Immunology 1993 **79** 341–347

Identification and quantification of complement regulator CD46 on normal human tissues

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Accepted for publication 25 February 1993

SUMMARY

CD46 is a cell-surface regulatory molecule that prevents lysis of autologous human cells by activated complement. It has been well characterized on leucocytes, reproductive cells and various cultured cell lines and is considered to be ubiquitously expressed. We now extend these analyses and describe CD46 in a variety of different human tissues. Strong expression was observed by immunohistology on epithelial cells lining exocrine ducts and glands, such as salivary gland and pancreas and on kidney tubules and glomerular epithelium. Quantitative tissue expression was measured by radioimmunoassay and confirmed histological observations. Thus, CD46 is highly expressed on cells in contact with extracellular fluids thought not to contain large quantities of complement but which may still be subjected to complement attack thereby necessitating the presence of complement regulators to prevent non-specific destruction of cells.

INTRODUCTION

CD46 [membrane cofactor protein (MCP)] is one of a family of complement regulatory molecules which prevent the destruction of autologous cells by activated complement.[1-3] It acts as a cofactor for the factor I-mediated cleavage of activated complement components C3b and C4b, thus preventing the cascade of reactions resulting in the formation of the membrane attack complex.[4,5] CD46 prevents complement amplification only on the cells on which it is expressed,[6] and does not protect neighbouring cells. Complement regulators C4 binding protein (C4bp) and Factor H are inhibitors that prevent spontaneous complement activation in the fluid phase, while CR1 can protect cells on which it is expressed and can further protect neighbouring cells.[7-9] CD46 exists on the surface of human peripheral blood lymphocytes (PBL) as several different protein isoforms which arise due to alternative splicing of the RNA, with two prevalent species of 66,000 MW (α) and 56,000 MW (β).[1,10,11] The expression of these two species is under autosomal codominant control and is assosicated with *Hin*dIII/*Pvu*II/*Bgl*II

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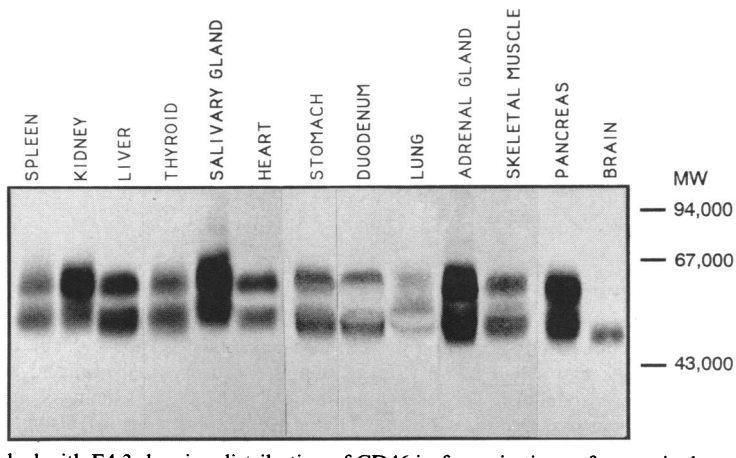

Figure 1. Western blot probed with E4.3 showing distribution of CD46 isoforms in tissues from a single cadaver. Relative molecular mass is shown on the right.

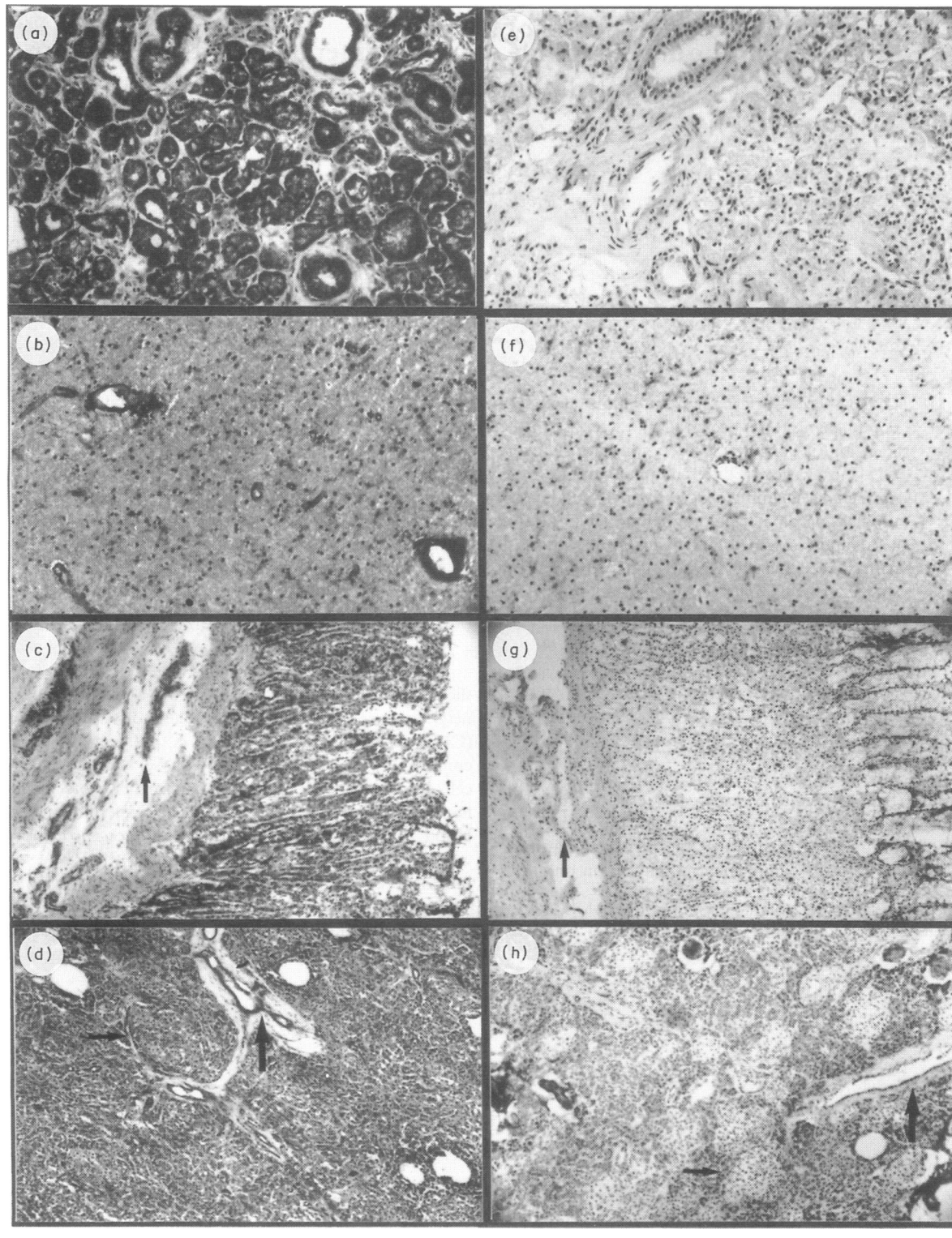

Figure 2. Tissues were examined immunohistochemically for CD46 using mAb E4.3 and isotype control antibody 1302, against mouse Ly-2.1 antigen. Anti-CD46-stained sections are indicated on the left hand panel (a–d ×90; i–l ×360), control anti-Ly-2.1-stained sections are on the right panels (e–h ×90; m–p ×360). (a) Very strong staining of salivary gland acini and ductile epithelium. (b) Faint staining of cerebral neurones with stronger staining of blood vessel endothelium. (c) Moderate staining of intestinal mucosal epithelium and blood vessel endothelium in the submucosa (arrowed) with little staining of connective tissue. (d) Strong staining of pancreatic acini and ductule epithelium (large arrow) and of islet cells (small arrow).

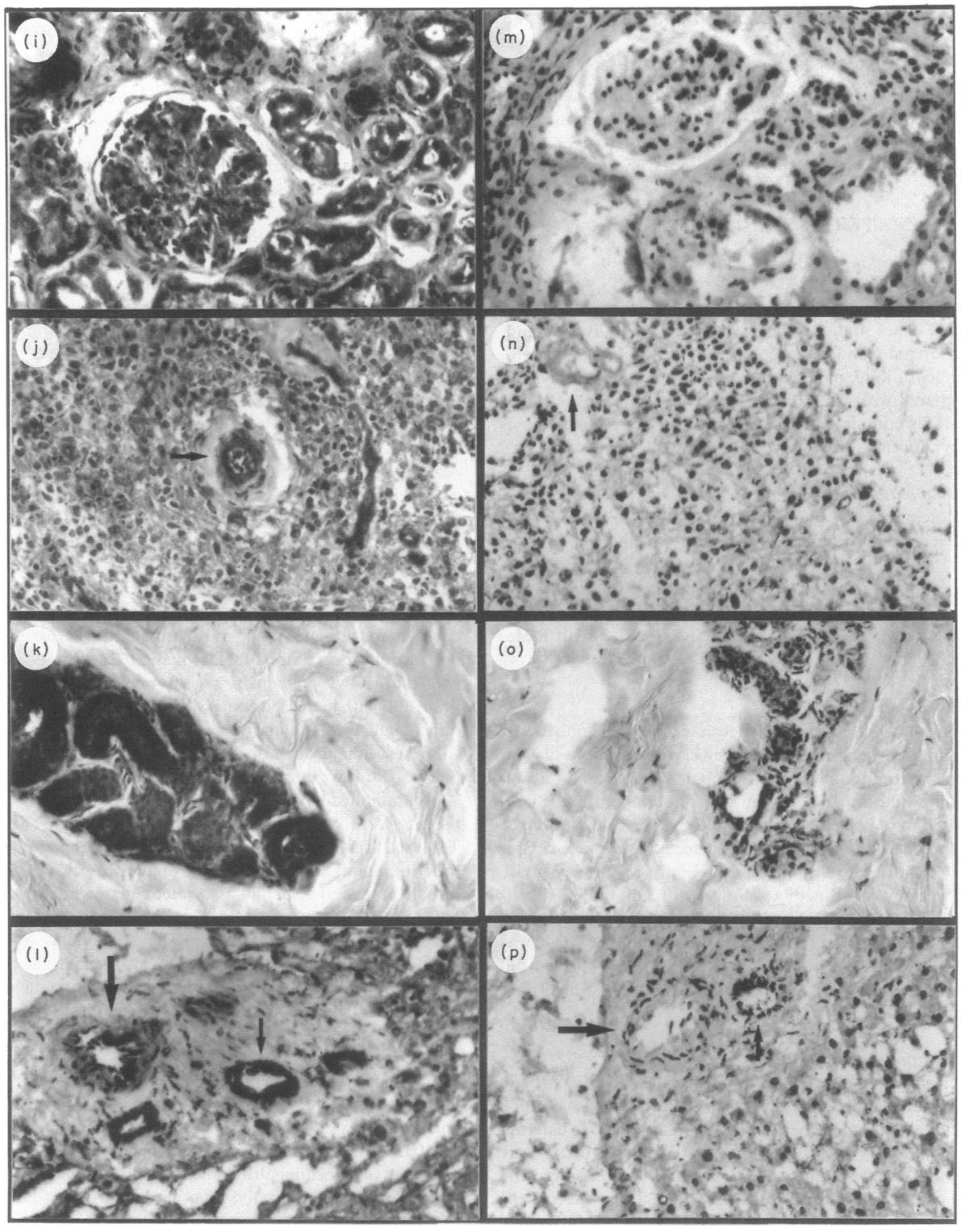

Figure 2. (*continued*) (i) Intense staining of glomerular endothelium and ductule epithelium in the kidney. (j) Weak staining of splenic lymphocytes was observed with stronger staining of vascular endothelium (arrowed). (k) Strong staining of sebaceous gland epithelium in skin with little/no staining of surrounding connective tissue. (l) Strong staining of bile duct epithelium in the liver (small arrow), weaker staining of hepatic artery endothelium (large arrow) and hepatocytes. Similar results were obtained with anti-CD46 mAb M177 and 1305 isotype control.

restriction fragment length polymorphism (RFLP) in genomic DNA.[12,13] The presence of CD46 has been reported on different cells including leucocytes,[5,14] placental cells,[15] human cell lines [16] and reproductive cells.[17,18]

A comprehensive study of the distribution of CD46 on tissue types from a single cadaver was carried out by immunostaining with monoclonal antibody E4.3. A sensitive radioimmunoassay was used to quantify the amount of CD46 for a given tissue sample and is shown to be consistent with the immunohistology data.

MATERIALS AND METHODS

Tissue

Tissue samples from a cadaver were obtained ~5 hr *post mortem* and tissue blocks were excised and snap frozen in liquid nitrogen. Tissues studied include: brain, spleen, salivary gland, kidney, adrenal gland, liver, colon, stomach, duodenum, skin, thyroid, heart, lung, pancreas and skeletal muscle. Cell lysates were prepared with 0·5% Triton X-100 in 10 mM Tris, 0·15 M NaCl, pH 7·4 containing 1 mM EDTA and 1 mM phenylmethylsulphonyl fluoride.

Monoclonal antibodies (mAb)

The following mAb were used: E4.3 (IgG2a)[19] and M177 (lgG1)[20] react with different epitopes on CD46; 1302 (IgG2a) and 1305 (IgG1) to mouse Ly-2.1[21] were used as non-reactive isotype controls. All antibodies were purified on protein A–Sepharose (Pharmacia, Uppsala, Sweden). For radioimmunoassays, M177 was labelled with ^{125}I (Amersham, North Ryde, Australia).[22]

Immunostaining

Small blocks of frozen tissue were placed in OCT-embedding compound (Lab-Tek, Eikhart, IN) and cryostat sections of 7 μm thickness were mounted and incubated with mAb at an appropriate dilution for 60 min at room temperature. Slides were rinsed in phosphate-buffered saline (PBS) and binding of primary mAb to tissue sections was detected using peroxidase-conjugated anti-mouse Lg (Sigma, St Louis, MO). Peroxidase activity was developed with a solution of 0·5 mg/ml 3,3′diaminobenzidine-tetrahydrochloride (DAB: Sigma) and 0·01% H_2O_2 in Tris–HCl buffer (0·5 M, pH 7·6). Staining for CD46 was quantified by independent researchers with a scale scoring from ++++ indicating very strong staining to +/− which indicates little or no staining.

Immunoblotting and immunoassay

Cell lysates from tissue samples were standardized for protein concentration[23] and analysed by immunoblotting and in a quantitative radioimmunoassay for CD46. For protein determination, 5 μl aliquots of lysates serially diluted in water were mixed with 200 μl Bradford Reagent[24] in a 96-well microtitre plate, incubated at room temperature for 20–60 min and the optical density was determined at A_{595} on a Titre Tek automated plate reader (Bio-Tek, Winooski, VT).

Cell lysates were separated by SDS-PAGE under non-reducing conditions.[24] Proteins were electroblotted onto Immobilon P membrane (Millipore, Bradford, MA) and protein binding sites were blocked with 2·5% w/v casein in PBS. CD46 was detected using mAb E4.3, followed by incubation with alkaline phosphatase-conjugated anti-mouse IgG (Silenus, Hawthorn, Australia) and visualized with 5-bromo-4-chloro-3-indoyl phosphate/nitro blue tetrazolium (Promega, Madison, WI).

For the radioimmunoassay, 50 μl/well of E4.3 mAb at 2 μg/ml in carbonate/bicarbonate buffer pH 9·6 was added to the wells of a microtitre plate and incubated overnight at 4°, washed in PBS pH 7·6 (×3) and blocked with 200 μl/well 4% bovine serum albumin (BSA) in PBS for 2 hr at 37°. Plates were then washed three times and 50 μl aliquots of serially diluted tissue lysates, quantified and equilibrated for total protein, were added. These were incubated at room temperature for 4 hr, washed 10 times and the bound CD46 was quantified by addition of radiolabelled M177 mAb using 100,000 c.p.m./well. Unbound M177 was removed by washing 10 times, plates were dried and individual wells were analysed for bound ^{125}I with a γ-counter (LKB-Wallac, Uppsala, Sweden).

RESULTS

CD46 protein isoforms were expressed in all tissues studied with bands at 56,000 and 66,000 MW by Western blot (Fig. 1). According to previous criteria,[25] this individual has been classified as having the αβ protein phenotype due to the equal expression of the 66,000 and 56,000 MW isoforms on the surface of PBL.[26] These CD46 isoforms occur due to alternative splicing of exons encoding extracellular protein rich in Ser/Tho/Pro residues[10,11] which are potential sites of O-linked glycosylation; they are not part of the complement binding region nor are they epitopes for either E4.3 or M177 mAb.[20,27] The tissue-specific polymorphic expression of CD46 α and β isoforms is consistent with that shown previously,[26] with only the β form present in brain and with increased expression of the α form in kidney and salivary gland. The relative intensities of CD46 bands range from very intense in salivary and adrenal gland, kidney and pancreas to least intense in lung and brain.

Immunohistochemical analyses of a range of different tissues showed varied cellular expression of CD46 (Fig. 2 and Table 1). CD46 is strongly expressed on epithelial cells lining exocrine glands and ducts. Very strong expression was observed on salivary gland acini and associated ducts, while very little or no staining of the connective tissue capsule or septa was apparent (Fig. 2a,e). The staining was most intense on internal luminal surfaces. Strong staining was also seen in the pancreas (Fig. 2d,h) where expression on the epithelium in exocrine glands and ducts was slightly stronger than that seen on the islet cells. In skin sections, strong to moderate expression of CD46 was observed on the glandular cells of the dermis, weak expression was seen on epithelial cells within the stratum basale and there was little staining of other epithelial cells of the epidermis (Fig. 2k,o). In the kidney, strong to moderate staining for CD46 was observed on glomerular epithelium and on epithelial cells forming distal and proximal convoluted tubules, the loop of Henle and collecting ducts (Fig. 2i,m).

Heterogeneous expression of CD46 was observed in liver tissue, with strong to moderate staining of hepatocytes and bile duct epithelium, moderate staining of hepatic artery endothelium and weak staining of portal vein endothelium (Fig. 2l,p). The expression of CD46 within the gastrointestinal tract was similar in the stomach, duodenum and colon with moderate staining of mucosal epithelium, weak staining of blood vessel endothelium in the submucosa and smooth muscle of the

Table 1. Expression of CD46 by immunohistochemistry

Tissue	Cells	Expression
Salivary gland	Ductal epithelium	++++
	Acinar cells	++++
Pancreas	Exocrine ducts	++++
	Islet of Langerhans'	+++
	Acinar cells	++++
Kidney	Glomerular capillaries	+++
	Glomerular epithelial cells	+++
	Proximal tubules	+++
	Distal tubules	+++
	Collecting ducts	+++
Liver	Hepatocytes	++/+++
	Bile duct	++++
	Hepatic artery endothelium	++
	Portal vein endothelium	+
Lung	Brochi/bronchioli	++
	Alveoli	++
Skin	Distal epithelium	+/−
	Basal epithelium	+
	Dermal glandular epithelium	
Gastrointestinal tract	Mucosal epithelium	+++
	Submucosal vascular endothelium	++
	Muscularis myofibres	+
	Adventitia	+/−
Endocrine glands	Adrenal epithelium	++/+++
	Thyroid epithelium	++
Spleen	Lymphocytes	+
	Vascular endothelium	++
Brain	Neurones	+/++
	Vascular endothelium	++
Muscle	Visceral	+
	Cardiac	+
	Skeletal	+

muscularis mucosa and little or no staining within the outer connective tissue layer (Fig. 2c,g).

CD46 was expressed within endocrine glands, with moderate staining of adrenal cortical epithelial tissue and the epithelial cells of the thyroid. Examination of normal lung sections revealed moderate staining of alveolar, bronchial and bronchiolar epithelium. There was faint staining of lymphocytes within the spleen, stronger staining of the central arterial endothelium and little or no staining of connective tissue trabeculae (Fig. 2j, n). In the brain, weak staining of the cell body, axons and dendrites of neurones was observed while there appeared stronger staining on blood vessel endothelium (Fig. 2b,f). Only faint staining of cardiac and skeletal muscle tissue was observed.

Quantitative radioimmunoassays on tissue lysates, standardized for total protein concentration, were performed to determine the relative amounts of CD46 present in each tissue sample (Fig. 3). The results are given as radioactive counts bound per 2 mg protein in the lysate. Briefly, salivary gland, pancreas, adrenal gland and kidney expressed the largest amounts of CD46 compared with the other tissues analysed. This compares well with the immunohistochemical findings presented above and with the immunoblot data of Fig. 1. Moderate expression was observed in liver and spleen. Tissues from the gastrointestinal tract (stomach, duodenum, colon) contained similar amounts of CD46. Very small amounts of CD46 were found in skin and skeletal and cardiac muscle samples. A high expressing transfected cell line (5.3) and negative Chinese hamster ovary (CHO) cell line were included as positive and negative controls.

DISCUSSION

In previous studies, we and others have shown that CD46 is present on the surface of leucocytes,[5,14] sperm,[17] ova,[18] on a range of cultured cell lines[16] and in body fluids.[28] This study represents the first comprehensive analysis of CD46 distribution on a range of different cell types in a variety of normal tissues. Western blot analysis revealed polymorphic expression of different molecular weight isoforms of CD46 as had been shown in preliminary studies.[26] Immunohistochemistry with mAb E4.3

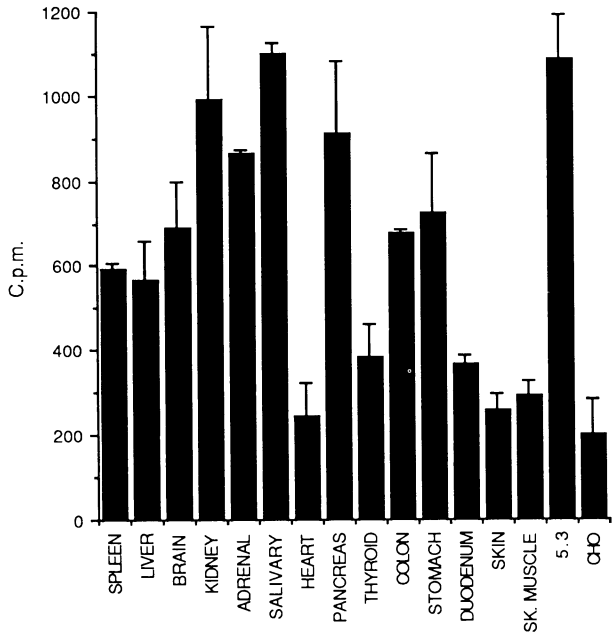

Figure 3. Quantitative radioimmunoassay on tissue lysates with mAb [^{125}I]E4.3 and M177. Lysates and equivalent total protein concentrations with results given as c.p.m. bound (means of triplicates). 5.3 was a positive control lysate of CHO cells transfected with CD46 cDNA, with a mock-transfected CHO cell lysate as a negative control.

and M177 revealed wide-ranging expression of CD46 with a distinct tissue-specific pattern from very strong to very little, though no tissues were definitely negative. There was strong staining on most epithelial tissues; acinar epithelium and cells lining exocrine ducts appeared to express the highest amounts of CD46, particularly in salivary gland and pancreas. Strong staining of glomerular and ductule epithelium was observed in kidney samples and on epithelial cells lining merocrine sweat glands and sebaceous glands. Very little or no staining of the connective tissue components collagen and elastin, or of fibroblasts, was seen and expression on skeletal, cardiac and visceral muscle cells was uniformly low. Epithelial cells lining blood vessels in all tissues were found to express moderate to low amounts of CD46.

Sections from tissues of the gastrointestinal tract, including stomach, small intestine and colon, had similar staining patterns. Moderate to strong staining of mucosal epithelial cells, weaker staining of submucosal endothelial and muscularis mucosal visceral muscle cells and little or no staining of connective tissue components was observed. Analysis of both thyroid and adrenal gland sections revealed strong to moderate staining of glandular epithelium and blood vessel endothelium.

The quantitative assessment of CD46 in each tissue sample by immunochemistry and immunoblotting was compared with a radioimmunoassay. This assay showed the presence of large amounts of CD46 in salivary gland, adrenal gland, pancreas and kidney with little CD46 found in skin and muscle samples. Thus, the tissues containing a high density of glandular epithelial cells contained the largest amounts of CD46 per mg total protein. The intensity of staining of exocrine gland epithelial cells was strongest on the luminal surfaces of the cells and may be due to cell differentiation and/or maturation, or due to polarization of membrane proteins to the exterior cell surface.[29]

The presence of CD46 on glandular and luminal epithelia may reflect the particular need for regulators of complement activation on cells exposed to extracellular fluids other than serum, or that CD46 has another, as yet unidentified, physiological role. There is very little soluble CD46 in saliva, or tears or urine compared to that present in plasma.[28] Thus, there appears to be little cleavage of CD46 from epithelial cells on the luminal surface of salivary gland acini or kidney tubules, and CD46 produced by these cells remains membrane bound.

The results presented in this study show similarities between the tissue distribution of CD46 and expression of the complement regulators CD55 (DAF)[30] and CD59.[31] In particular, there appears to be high expression of CD46, CD55 and CD59 on exocrine glandular cells, especially on the luminal surfaces of these cells. Thus, there is similar tissue expression of at least three of the regulators of complement activation which could act co-ordinately to prevent complement-mediated injury to epithelial and glandular cells. These cells are often exposed to microbial organisms and must survive the body's (relatively non-specific) immune mechanisms that constitute the first line of defence.

ACKNOWLEDGMENTS

Tissue samples were supplied by Professor R. Sinclair from the Department of Pathology at the Austin Hospital. This work was supported by the National Health and Medical Research Council of Australia. R. Johnstone is a recipient of an Australian Postgraduate Research Award.

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