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Expression of integrin αvß3 in pig, dog and cattle

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Summary. The αvß3 integrin, also known as vitronectin receptor, is an adhesive glycoprotein that promotes angiogenesis in the embryo and tumors such as melanoma. Integrin αvß3 is one of the receptors for adenovirus and hantavirus. There is little information on the constitutive expression of this integrin especially in animal species that are used for biomedical research. We used light and electron microscope immunocytochemistry and western blots to determine integrin αvß3 expression in seven organs in the pig, dog and cattle. Immunohistology showed the integrin expression on the epithelium of small intestine, bile duct and renal proximal convoluted tubules in three species. The airway epithelium revealed a weak reaction for integrin αvß3. Skin showed the integrin in occasional extravascular cells while skeletal muscles were negative. The integrin was expressed only in bronchial vasculature in the lung and occasional dermal microvessels. Many mononuclear cells in the lung and spleen stained for integrin αvß3. Immunogold electron microscopy revealed the expression on the epithelium but not on the vasculature of the small intestine. Western blots detected integrin αvß3 in small intestine and lung but not in skeletal muscles. We conclude the integrin is expressed on the epithelium but not in the vasculature. The expression differs strikingly among organs in the same species although the inter-species differences are minor. Restriction of the integrin to absorptive epithelia of small intestine and kidney may suggest its putative role in endocytosis. Because the integrin is a receptor for adenovirus, these data may be relevant to gene therapy studies.

Key words: Integrin, αvß3, adhesion molecules, epithelium

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Introduction

Adhesion molecules play critical roles in cell physiology and pathology (Albelda et al., 1994; Springer, 1994). These molecules are grouped into three major families i.e. integrins, selectins, and immunoglobulin superfamily members (Albelda and Buck, 1990; Pilewski and Albelda, 1993). Integrins are heterodimers composed of various α and ß units that generate more than 20 distinct forms expressed on a variety of vascular and extravascular cells (Springer, 1994). Integrin subunits are composed of cytoplasmic, transmembrane and extra-cytoplasmic domains (Albelda and Buck, 1990). Integrins have a highly diverse functional repertoire that influences signaling, recruitment, differentiation and death of cells (Giancotti, 1997). For example, ß2 integrins facilitate recruitment of inflammatory cells and αv integrins are implicated in vascular remodeling (Gahmberg et al., 1998).

The αv subunit forms heterodimers with ß1, ß3, ß5, ß6 and ß8 chains (Shattil and Ginsberg, 1997). The αvß3 integrin, also referred to as vitronectin receptor, was identified as an Arg-Gly-Asp (RGD)-binding peptide in 1987 (Cheresh and Spiro, 1987; Lam et al., 1989). This integrin is expressed on vascular and extravascular cells and facilitates their adherence to extracellular matrix components such as fibronectin and vitronectin. Integrin αvß3 drives angiogenesis in embryos and melanoma tumors by down-regulating endothelial cell apoptosis via suppression of p53 activity (Strombald et al., 1996; Peticlerc et al., 1999). This was further supported by the finding that anti-αvß3 monoclonal antibody, LM609, or specific RGD-containing peptides induce endothelial apoptosis to inhibit angiogenesis and growth of chicken embryo and melanoma tumors (Eliceiri and Cheresh, 1999; Storgard et al., 1999). Recent data show that ligation of the integrin with vitronectin activates tyrosine kinase-mediated cell signaling and induces rapid transient increases in lung microvascular permeability (Tsukada et al., 1995). The αvß3 integrin is a cellular receptor for adenovirus, hantavirus, and foot-and-mouth disease virus (Gavrilovskaya et al., 1998; Neff et al., 1998). These data show the functional diversity of the integrin and its implications in various pathological

conditions. However, there is little information available on physiological functions of the integrin. This may partly be because of a lack of precise information on its constitutive expression.

Recently, we reported localization of integrin αvß3 mRNA and protein in the vasculature of seven organs of the rat (Singh et al., 2000); the integrin was found in the microvasculature of lung but not of other organs such as liver, brain and skeletal muscles. These results showed that the integrin is expressed in resting microvessels, contradicting the existing belief that it is present only in dividing microvessels (Damjanovich et al., 1992; Mette et al., 1993). Furthermore, our observations highlighted the inter-organ and inter-species differences in the expression of integrin αvß3. In the present study, we report the αvß3 integrin expression in seven organs of pig, dog and cattle. Our data show striking diversity in the αvß3 integrin expression in various organs of the same species but minor inter-species distinctions.

Materials and methods

Animal and reagents

Small intestine, kidney, liver, lung, skin, spleen and skeletal muscle were collected from dog, cattle and pig (n=2 each). Primary anti-αvß3 (LM609; Chemicon Inc., Temecula, USA) and anti-von Willebrand Factor (vWF; DAKO Corp. Carpinteria, USA) monoclonal antibodies recognize respective antigens in bovine, canine and porcine tissues. The vWF antibody was used to delineate the vasculature which also served as a control. Secondary antibodies conjugated to horseradish peroxidase (HRP) and gold particles were purchased from DAKO Corporation, Carpinteria, USA and Jackson Labs, West Grove, USA, respectively. Color development kits and methyl green stain were obtained from Vector Laboratories, Burlingame, USA.

Light microscopy

Tissue pieces were fixed in 4% paraformaldehyde for 16 hours followed by three washes in phosphate buffer saline (PBS). Tissues were dehydrated in ascending concentrations of ethanol and xylene, and embedded in paraffin. Sections, 5-7 μm, were prepared from three randomly selected tissue blocks and three consecutive sections were placed on slides coated with Vectabond (Vector Laboratories, Burlingame, USA). The tissue slides were kept at 55 °C for 45 minutes in an oven to improve the adherence of sections to glass.

Immunohistology

The sections were deparaffinized and rehydrated in xylene and descending concentrations of ethanol, respectively. Endogenous peroxide was quenched by a 30 minute incubation in 5.0% hydrogen peroxide in methanol. The antigens were unmasked by treating the sections with 2 mg of pepsin/ml of 0.01N HCl for 45 minutes; this incubation time was determined with a series of trials and was found to yield maximum staining. The sections were blocked with 1% bovine serum albumin in PBS for 30 minutes. Adjacent sections were incubated for 90 minutes with monoclonal antibodies against integrin αvß3 (1:75) and vWF (1:300) followed by a 45 minute exposure to appropriate HRP-conjugated secondary antibodies (1:100 to 1:400). The reactions were color developed with a commercial kit. Controls were maintained by omitting either the primary antibody or both primary and secondary antibodies to determine non-specific binding of the secondary antibody and inhibition of endogenous tissue peroxidase, respectively. Localization of blood vessels with anti-vWF antibody provided another control. Some of the sections were counter-stained for 2-4 minutes with methyl green. The slides were examined and images were captured on an image analyses system (Northern Eclipse, Empix Imaging, Toronto, Canada).

Electron microscopy

Small intestine and lungs from the three species were processed for electron microscopy (EM). The tissues were fixed in freshly prepared 0.1% glutaraldehyde plus 2.0% paraformaldehyde in 0.1M sodium cacodylate buffer for 3 hours at 4 °C. After three rinses in the buffer, the tissues were dehydrated in a graded ethanol series at -10 °C and infiltrated with LR White resins (London Resin Company, USA). Then the tissues were embedded in gelatin capsules and polymerized under ultraviolet light at -8 °C for 72 hours. The tissue blocks were trimmed and sectioned to identify representative areas for ultrathin sections that were subsequently placed on nickel grids.

Immunogold labeling

The section grids were floated face-down on drops of 1% ovalbumin for 30 minutes. The sections then were exposed to primary antibody (1:100) for 60 minutes and gold-conjugated secondary antibodies (1:60) for 30 minutes followed by three washes in PBS. Controls were maintained as follows: labeling with anti-vWF antibody (1:200) to mark the endothelial cells; omitting the primary antibody to determine the non-specific binding of the gold conjugate. Some of the sections were stained with uranyl acetate and lead citrate, and were examined at 80 kV with a Philips 410LS transmission electron microscope.

Western Blots

Small intestine, lung and skeletal muscle of pig, stored at -70 °C, were processed for western blots. The tissues were thawed at 20 °C and known weights were homogenized in Hank's Balanced Salt Solution with 2 mM EDTA and 0.1% of polyoxyethylene sorbitan

monolaurate (Tween20; Sigma Co. St. Louis, USA). Homogenates were centrifuged at 10,000 rpm for 10 minutes and the supernatant was collected. Protein concentrations were determined by Bradford method (Bradford, 1976). Equal amounts of proteins were separated on 10% sodium dodecyl sulphate polyacrylamide gel system together with prestained molecular weight standards (Gibco Co. USA). The proteins on the gel were then transferred to 0.2 mm nitrocellulose membrane (Sigma Co. St. Louis, USA). Blots were blocked overnight with Tris-buffered saline containing 0.1% Tween 20 and 5% skim milk powder. Primary anti-αvß3 antibody was added at a dilution of 1:300 in PBST for 1 hr at 20 °C followed by three washes of five minutes each with PBST. The membranes were incubated with goat anti-mouse HRP conjugate (1:1000 in PBST) for 90 minutes, washed three times and exposed to the substrate (4-chloro-1-napthol solution; Sigma Co. St. Louis, USA) for color development. The blots were air dried and stored.

Results

Light microscopy

Small intestine

Negative controls with omission of the primary antibody resulted in the absence of any staining reaction (Fig. 1a). Small intestines of pig, dog and calf revealed intense staining with anti-αvß3 integrin antibody. The integrin was detected on the epithelium but not in other tissue layers such as the muscle (Fig. 1c,e,f). The blood vessels were negative for the integrin. This was further confirmed by delineation of vasculature with anti-vWF antibody in an adjacent section and its comparison with the integrin-stained section (Fig. 1b-e).

Kidney

Glomerulus and vWF-stained branches of renal artery and vein (Fig. 2a) were negative for the integrin (Fig. 2b). The integrin staining was restricted to the proximal convoluted tubules of pig, dog and calf (Fig. 2b-d). Cattle and dog kidneys also showed a similar distinction in the integrin staining of vascular and extra-vascular structures (data not shown).

Liver

Central vein and its branches in the liver of pig reacted with vWF antibody (Fig. 2e) but not with the integrin antibody. Integrin αvß3 was detected on bile duct epithelium of pig and calf but the parenchyma was negative (Fig. 2f,g). Occasional mononuclear cells, probably Kupffer cells, stained positive for the integrin. Dog liver exhibited a staining pattern similar to that in the pig (data not shown).

Lung

Calf and dog lungs demonstrated the αvß3 integrin in systemic (bronchial) blood vessels; bronchiolar airway epithelium showed a faint reaction (Fig. 3a,b). Occasional systemic blood vessels in pig lung stained faintly for the integrin (Fig. 3c). Few mononuclear cells in the parenchyma and alveolar spaces were positive for the αvß3 integrin, which was not detected in pulmonary microvasculature of three species.

Spleen

Only the mononuclear cells in spleen of pig (Fig. 3d), dog and calf (data not shown) stained positive for the integrin.

Skin

Pig skin showed a weak reaction for integrin αvß3 in an occasional microvessel (Fig. 3f) that was highlighted by vWF antibody in an adjacent section (Fig. 3e). Dog and calf showed a similar staining pattern (data not shown). A few extravascular connective tissue cells in three species were positive.

Muscle

The integrin expression was absent in vascular or extravascular components of skeletal muscles of dog (Fig. 3h), pig and calf (data not shown).

Electron microscope immunocytochemistry

Immunogold EM revealed labeling with gold particles in the epithelium of the small intestine of pig, dog (Fig. 4a,b) and calf (data not shown). Gold particles were present in the cytoplasmic vesicles and on the apical surface of the epithelium. Vascular endothelium in small intestine was negative for the integrin (Fig. 4c). In contrast, bronchial blood vessels were positive and mostly a negative reaction was noted in airway epithelium (data not shown).

Western blots

To further confirm the microscopy data and the specificity of the antibody, we performed western blots on small intestine, lung and skeletal muscles of pig. Western blots probed with anti-αvß3 monoclonal antibody showed a single band of approximately 170 kD for the integrin in small intestine and lung. Skeletal muscle revealed a very faint or no band (Fig. 5).

Discussion

We report diversity in the organ expression of integrin αvß3 in pig, dog and cattle. To our knowledge, this is the first survey of constitutive expression of the

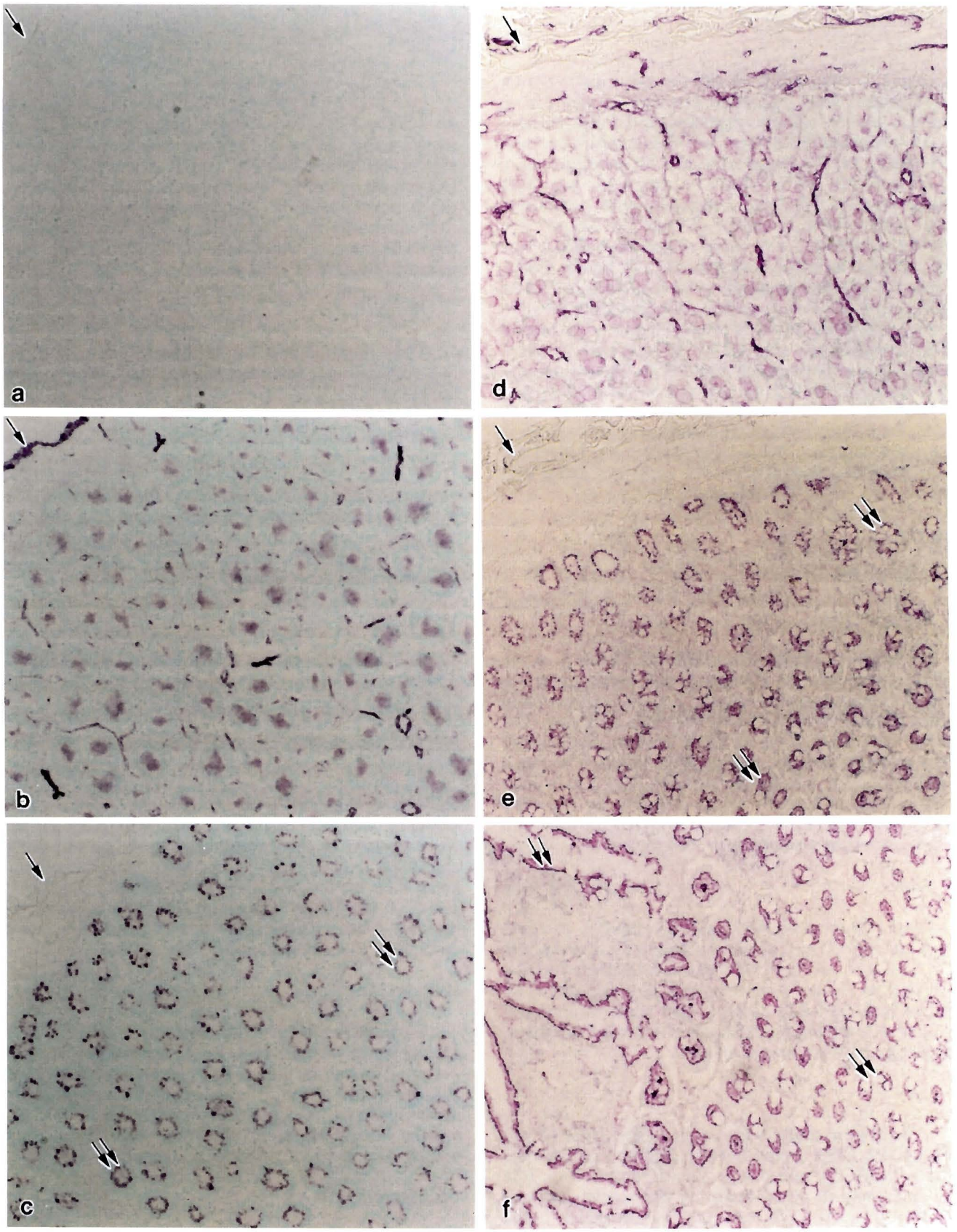

Fig. 1. Negative control demonstrates absence of reaction after omission of primary antibody **(a)**. Integrin αvß3 is depicted on the epithelium (double arrows) of small intestine from a pig, cattle and dog **(c, e and f)**. Adjacent sections from pig and cattle small intestine show blood vessels (single arrows) reactive with vWF **(b, d)**. x 100

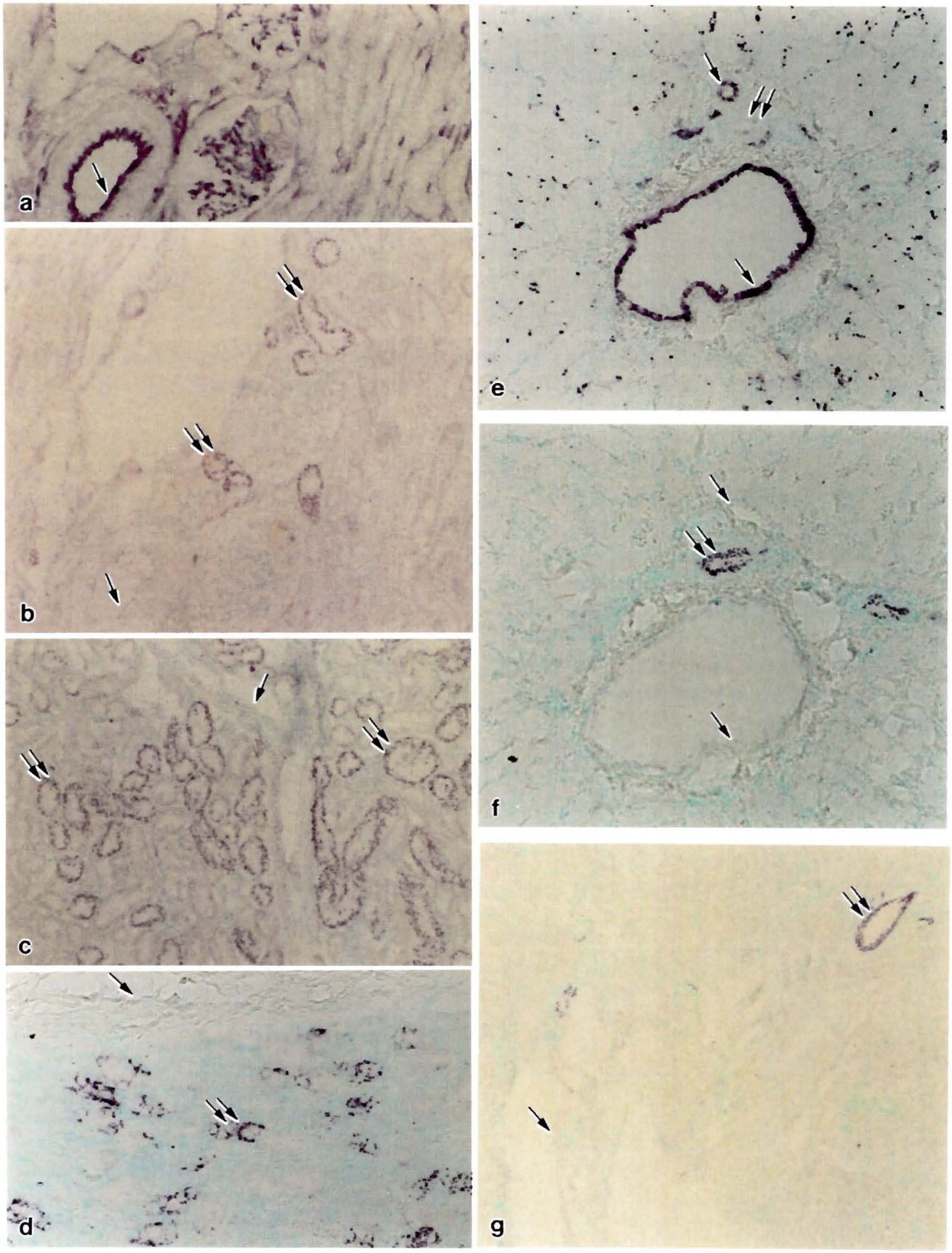

Fig. 2. Vasculature (single arrows) in pig kidney reacted with vWF antibody **(a)** but with the integrin antibody in an adjacent section **(b)**. Integrin αvß3 is present on convoluted tubule epithelium of pig **(b)**, dog **(c)** and calf **(d)**. Pig liver **(f)** shows integrin avß3 in bile duct (double arrows) but not in vasculature (single arrow) that is reactive with vWF antibody in an adjacent section **(e)**. Calf liver **(g)** also revealed the integrin in bile duct epithelium (double arrows) but not in blood vessels (single arrows). x 100 (a-d), x 200 (e-g)

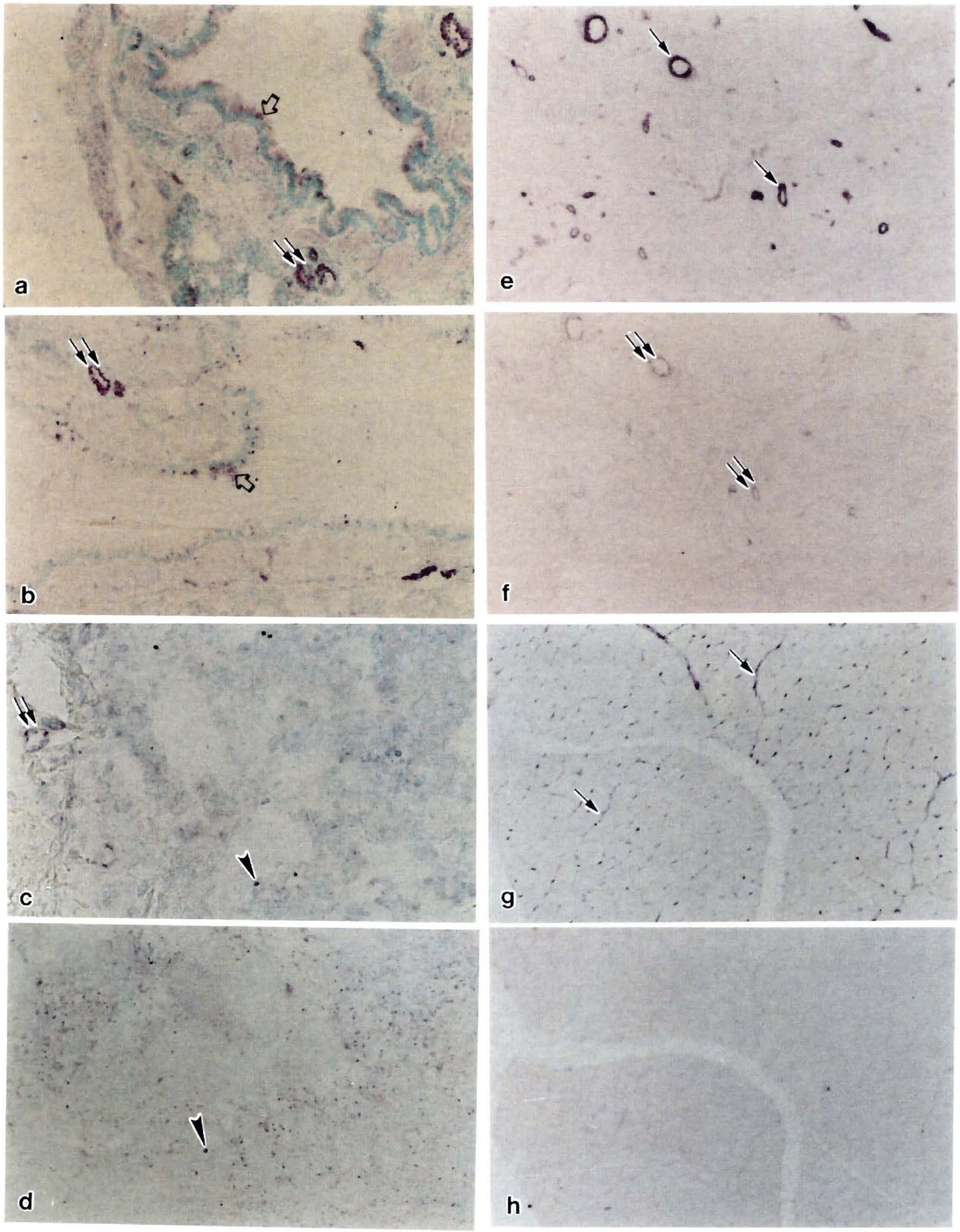

Fig. 3. The αvß3 integrin staining is intense in bronchiolar vasculature (double arrows) of cattle and dog **(a, b)** but weak in pig lung **(c).** Airway epithelium shows faint staining (hollow arrows). Mononuclear cells (arrowheads) in lung **(c)** and spleen **(d)** of pig reacted with the integrin antibody. Skin vasculature that reacted with vWF (single arrow, **e**) also stained with the integrin (double arrows, **f**). Vasculature in the skeletal muscle reacted for vWF **(g)** but not for the integrin **(h)**. x 100

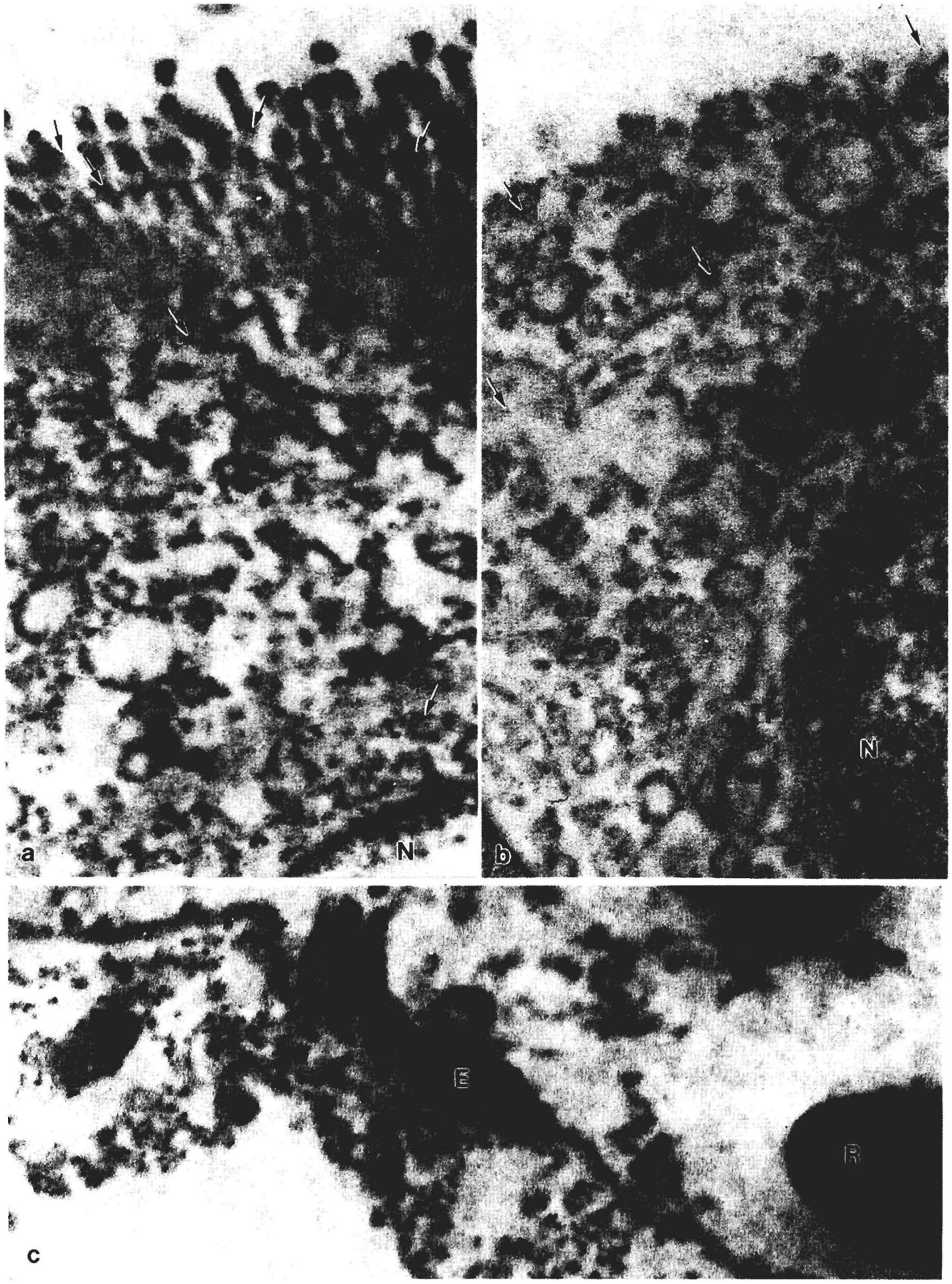

Fig. 4. Immunogold electron microscopy showed the integrin in pig **(a)** and dog **(b)** small intestine epithelium (single arrows). Endothelial cells (E) of the vasculature were negative **(c)**. N: nucleus; R: red blood cell. a, x 25,000; b, x 40,000, c, x 25,000

integrin in seven organs of three animal species that have distinct physiological and immune responses such as those in the lung (Longworth et al., 1992; Staub, 1994). Pig is an omnivore, cattle are herbivores and the dog is primarily a carnivore. While dog and pig are monogastric species, cattle are polygastric (ruminant). These species are also of significant economic and biomedical importance; pigs are one of the preferred models to study organ transplantation and adenovirus-based gene therapy (Patel et al., 1999).

The integrin expression was mostly restricted to the epithelium of small intestine, kidney and bile duct of these species. Immunogold EM confirmed extensive expression of integrin αvß3 in small intestinal epithelium. The integrin was generally absent in blood vessels in these organs except bronchial vasculature. Skeletal muscles were negative for the anti-αvß3 antibody. Western blot data provided further evidence for the heterogeneity in the integrin expression in small intestine, lung and muscle of pig.

A novel finding in the present study is the intense expression of the αvß3 integrin restricted to the epithelium of small intestine, renal proximal convoluted tubules and bile ducts. Small intestine and convoluted tubules constitute major absorptive surfaces in digestive and urinary tracts, respectively. The absorptive and endocytic events are either receptor-mediated or are constitutive (Sandvig et al., 1993). The receptors present on the apical surface of cells transport ligands rapidly to intracellular sites and cycle between plasma membrane and cytoplasmic organelles (Dunn and Maxfield, 1992). The integrin expressed on the apical surface under physiological conditions may facilitate transport of various molecules into, and across the epithelial cells (Giancotti, 1997; Giancotti and Ruoslahti, 1999; Parolari et al., 1999). The association of the integrin with caveolin-1 further strengthens its putative involvement in endocytosis since caveolin-1 is intimately involved in this process (Parton et al., 1994; Giancotti and Ruoslahti, 1999; Minshall et al. 2000). Moreover, this integrin is known to be a receptor for a diverse range of ligands such as viruses and vitronectin (Gavrilovskaya et al., 1998). Currently, these roles of the integrin remain to be clarified in the context of the epithelium.

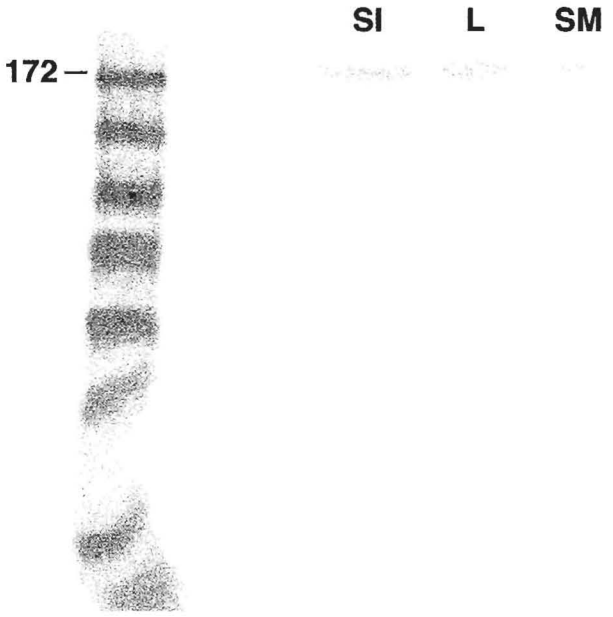

Fig. 5. Western blots detected the integrin in small intestine (SI), lung (L) but showed a very faint band in skeletal muscle (SM) of pig.

The proteins expressed on various cells impart them distinct antigenic characteristics. Selective expression of integrin αvß3 on the epithelium may represent one such antigenic distinction. These antigenic distinctions can be exploited for gene and protein delivery (Herari et al., 1999). This integrin is a cellular receptor for adenoviruses that are employed as vectors for gene transfection (Gavrilovskaya et al., 1998; Neff et al., 1998; Patel et al., 1999). Abundant expression of the integrin in small intestinal epithelium and renal convoluted tubules makes it an attractive target for adenovirus-mediated gene transfection in these organs. Interestingly, some of the gene delivery trials via the oral route, also a convenient route of administration, have shown a high rate of transfection in the gut epithelium (Kaner et al., 2000). On the other hand, inefficient gene transfer in airway epithelium in cell culture with the same vectors has been attributed to the absence of αvß3/αvß5 integrins (Pickels et al., 1998). Now, we report in situ data to demonstrate that the αvß3 integrin is nearly absent in the airway epithelium but abundantly present in the gut epithelium of pig, cattle and dog. Therefore, vectors such as adenoviruses that use integrin αvß3 as a receptor may be more suitable for gene transfer in the gut epithelium of these species.

Surface expression of distinct proteins may prove useful in directing drugs or proteins to specific organs. Recently, platelet endothelial cell adhesion molecule-1 (PECAM-1; CD31) present on the apical surface of lung microvascular endothelium was used as a target. In these experiments, glutathione oxidase conjugated to an anti-PECAM-1 antibody was successfully delivered to lung microvascular endothelium (Christofidou-Solomidou et al., 2000). The organ differences in the integrin expression, skin and skeletal muscle versus small intestine and kidney, may be suitable for organ-specific delivery of genes, drugs or other molecules under physiological and pathological states. These comparative data on the constitutive expression of the integrin may provide a more rational basis for gene and drug delivery experiments.

The integrin was absent in the vasculature of small intestine, kidney, liver, skeletal muscles and spleen in our study. However, some expression was noted in bronchial blood vessels and a few microvessels in the skin. The findings support the prevailing views that the integrin is mostly expressed on proliferating microvascular endothelium but not on resting

endothelium (Friedlander et al., 1995; Eliceiri et al., 1998; Peticlerc et al., 1999). Previous reports show the presence of αvß3 integrin on podocytes and Bowman's capsule and the αv subunit was localized on endothelial cells of glomeruli and major blood vessels (Patey et al., 1994; Kreidberg and Symons, 2000). Because we have not noticed expression of integrin αvß3 in renal vasculature, the αv localization reported previously may be associated with a subunit other than ß3. This calls for cautious interpretation of data on the expression of a single chain, αv, as indicative of a specific dimer since it can combine with multiple ß chains. However, absence of the integrin in lung microvasculature in three species differs from our recent observations in that it is present mainly in lung microvascular endothelium of rats (Singh et al., 2000). We do not know the precise reasons for this discrepancy but it may be because of inter-species differences. Further, we used retired male breeder rats in the previous study compared to young animals in the present experiments. It is possible that the age may alter constitutive expression of the integrin in lung and other organs.

Considerable evidence exists for the involvement of integrin αvß3 in microvascular pathology in various organs (Roy-Choudhury et al., 1997). The integrin activates signals in endothelial cells that have profound implications for their survival and microvascular permeability (Tsukada et al., 1995; Strombald et al., 1996). The integrin-mediated cells signals are initiated following ligation by molecules such as vitronectin and use tyrosine kinase pathway and proteins such as Shc (Tsukada et al., 1995; Giancotti and Ruoslahti, 1999; Bhattacharya et al., 2000). However, very little is known about similar physiological and pathological functions of the integrin expressed on epithelia. Recent data show that integrins such as α4ß7 along with cytokines facilitate recruitment and retention of gut epithelium-associated T lymphocytes (Hayday and Viney, 2000). It is possible that integrin αvß3 present in the epithelium may regulate functions such as cell death and barrier integrity, and thus it requires further investigations.

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References

Albelda S.M. and Buck C.A. (1990). Integrins and other cell adhesion molecules. FASEB J. 4, 2868-2880.

Albelda S.M., Smith C.W. and Ward P.A. (1994). Adhesion molecules and inflammatory injury. FASEB J. 8, 504-512.

Bhattacharya S., Ying X., Fu C., Patel R., Kuebler W.M., Greenberg S. and Bhattacharya J. (2000). alpha(v)beta(3) integrin induces tyrosine phosphorylation-dependent Ca(2+) influx in pulmonary endothelial cells. Circ. Res. 86, 456-462.

Bradford M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem. 72, 248-254.

Cheresh D.A. and Spiro R.C. (1987). Biosynthetic and functional properties of an arg-gly-asp-directed receptor involved in human melanoma cell attachment to vitronectin, fibrinogen, and von Willebrand factor. J. Biol. Chem. 262, 17703-17711.

Christofidou-Solomidou M., Pietra G.G., Solomides C.C., Arguiris E., Harshaw D., Fitzgerald G.A., Albelda S.M. and Muzykantov V.R. (2000). Immunotargeting of glucose oxidase to endothelium in vivo causes oxidative vascular injury in the lungs. Am. J. Physiol. 278, L794-L805.

Damjanovich L., Albelda S.M., Mette S.A. and Buck C.A. (1992). Distribution of integrin cell adhesion receptors in normal and malignant lung tissue. Am. J. Respir. Cell. Mol. Biol. 6, 197-206.

Dunn K.W. and Maxfield F.R. (1992). Delivery of ligands from sorting endosomes to late endosomes occurs by maturation of sorting endosomes. J. Cell Biol. 117, 301-310.

Eliceiri B.P. and Cheresh D.A. (1999). The role of alpha-v integrins during angiogenesis: insights into potential mechanisms of action and clinical development. J. Clin. Invest. 103, 1227-1230.

Eliceiri B.P., Klemke R.L., Strombald S. and Cheresh D.A. (1998). Integrin αvß3 requirement for sustained mitogen-activated protein kinase activity during angiogenesis. J. Cell Biol. 140, 1255-1263.

Friedlander M., Brooks P.C., Shaffer R.W., Kincaid C.M., Varner J.A. and Cheresh D.A. (1995). Definition of two angiogenic pathways by distinct αv integrins. Science 270, 1500-1502.

Gahmberg C.G., Valmu L., Kotovuori P., Ihanus E., Tian L. and Pessa-Morikawa T. (1998). Leukocyte integrins in inflammation. Cell Mol. Life Sci. 54, 549-555.

Gavrilovskaya I.N., Shepley M., Shaw R., Ginsberg M.H. and Mackow E.R. (1998). ß3 integrins mediate the cellular entry of hantaviruses that cause respiratory failure. Proc. Natl. Acad. Sci. USA 95, 7074-7079.

Giancotti F.G. (1997). Integrin signaling: specificity and control of cell survival and cell cycle progression. Curr. Opin. Cell Biol. 9, 691-700.

Giancotti F.G. and Ruoslahti E. (1999). Integrin signaling. Science 285, 1028-1033.

Hayday A. and Viney J.L. (2000). The ins and outs of body surface immunology. Science 290, 89-92.

Herari O.A., Wickham T.J., Stocker C.J., Kovesdi I., Segal D.M., Huehns T.Y., Serraf C. and Haskard D.O. (1999). Targeting an adenoviral gene vector to cytokine-activated vascular endothelium via E-selectin. Gene Ther. 6, 801-807.

Kaner R.J., Ladetto J.V., Singh R., Fukuda N., Matthay M.A. and Crystal R.G. (2000). Lung over-expression of the vascular endothelial growth factor gene induces pulmonary edema. Am. J. Respir. Cell Mol. Biol. 22,657-664.

Kreidberg J.A. and Symons J.M. (2000). Integrins in kidney development, function, and disease. Am. J. Physiol. 279, F233-F242.

Lam S.C-T., Plow E.F., D'Souza S.E., Cheresh D.A., Frelinger A.L. and Ginsberg M.H. (1989). Isolation and characterization of a platelet membrane protein related to the vitronectin receptor. J. Biol. Chem. 264, 3742-3749.

Longworth K.E., Westgate A.M., Grady M.K., Westcott J.Y. and Staub N.C. (1992). Development of pulmonary intravascular macrophages function in newborn lambs. J. Appl. Physiol. 73, 2608-2615.

Mette S.A., Pilewski J., Buck C.A. and Albelda S.M. (1993). Distribution

of integrin cell adhesion receptors on normal bronchial epithelial cells and lung cancer cells in vitro and in vivo. Am. J. Respir. Cell Mol. Biol. 8, 562-572.

Minshall R.D., Tiruppathi C., Vogel S.M., Niles W.D., Gilchrist A., Hamm H.E. and Malik A.B. (2000). Endothelial cell-surface gp60 activates vesicle formation and trafficking via G(i)-coupled Src kinase signaling pathway. J. Cell Biol. 150, 1057-1070.

Neff S., Sa-Carvalho D., Rieder E., Mason P.W., Blystone S.D., Brown E.J. and Baxt B. (1998). Foot-and-Mouth disease virus virulent for cattle utilizes the integrin αvß3 as its receptor. J. Virol. 72, 3587-3594.

Parolari A., Guarnieri D., Alamanni F., Toscano T., Tantalo V., Gherli T., Colli S., Foieni F., Franze V., Stanghellini M., Giancotti G.A., Bigioli P. and Tremoli E. (1999). Platelet function and anesthetics in cardiac surgery: An in vivo and ex vivo study. Anesth. Analg. 89, 26-31.

Parton R.G., Joggerst B. and Simons K. (1994). Regulated internalization of caveolae. J. Cell Biol. 127, 1199-1215.

Patel S.R., Lee L.Y., Mack C.A., Polce D.R., El-Sawy T., Hackett N.R., Ilercil A., Jones E.C., Hahn R.T., Isom O.W., Rosengart T.K. and Crystal R.G. (1999). Safety of direct myocardial administration of an adenovirus vector encoding vascular endothelial growth factor 121. Hum. Gene Ther. 10, 1331-1348.

Patey N., Halbwachs M., Droz D., Lesavre P. and Noel L.H. (1994). Distribution of integrin subunits in normal human kidney. Adhesion Commun. 2, 159-167.

Peticlerc E., Strombald S., von Schalscha T.L., Mitjans F., Piulats J., Montgomery A.M.P., Cheresh D.A. and Brooks P.C. (1999). Integrin alpha-v beta-3 promotes M21 melanoma growth in human skin by regulating tumor cell survival. Cancer Res. 59, 2724-2730.

Pickels R.J., McCarty D., Matsui H., Hart P.J., Randell S.H. and Boucher R.C. (1998). Limited entry of adenovirus vectors into well-differentiated airway epithelium is responsible for inefficient gene transfer. J. Virol. 72, 6014-6023.

Pilewski J. and Albelda S.M. (1993). Adhesion molecules in the lung: an overview. Am. Rev. Resp. Dis. 148, S31-S37.

Roy-Choudhury P., Hillis G., McDonald S., Simposon J.G. and Power D.A. (1997). Importance of the tubulointerstitium in human glomerulonephritis. II. Distribution of integrin chains beta 1, alpha 1 to 6 and alpha V. Kidney Int. 52, 103-110.

Sandvig K., Garred O., Holm P.K. and van Deurs B. (1993). Endocytosis and intracellular transport of protein toxins. Biochem. Soc. Trans. 21, 707-715.

Shattil S.J. and Ginsberg M.H. (1997). Integrin sigaling in vascular biology. J. Clin. Invest. 100, 1-5.

Singh B., Fu C. and Bhattacharya J. (2000). Vascular expression of the αvß3 integrin in lung and other organs. Am. J. Physiol. 278, L217-L226.

Springer T.A. (1994). Traffic signals for lymphocyte recirculation and leukocyte emigration: The multistep paradigm. Cell 76, 301-314.

Staub N.C. (1994.) Pulmonary intravascular macrophages. Annu. Rev. Physiol. 56, 47-67.

Storgard C.M., Stupack D.G., Jonczyk A., Goodman S.L., Fox R.I. and Cheresh D.A. (1999). Decreased angiogenesis and arthritic disease in rabbits treated with an αvß3 antagonists. J. Clin. Invest. 103, 47-54.

Strombald S., Becker J.C., Yebra M., Brooks P.C. and Cheresh D.A.. (1996). Suppression of p53 activity and $p21^{WAF/CIP1}$ expression by vascular cell integrin αvß3 during angiogenesis. J. Clin. Invest. 98, 426-433.

Tsukada H., Ying X., Fu C., Ishikawa S., McKeown-Longo P.J., Albelda S.M., Bhattacharya S., Bray B.A. and Bhattacharya J. (1995). Ligation of endothelial αvß3 integrin increases capillary hydraulic conductivity of rat lung. Circ. Res. 77, 651-659.

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