Layer
0: negative; 1: slight; 2: moderate; 3: strong

DBA: The endothelium and the smooth muscle layer showed slight or traceable staining for DBA binding in the normal vessels and haemangiomas.

Discussion

Factor VIII (antihemophiliac factor) consists of three functional components F VIII RAG, F VIII clot promoting factor and von Willebrand factor. Endothelial cells from the blood vascular system have been identified as a site of immunohistochemically detectable F VIII RAG, vascular endothelium is a synthetic site of the factor (Jaffe 1977). Significant staining of F VIII RAG may indicate platelets, megakaryocytes and tissue mast cells in addition to vascular endothelial cells (Kindblom 1982). It is interesting to find that F VIII RAG staining in endothelial cells was most prominent in the capillary vessels, that it was more marked in veins than in arteries and that in small vessels it was stronger when compared to large vessels. These different reactions in normal vessels might be related to differing functions in coagulation. It is reported that F VIII RAG in benign vascular lesions including haemangioma was positive with less intensity in their endothelial cells, however malignant vascular lesions had negative responses (Feigl et al. 1976; Guarda et al. 1981; Nadji et al. 1981; Sehested and Hou-Jensen 1981; Guarda et al. 1982; Jurco et al. 1982; Said et al. 1982; Pfaltz et al. 1983). In the present result, including several types of haemangiomas, endothelial cells in haemangiomas were characterized by marked staining of F VIII RAG in luminal site of the cells, whereas those in proliferating cells at the periphery contained the factor in the whole of the cellular plasma, which was lightly stained. In some parts of angiokeratomas, tumour endothelial cells showed loss of F VIII RAG stainability. These findings probably indicate that if the endothelial cells tend to proliferate or are neoplastic in nature, reduced staining of F VIII RAG is due to dedifferentiation or disorganization of biosynthesis in these abnormal cells.

Microfilaments are distributed at the cellular periphery and are related to cell contraction and motility. Vimentin is a smooth muscle marker, and desmin specifically exists in connective tissue (Franke et al. 1978; Frank and Warren 1981; Gabbiani et al. 1981; Quinlan and Franke 1982; Schmid et al. 1982; Travo et al. 1982; Holthöfer et al. 1983; Kocher et al. 1984). These filament proteins were both expressed in vein and artery and small vessels walls. Small vessels showed rather stronger actin staining when compared with large ones. No specific distribution patterns in expression of microfilaments and intermediate filaments were obtained in the wall of haemangiomas.

Lectins isolated from plant sources have been specifically linked to corresponding sugar residues in cellular macromolecules of mammalian tissues. UEA-1 lectin (fucose binding lectin) is a particular marker of vascular endothelial cells in human tissues (Pereira et al. 1978; Holthöfer et al. 1982, 1983; Kariniemi et al. 1983; Soda et al. 1983; Möller and Lennert 1984). The present study examined comparative binding affinities in different lectins which specifically interact to glucose, mannose (Con A), Gal, GalNAc (SBA, PNA), Gal (RCA-1), GalNAc (DBA), GlcNAc, NANA (WGA), and fucose (UEA-1) in the endothelial cells of haemangiomas. Endothelial cells in varying histological types of haemangiomas show binding to SBA and WGA lectins as well as UEA-1 lectin. Positive stainings to Con A, PNA, and RCA-1 lectins were found in the cytoplasm of endothelial cells. The vascular smooth muscle zone was also positively stained to Con A, PNA and RCA-1 lectins at with slight to moderate levels. UEA-1 distribution and staining in normal blood vessels were different by location, capillary endothelial cells had the most intense UEA-1 staining while endothelial cells in both arteries and vein had less. In the present study, the endothelial cells from haemangiomas showed generally regular staining for UEA-1 lectin at *strong level*, and were constantly positive in luminal border to SBA and WGA lectins at *slight to moderate levels* . It is concluded that the surface lining in the endothelial cells of the normal capillary vessels and small vessels, either vein or artery, and haemangiomas contained Gal and GalNAc (SBA) binding sugar) and GlcNAc (WGA binding sugar), as well as L-fucose residues (UEA-1 binding sugar). SBA and WGA binding profiles in endothelial cells are histochemical markers for detecting endothelium, in addition to UEA-1 lectin and F VIII RAG.

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