

ORIGINAL ARTICLE

O. Naňka · W.J. Peumans · E.J.M. Van Damme
 U. Pfüller · P. Valášek · Z. Halata · U. Schumacher
 M. Grim

Lectin histochemistry of microvascular endothelium in chick and quail musculature

Accepted: 27 July 2001

Abstract The lectin binding pattern of muscular microvessels in chick, quail and chick/quail chimeras was analysed. Paraffin wax sections of muscles from embryonic and adult animals were used. The biotin-labelled lectins were detected by avidin-alkaline phosphatase complex. The following lectins bound to muscular microvessels including arterioles, capillaries and venules of both species: SNA-I (*Sambucus nigra* agglutinin), MAA (*Maackia amurensis* agglutinin), AIA (*Artocarpus integrifolia* agglutinin), VAA-I, VAA-II and VAA-III (*Viscum album* agglutinin I–III), WGA (wheat germ agglutinin), LEA (*Lycopersicon esculentum* agglutinin). Endomysium and basement membranes of muscle fibres were also stained to a variable extent and intensity. Only SNA-I stained almost exclusively the endothelium of blood vessels. WFA (*Wisteria floribunda* agglutinin) bound to the quail endothelium only. MPA (*Maclura pomifera* agglutinin) marked vessels in adult muscles of chick and quail, but embryonic vessels were stained in quail only. Our results show that lectin histochemistry is a useful tool for visualisation of microvasculature in avian species. In particular, WFA and MPA can be used to determine the origin of endothelia in chick/quail chimeras.

Key words Muscle capillaries · Lectin staining · Avian muscles · Chick/quail chimera

Introduction

The evaluation of the capillary density in skeletal muscles requires the selective visualisation of endothelial cells. A number of histochemical staining methods for muscle capillaries were introduced, including the reaction for alkaline phosphatase (Romanul and Banister 1962), alkaline phosphatase in combination with dipeptidyl peptidase IV (Lojda 1979; Mrázková et al. 1986) or adenosine triphosphatase (ATPase, Sillau and Banchemo 1977). For immunohistochemical labelling of endothelium antibodies against vascular endothelial cells such as MRC OX 43 (Robinson et al. 1986), antibody against von Willebrand's factor (Wall et al. 1980) or QH-1 antibody (Pardanaud et al. 1987) were used. In addition, the expression of VE-cadherin (Breier et al. 1996), and VEGF receptors has been used to specifically label endothelial cells (Eichmann et al. 1996). However, each method has certain limitations and can not be used for all purposes. ATPase staining is sensitive to acid preincubation (Hansen-Smith et al. 1992) while alkaline phosphatase staining is affected by fixation and paraffin embedding. In addition, alkaline phosphatase staining pattern exhibited a considerable variability among species (Grim and Carlson 1990).

Excellent results of labelling capillaries in muscles of rats and mice were obtained by Hansen-Smith and collaborators (Hansen-Smith et al. 1988, 1989) using lectin histochemistry with *Griffonia simplicifolia* agglutinin I (GSA-I). These authors have shown that GSA-I binds to capillaries in muscles of a number of mammalian species; more capillaries were detected by this method than by any other. In contrast to mammalian species, lectin staining of endothelium of blood vessels of chick and quail, two species widely used in experimental embryology, has so far only been infrequently used (Henry and DeFouw 1995; Nico et al. 1998). We therefore tested a

O. Naňka (✉) · P. Valášek · M. Grim
 Institute of Anatomy, Charles University First Medical Faculty,
 U nemocnice 3, 128 00 Prague 2, Czech Republic
 e-mail: Ondrej.Nanka@lf1.cuni.cz
 Fax: +420-2-24965770

W.J. Peumans · E.J.M. Van Damme
 Laboratory for Phytopathology and Plant Protection,
 Catholic University Leuven, Willem de Croylaan 42,
 3001 Heverlee-Leuven, Belgium

U. Pfüller
 Institute for Phytochemistry, University Witten-Herdecke,
 Stockumer Strasse 10, 58448 Witten, Germany

Z. Halata · U. Schumacher
 Institute for Anatomy, University Hospital Hamburg-Eppendorf,
 Martinistrasse 52, 20246 Hamburg, Germany

Table 1 Lectin staining of vascular endothelium in adult (*a*) and embryonic (*e*) muscles of chick (*C*) and quail (*Q*)

Lectin	Lectin origin	Sugar specificities	Endothelium				Conn. tissue	Reaction after inhibition
			Ca	Ce	Qa	Qe		
SNA-I	<i>Sambucus nigra</i>	α -NeuNAc(2,6)Gal	+	+	+	+	-	Neuraminidase pretreatment -
MAA	<i>Maackia amurensis</i>	α -NeuNAc(2,3)Gal	+	+	+	+	+	
AIA	<i>Artocarpus integrifolia</i>	α -Gal	+	+	+	+	+	Gal \pm
GSA -I/ BSA- I	<i>Griffonia simplicifolia/ Bandeiraea s.</i>	α -Gal/ Gal NAc	-	-	-	-	-	
VAA I	<i>Viscum album</i>	Gal	+	+	+	+	+	Gal -
VAA II	<i>Viscum album</i>	Gal/GalNAc	+	+	+	+	+	Gal \pm /GalNAc-
VAA III	<i>Viscum album</i>	β -GalNAc	+	+	+	+	+	GalNAc -
WGA	Wheat germ	GlcNAc	+	+	+	+	+	GlcNAc -
LEA	<i>Lycopersicon esculentum</i>	(GlcNAc) ₃	+	+	+	+	+	GlcNAc -
MPA	<i>Maclura pomifera</i>	Gal/GalNAc	+	-	+	+	+	Gal \pm /GalNAc -
WFA	<i>Wisteria floribunda</i>	GalNAc	-	-	+	+	+	GalNAc -

panel of selected lectins for their binding specificity to endothelium in prenatal and adult muscles of chick and quail.

Material and methods

This study was performed on muscles of embryos at the day of hatching and adult chick (White Leghorn) and Japanese quail (*Coturnix coturnix japonica*) and their embryonic chimeras. Chimeric legs were prepared by the grafting of the quail leg bud in the place of the removed leg bud of the chick on embryonic day 3 (stage 19 according to Hamburger and Hamilton 1951). After 11 days of re-incubation, the grafted leg was used for the study. Adult animals were terminated with an overdose of pentobarbital and embryos by decapitation. Principles of laboratory animal care were applied (NIH publication No. 86-23, revised 1985). The extensor digitorum longus and plantaris muscles from two embryonic and two adult animals of both species were investigated. From chimeric legs crural muscles were used. All specimens were fixed *in situ* in 3.5% paraformaldehyde in 0.1 M phosphate buffer for 1-2 h. Muscles were then removed, further fixed overnight, washed and embedded in paraffin wax. Transverse, 7- μ m sections were treated with 0.1% trypsin in TRIS buffer with 1 mM MgCl₂ and 1 mM CaCl₂, pH 7.6, for 10 min at 37°C, then washed in the same TRIS buffer and incubated with biotin-labelled lectin (10 μ g/1 ml) for 60 min at room temperature. For lectins, their abbreviations and sugar specificities see Table 1. The specificity of lectin binding was assessed by incubation with the appropriate inhibitory sugar (0.2 M) or in the case of neuraminic acid by neuraminidase pre-digestion. After washing, the avidin-alkaline phosphatase (Vector ABC kit) was used for visualisation of binding sites according to the manufacturer's instructions. Finally, the sections were counterstained with haematoxylin and mounted in Crystal Mount. Parallel sections from chimeric muscles were stained with QH-1 antibody that marks quail endothelial cells, leaving chick endothelium unstained (Pardanaud et al. 1987).

Results

SNA- I, MAA, AIA, VAA I- III, WGA and LEA (see Table 1) bind to the endothelium of capillaries, arteries and veins in the entire vascular bed of muscles of embryonic and adult chick as well as that of quail (Figs. 1-4, 9-12). In contrast, WFA stained the endothelium in embryonic and adult quail only and did not label it at all in

the chick (Figs. 5-8). MPA was less species specific. It bound to the endothelium of adult chick and quail, but not to embryonic chick muscle (Figs. 13, 14). In chimeric leg vessels that contained endothelial cells of both species (Grim et al. 1997), WFA stained the quail endothelial cells only (Fig. 15). It was verified by QH-1 staining in parallel sections (Fig. 16).

GSA-I, which stains the endothelium of blood vessels in many mammalian species, exhibited no binding to the endothelium of the two avian species.

The endothelium was almost exclusively stained by SNA-I (Figs. 1-4). Other lectins also stained the connective tissue structures of muscle (endomysium, perineurium, capsule and the inner space of muscle spindles) to variable extent (Figs. 8, 9). In addition, some lectins (VAA I, VAA III, AIA, MAA) bound to the basement membrane of muscle fibres (Figs. 9, 11). The staining of connective tissue was most intense using AIA (Fig. 9). This staining was reduced by pre-incubation with galactose, whereas the labelling of endothelium was unaffected in quail (Fig. 10) and only slightly inhibited in chick.

Discussion

The vascular endothelium is a highly specialised semi-permeable barrier whose luminal surface contains specific domains with characteristic carbohydrate residues (Simionescu and Simionescu 1986). In the present study,

Figs. 1-15 Lectin staining of endothelium in transverse paraffin sections of skeletal muscles of adult chick (*Ca*), embryonic chick (*Ce*), adult quail (*Qa*) and embryonic quail (*Qe*)

Figs. 1-4 SNA-I

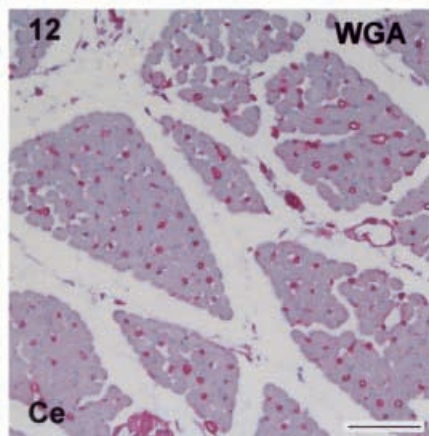
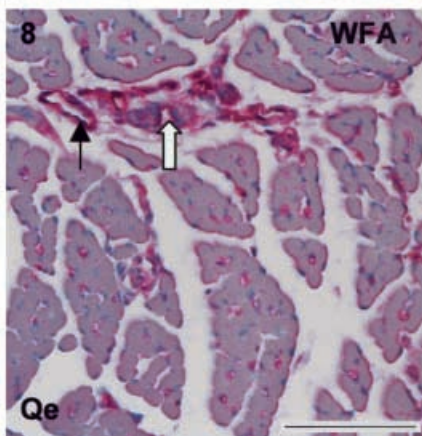
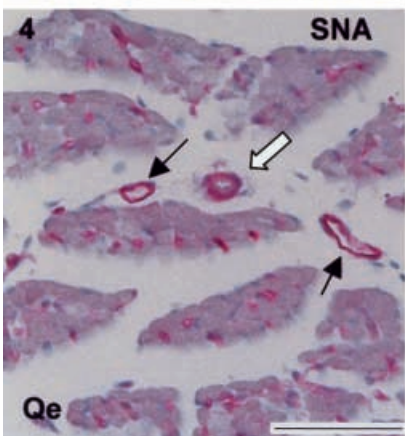
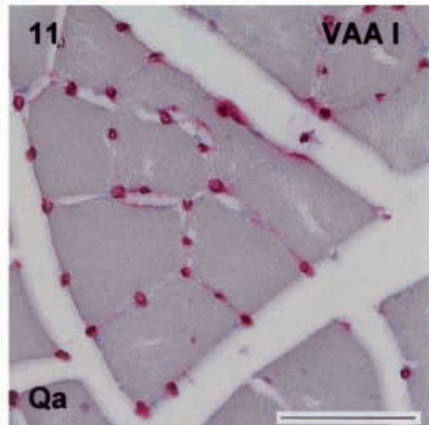
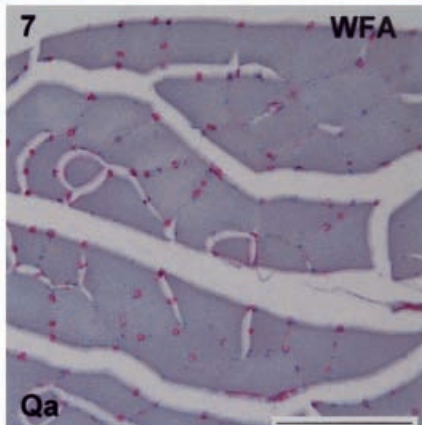
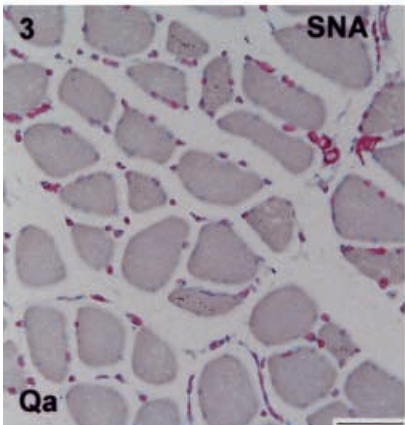
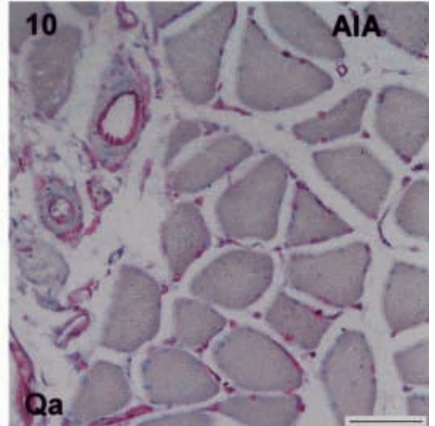
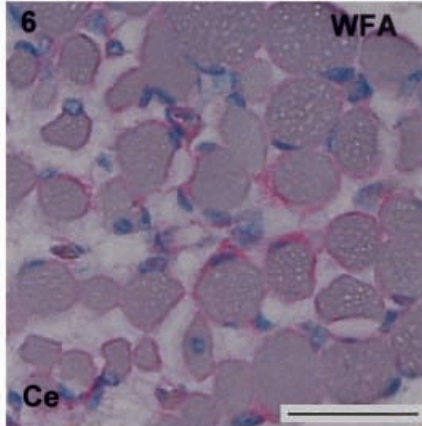
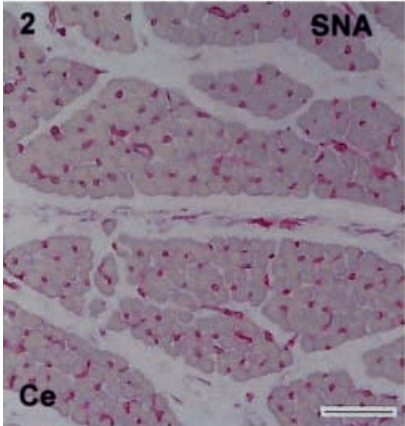
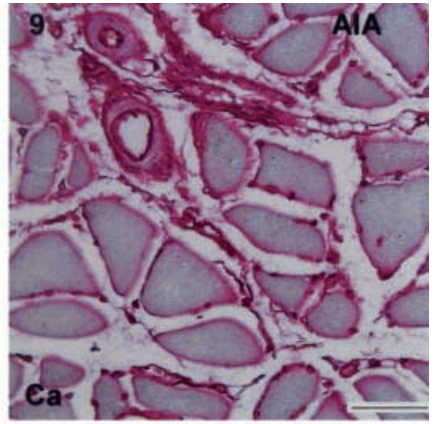
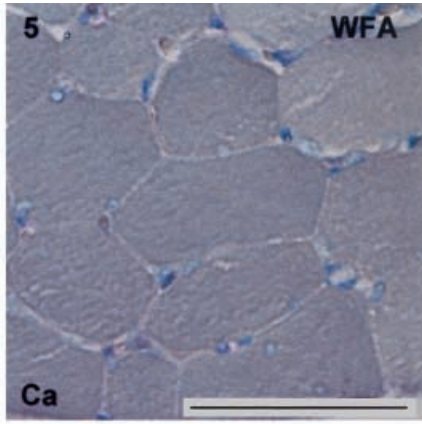
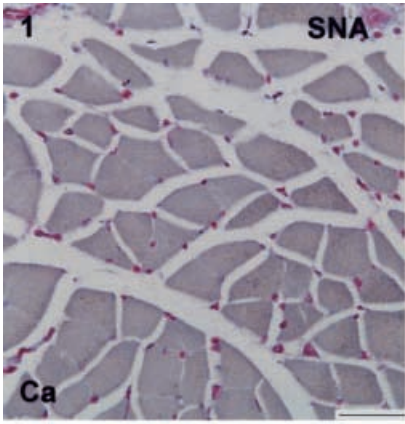
Figs. 5-8 WFA

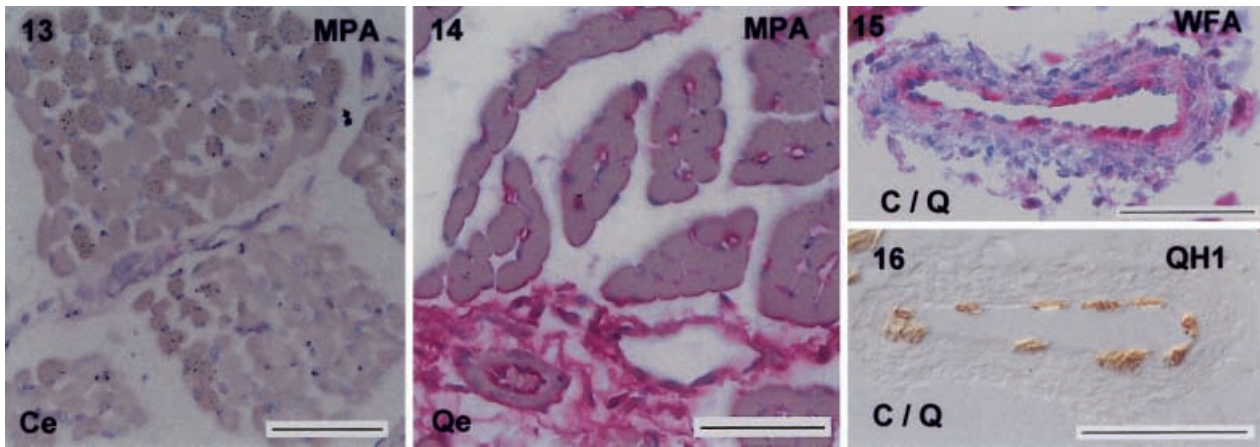
Fig. 9 AIA

Fig. 10 The staining with AIA after inhibition with galactose; the staining of connective tissue is inhibited

Fig. 11 VAA I

Fig. 12 WGA





Figs. 13–14 MPA

Fig. 15 WFA in chick/quail chimera, embryonic day 14; endothelial cells of chick origin are not stained

Fig. 16 A parallel section to Fig. 15 at a distance of 30 μm . Endothelial cells of quail origin are stained with QH-1 Ab (*Nomarski optics*)

Sections on Figs. 1–15 were counterstained with haematoxylin; *black arrows* venules, *white arrows* arterioles. Bar 50 μm

eight lectins (SNA-I, MAA, AIA, VAA-I, VAA-II, VAA-III, WGA and LEA) labelled the endothelium of muscle vessels in both embryonic and adult chick and quail. These lectins are specific for four different terminal monosaccharides (*N*-Acetyl-galactosamine, *N*-Acetylglucosamine, galactose and neuraminic acid). They thus indicate the carbohydrate residues present in endothelial cells and their basal lamina in chick and quail.

Ultrastructural lectin histochemistry carried out in the microvasculature of the optic tectum in the chick (Nico et al. 1998) has shown that *N*-acetylglucosamine and sialic residues are located on the luminal surface of the endothelium. It is well-known that sialic acid is a constant component of the endothelium in many species including bird species (De Bruyn et al. 1978; Henry and DeFouw 1995, 1996). In contrast, glycoconjugates containing β -galactose residues that are involved in the tight junctions and basement membrane were located on the abluminal surface of vessels (Nico et al. 1998).

In 7- μm paraffin wax sections, which were used in our study, it was not possible to distinguish with certainty whether the lectins bind to the endothelial cell only or to the basement membrane or to both of them. Therefore the term endothelium for the endothelial cell and its adjacent basement membrane was used here.

GSA- I (also named BSA-I), which stains the endothelium of blood vessels in many mammalian species (Hansen-Smith et al. 1988), did not bind to the endothelium of the two avian species. However, WGA, which stained the vascular endothelium in ten mammalian species (Alroy et al. 1987), also bound to the endothelium of chick and quail. These results thus show that the lectin-binding pattern of vascular endothelium exhibits

some common features as well as great individual differences across species barriers.

MPA stained the endothelium of the adult but not that of embryonic chick. This difference in staining probably reflects developmentally regulated changes in the distribution of cell surface oligosaccharides and our findings thus corroborate those of Griffith and Sanders (1991) and Nico et al. (1998) in earlier developmental stages.

In addition to the endothelium, the majority of the tested lectins also stained the connective tissue and basement membranes of muscle fibres. The connective tissue was least labelled by SNA-I recognizing sialic acid in the α 2–6 glycosidic linkage (Lawrenson et al. 2000). However, MAA that labels sialic acid in α 2–3 linkage to β -galactose stained connective tissue and basement membranes of muscle fibres intensely. These findings indicate the presence of a delicate tissue specific glycosylation within one organ.

WFA bound to the endothelium of muscle microvessels in embryonic and adult quail, leaving endothelium in embryonic and adult microvessels of the chick muscles unstained. The application of this lectin makes it thus possible to distinguish between endothelium of quail and chick origin and is therefore useful in studies on chick/quail chimeras. During the embryonic period only, MPA could serve the same purpose.

Acknowledgements The authors wish to thank Ms. Maike Ziesenitz, Ms. Eva Kluzáková and Ms. Lenka Hrejsemnou for excellent technical assistance. Our study was supported by the projects VS 97108 and 1111 00003–3G of The Ministry of Education of The Czech Republic and in part by DAAD (Deutscher Akademischer Austauschdienst).

References

- Alroy J, Goyal V, Skutelsky E (1987) Lectin histochemistry of mammalian endothelium. *Histochemistry* 86: 603–607
- Breier G, Breviaro F, Caveda L, Berthier R, Schnurch H, Gotsch U, Vestweber D, Risau W, Dejana E (1996) Molecular cloning and expression of murine vascular endothelial-cadherin in early stage development of cardiovascular system. *Blood* 87: 630–641
- De Bruyn PP, Michelson S, Becker RP (1978) Nonrandom distribution of sialic acid over the cell surface of bristle-coated en-

- docytic vesicles of the sinusoidal endothelium cells. *J Cell Biol* 78: 379–389
- Eichmann A, Marcelle C, Breant C, Le Douarin NM (1996) Molecular cloning of Quek 1 and 2, two quail vascular endothelial growth factor (VEGF) receptor-like molecules. *Gene* 174: 3–8
- Griffith CM, Sanders EJ (1991) Changes in glycoconjugate expression during early chick embryo development: a lectin-binding study. *Anat Rec* 231: 238–250
- Grim M, Carlson BM (1990) Alkaline phosphatase and dipeptidyl-peptidase IV staining of tissue components of skeletal muscle: a comparative study. *J Histochem Cytochem* 38: 1907–1912
- Grim M, Jeřábková G, Tichý M, Smetana K Jr, Gabius HJ (1997) Migration of angiogenic cells from somites and limb primordia of avian embryos. *Histochem J* 29: 715
- Hamburger V, Hamilton HL (1951) A series of normal stages in the development of the chick embryo. *J Morphol* 88: 49–92
- Hansen-Smith FM, Watson L, Lu DY, Goldstein I (1988) *Griffonia simplicifolia* I: fluorescent tracer for microcirculatory vessels in nonperfused thin muscles and sectioned muscle. *Microvasc Res* 36: 199–215
- Hansen-Smith FM, Watson L, Joswiak GR (1989) Postnatal changes in capillary density of rat sternomastoid muscle. *Am J Physiol* 257: H 344–347
- Hansen-Smith FM, Banker K, Morris L, Joswiak G (1992) Alternative histochemical markers for skeletal muscle capillaries: a statistical comparison among three muscles. *Microvasc Res* 44: 112–116
- Henry CB, DeFouw DO (1995) Differential lectin binding to microvascular endothelial glycoconjugates during normal angiogenesis in the chick chorioallantoic membrane. *Microvasc Res* 49: 201–211
- Henry CB, DeFouw DO (1996) Distribution of anionic sites on microvascular endothelium of the chick chorioallantoic membrane. *Tissue Cell* 28: 449–454
- Lawrenson JG, Cassella JP, Hayes AJ, Firth JA, Allt G (2000) Endothelial glycoconjugates: a comparative lectin study of the brain, retina and myocardium. *J Anat* 196: 55–60
- Lojda Z (1979) Studies of dipeptidyl (amino) peptidase IV (glycyl-proline naphthylamidase) II. Blood vessels. *Histochemistry* 59: 153–166
- Mrázková O, Grim M, Carlson BM (1986) Enzymatic heterogeneity of the capillary bed of rat skeletal muscles. *Am J Anat* 177: 141–148
- Nico B, Quondamatteo F, Ribatti D, Bertossi M, Russo G, Herken R, Roncali L (1998) Ultrastructural localization of lectin binding sites in the developing brain microvasculature. *Anat Embryol (Berl)* 197: 305–315
- Pardanaud L, Altmann C, Kitos P, Dieterlen-Lievre F, Buck CA (1987) Vasculogenesis in the early quail blastodisc as studied with a monoclonal antibody recognizing endothelial cells. *Development* 100: 339–349
- Robinson AP, White TM, Mason DW (1986) MRC OX 43: A monoclonal antibody which reacts with all vascular endothelium in the rat except that of brain capillaries. *Immunology* 57: 231–237
- Romanul FDA, Banister G (1962) Localized area of high alkaline phosphatase activity in the terminal arterial tree. *J Cell Biol* 15: 73–84
- Sillau AH, Banchemo H (1977) Visualization of capillaries in skeletal muscle by the ATPase reaction. *Pflugers Arch* 369: 269–271
- Simionescu M, Simionescu N (1986) Functions of the endothelial cell surface. *Annu Rev Physiol* 48: 279–293
- Wall RT, Counts RB, Harker LA, Striker GE (1980) Binding and release of factor VIII-von Willebrand's factor by human-endothelial cells. *Brit J Haematol* 46: 287–298