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Tissue distribution of the guinea-pig decay-accelerating factor

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SUMMARY

MCA44 is a monoclonal antibody (mAb) to guinea-pig decay-accelerating factor (DAF) and, using this mAb, tissue distribution of guinea-pig DAF was studied by immunofluorescence. Guinea-pig DAF was found to be expressed not only on the vascular endothelium but also on different types of cells, such as the tubular epithelium of the kidney, epidermal cells of the skin and synovial lining cells. As there was no significant reduction in staining intensity with MCA44 following treatment with phosphatidylinositol-specific phospholipase C, many guinea-pig DAF molecules expressed in these tissues may be of the transmembrane form.

INTRODUCTION

Prevention of complement activation on self cell membranes by species-specific membrane inhibitors of complement results in discrimination of invading micro-organisms on which complement can react without such restriction. In humans, decay-accelerating factor (DAF)[1–3] and membrane cofactor protein (MCP)[4] restrict amplification of complement activation at the C3 convertase steps, and homologous restriction factor/C8-binding protein[5,6] and CD59 (20-kDa homologous restriction factor; HRF20)[7–9] restrict formation of membrane attack complexes at the terminal step of the complement cascade.

We have produced monoclonal antibodies (mAb) that suppress the function of complement regulatory membrane proteins of humans (1F5),[10,11] rats (5I2)[12,13] and guinea-pigs (MCA44).[14] The target molecules of these mAb have previously been characterized as HRF20 (CD59), 5I2Ag (rat Crry) and guinea-pig DAF, respectively. The mAb against rat Crry enabled us to study the effects of functional suppression of the complement regulatory membrane protein, 5I2Ag, *in vitro* and *in vivo*.[15–19] MCA44 has the capacity to sensitize neuraminidase-treated guinea-pig erythrocytes for haemolysis by homologous guinea-pig complement,[14] and recognizes membrane glycoproteins of 55 kDa, 70 kDa and 88 kDa, which are isotypes of guinea-pig DAF generated by alternative splicing of mRNA.[20] Although human DAF is present on cell membranes with a glycosyl phosphatidylinositol (GPI) anchor, more than one-half of MCA44 antigen (guinea-pig DAF) molecules on lymphocytes were found to be resistant to treatment with phosphatidylinositol-specific phospholipase C (PIPLC).[14] This is confirmed to be caused by the presence of a membrane-spanning type of guinea-pig DAF in addition to the GPI-anchored type generated by the alternative splicing.[20]

We describe here the tissue distribution of guinea-pig DAF in various organs as determined by immunofluorescence using MCA44 mAb.

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Abbreviations: DAF, decay-accelerating factor; GPI, glycosyl phosphatidylinositol; MCP, membrane cofactor protein; PIPLC, phosphatidylinositol-specific phospholipase C.

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MATERIALS AND METHODS

Specimens

Organs were taken from female Hartley guinea-pigs purchased from Chubu Kagaku Shizai (Nagoya, Japan). Tissues of brain, lung, heart, thymus, liver, pancreas, spleen, stomach, small intestine, colon, kidney, adrenal gland, ovary, uterus, skin and synovium were snap-frozen in liquid nitrogen and kept at −80° until used.

Immunofluorescence analysis

Frozen sections were cut at 4 μm thickness and fixed in acetone at room temperature for 10 min. They were incubated with MCA44 (20 μg/ml, immunoglobulin G1 (IgG1) isotype) followed by fluoresceinated rabbit anti-mouse IgG antibodies (Cappel Laboratories, Westchester, PA). For control experiments, an isotype-matched mAb directed against rat Crry (5I2) was used. Sections were examined with an Olympus epifluorescence microscope (Olympus Optics Co. Ltd., Tokyo, Japan).

PIPLC treatment of sections

To analyse the sensitivity of guinea-pig DAF to PIPLC treatment, sections were incubated in phosphate-buffered saline (PBS) containing up to 300 mU/ml PIPLC (Toa Gousei Chemical Industry Co. Ltd., Tokyo, Japan) at 37° for 1 hr prior to fixation in acetone. The effect of PIPLC was

determined by examining the level of human DAF on peripheral lymphocytes following staining with anti-human DAF mAb 1C6 (kindly provided by Dr T. Fujita of Fukushima Medical University). The effect of PIPLC was studied further by incubating rat kidney sections with 300 mU/ml PIPLC at 37° for 1 hr before treatment with OX-7, a mAb against rat Thy-1 and, following treatment with fluorescein-labelled anti-mouse IgG, examining the level of Thy-1.

RESULTS

Distribution of guinea-pig DAF

Guinea-pig DAF was expressed on the endothelium of all organs studied (see Tables 1 and Figs 1, 2 and 3).

The distribution of DAF on tissues, other than the endothelium, in various organs is listed in Table 2. Organs such as brain, lung, heart, liver and pancreas were stained with MCA44 on the endothelium, and faint staining on hepatocytes was observed (Fig. 1). In addition to the endothelium MCA44 stained glomerular epithelium, mesangial cells and some types of tubular epithelium of the kidney (Fig. 2). The epithelial cells of the bladder (Fig. 3a), the oocyte of the ovary (Fig. 3b) and endometrium of the uterus were stained with MCA44. MCA44 stained skin epidermal cells, sebaceous glands and hair roots as well as the endothelium (Fig. 3c). Synovial lining cells strongly expressed DAF (Fig. 3d). No staining was observed in any control experiments where an isotype-matched mAb 5I2 directed to rat Crry was used instead of MCA44.

PIPLC treatment

Tissue sections of all the organs studied were treated with PIPLC before staining with MCA44. No significant reduction in staining was observed. However, PIPLC treatment of rat kidney sections with monoclonal anti-Thy-1 antibody (OX-7), whose antigen is anchored via GPI to the cell membranes, significantly reduced the staining intensity.

DISCUSSION

We successfully obtained a mAb that sensitizes guinea-pig erythrocytes for haemolysis by homologous guinea-pig complement.[21] The mAb, MCA44, was demonstrated to be directed to guinea-pig DAF, which is the guinea-pig counterpart of human DAF.[14] Although human DAF is a GPI-anchored

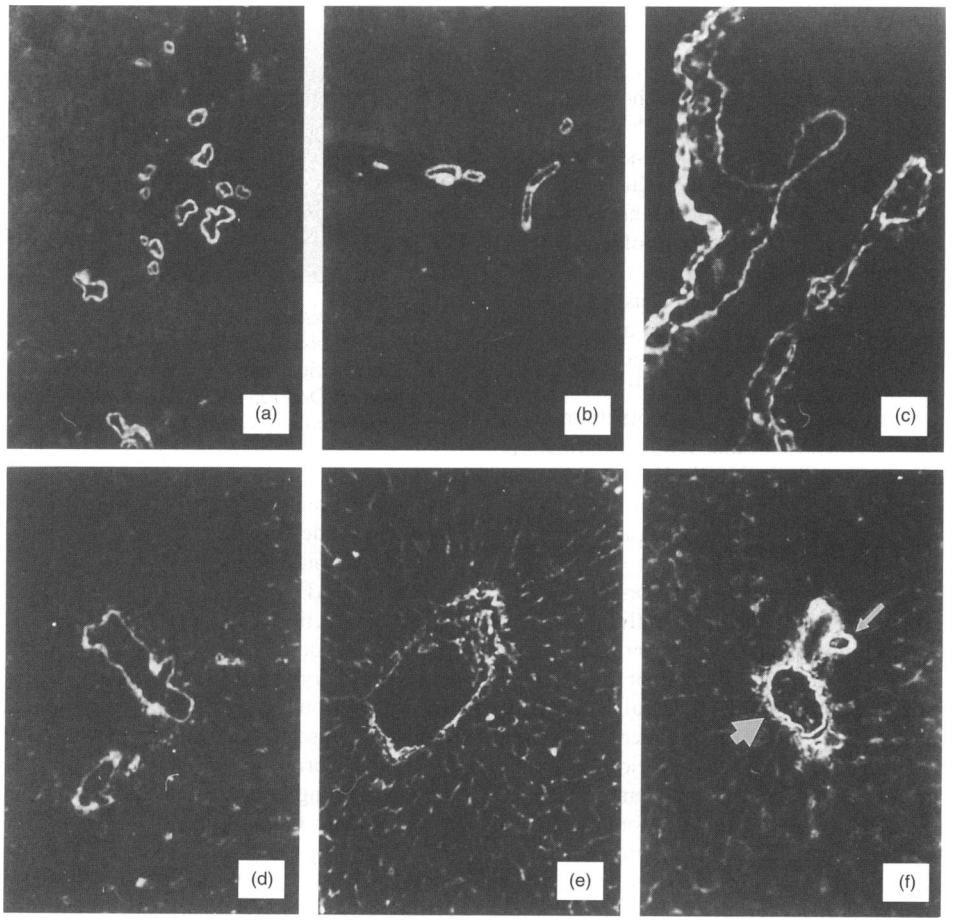

Figure 1. Guinea-pig DAF is expressed on the vascular endothelium of the cerebrum (a), cerebellum (b) and capillaries of the choroid plexus (c) in the brain. Expression of guinea-pig DAF is seen on the vascular endothelium and capillaries of the heart (d). Guinea-pig DAF is faintly detectable on hepatocytes (e) and (f). DAF is expressed on the central vein (e), the portal vein (large arrow) and hepatic arteries (small arrow) (f) as well as the sinusoidal endothelium in the liver. (a) and (b) ×200; (c), (d), (e) and (f) ×400.

Table 1. Summary of positive expression of guinea-pig delay-accelerating factor (DAF) on the endothelium in organs

Organ	Location of DAF
Brain	Vessels in cerebrum, cerebellum, choroid plexus
Heart	Coronary artery, capillary
Aorta	Endothelium, vasa vasorum
Lung	Alveolar capillary
Thymus	Vessels
Liver	Sinusoid capillary, hepatic artery, potal vein central vein,
Pancreas	Capillary
Spleen	Central artery, travecular vein
Stomach	Submucosal vessels
Small intestine	Submucosal vessels
Colon	Submucosal vessels
Kidney	Glomerular endothelium, peritubular capillary (weak), vascular bandles in medulla
Bladder	Submucosal vessels
Adrenal gland	Capillary in cortex
Ovary	Vessels
Uterus	Vesses
Skeletal muscle	Capillary
Skin	Vessels in dermis
Synovium	Vessels

membrane protein, guinea-pig DAF has been shown to have both GPI-anchored and transmembrane forms.[14,20] Therefore, it will be of value to determine the tissue distribution of guinea-pig DAF by comparison with that of human DAF. Human DAF is present on some types of epithelial cells as well as on vascular endothelium and the surface of blood cells. Medof *et al.* reported that DAF was present on the epithelial surface of cornea, conjunctiva, oral and gastrointestinal mucosa, exocrine glands, renal tubules, ureter and bladder, cervical and uterine mucosa, and synovial serosa in humans, as determined by immunoperoxidase staining.[22] Guinea-pig DAF was present not only on the vascular endothelium in all of the organs examined, but also on renal tubular epithelium, epithelium of the bladder, epidermal cells in the skin and synovial lining cells. However, the distribution of DAF in guinea-pigs was more restricted than in humans, although the significance of this difference remains unclear.

DAF is considered to be present on the vascular endothelium, as well as on the surface of blood cells, to protect these cells from plasma complement.[23] However, guinea-pig DAF was detected on some cells that are not in intimate contact with plasma complement under normal conditions. Plasma proteins including complement components and even immune complexes under pathological conditions may be trapped in glomeruli in the kidney, and complement may be activated locally in the glomeruli. DAF on glomerular epithelial cells and mesangial cells may play a protective role against locally activated complement.[24] DAF's presence on the renal tubular epithelium may be necessary to protect the cells from complement components in urine under proteinuric conditions or from complement easily activated through the alternative pathway in the medulla under conditions of physiologically high concentrations of ammonia.[25,26] Guinea-pig DAF was found to be distributed on the epithelial cells of the bladder. Upon bacterial invasion of the bladder, complement can act to destroy the foreign organisms while the DAF-expressing epithelial cells are protected from complement. In addition, DAF on the bladder epithelium may also play a role in self-protection from uric complement components under proteinuric conditions. Skin is the outermost barrier to invasion by foreign micro-organisms. As frequent complement activation would occur here to prevent infection with invasive micro-organisms, DAF expression on epidermal cells may be essential to protect these cells from complement components. Synovial lining cells are in contact with synovial fluid but not with plasma. Complement components exist in the synovial fluid and are activated in cases of synovitis.[27] Moreover, synovial cells are able to synthesize the complement components by themselves.[28,29] DAF expression on the synovial lining cells may protect these cells from complement components in the synovial fluid.

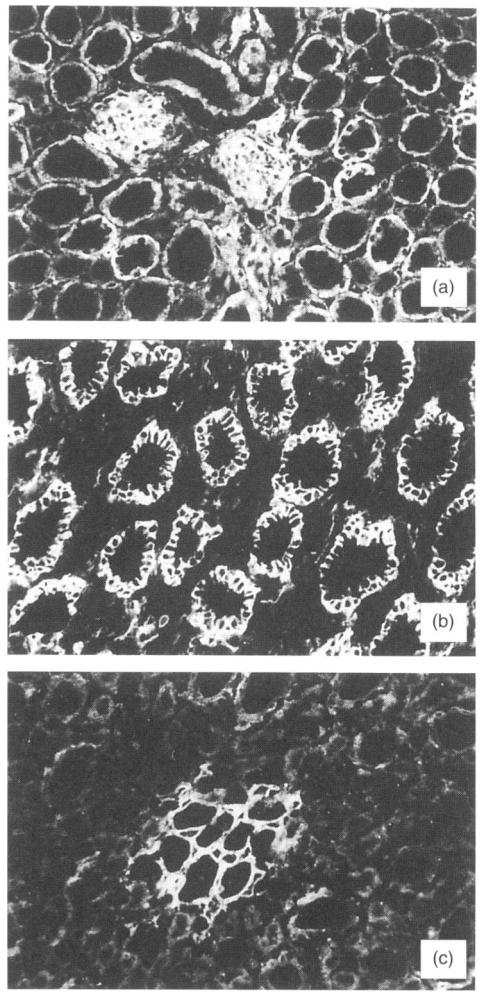

Figure 2. Expression of guinea-pig DAF in the kidney. Binding of mAb MCA44 is seen in the glomeruli, on the proximal tubules and weakly on peritubular capillaries in the renal cortex (a). There is expression of DAF on the collecting tubules (b) and vascular bundles (c) in the medulla of the kidney. (a), ×200; (b) and (c), ×400.

Experimental models are required to examine the physiological and/or pathological roles of complement regulatory membrane proteins. In mice, in addition to DAF,[30,31] Crry/p65

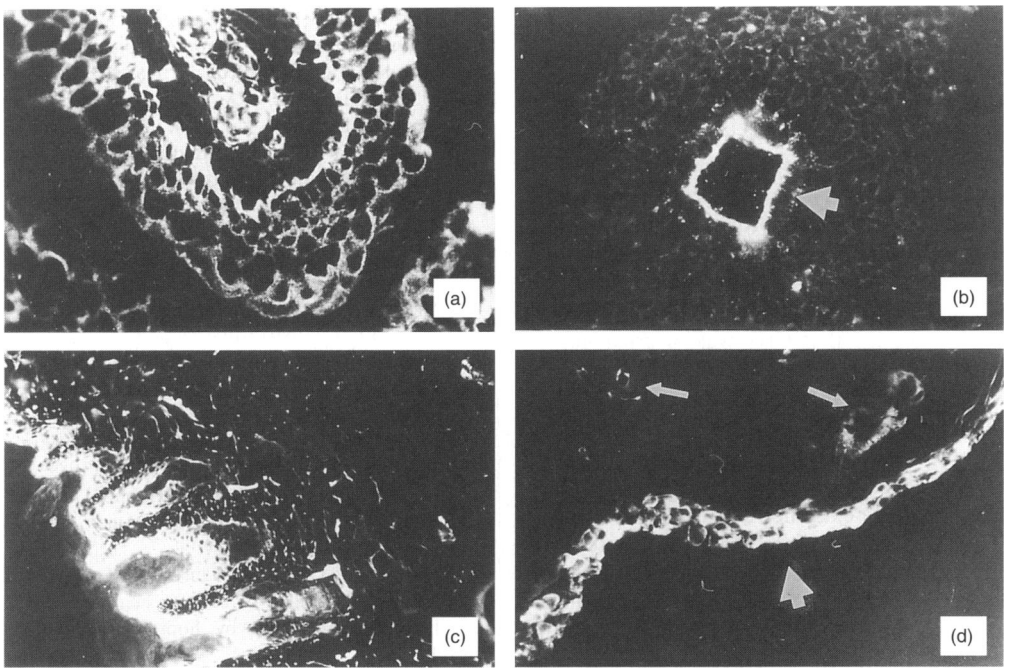

Figure 3. Expression of guinea-pig DAF in the bladder (a), the ovary (b), the skin (c) and the synovium of the knee joint (d). DAF is detectable on the epithelial cells of the bladder (a). There is expression of DAF on the oocyte (arrow) but not on the follicular cells of the ovary (b). DAF is expressed on epidermal cells and capillaries of the dermis of the skin (c). DAF is expressed on the synovial lining cells (large arrow) and vascular endothelium (small arrows) in the synovium (d). (a) and (b), ×400; (c) and (d), ×200.

Table 2. Summary of the distribution of guinea-pig delay-accelerating factor (DAF) in tissues other than endothelium

Tissue	Location	Presence/absence
Brain	Cerebral and cerebellar cortex	−
	Epithelium of choroid plexus	−
Heart	Heart muscle cells	−
Aorta	Tunica media	−
Lung	Alveoli	−(capillary: +)
	Bronchiole	−
	Bronchus (smooth muscle)	−
Thymus	Thymocyte	+(Hassal's body: ++)
	Septa	++
Liver	Hepatocyte	+/−
Pancreas	Islets of Langerhans	−
	Exocrine portion	−
Stomach	Mucosal layer	−
Small intestine	Mucosal layer	−
Colon	Mucosal layer	−
Kidney	Glomerular epithelium and mesangium	++
	Tubular epithelium	++
Bladder	Epithelium	++
Adrenal gland	Glandular cells	−
Ovary	Oocyte	++
	Follicular cells	−
Uterus	Endometrium	+
Skeletal muscle	Muscle cells	−
Skin	Sebaceous gland	+(epidermal cells: ++)
Synovium	Synovial lining cells	++

−, negative; +/−, faintly positive; +, positive; ++, strongly positive.

was identified as a functional counterpart of human MCP and DAF.[32,33] These have all been identified as complement regulatory membrane proteins that act at the C3 convertase level. We have produced a mAb, 5I2,[12,34] that suppresses the function of 5I2Ag, which is a rat counterpart of mouse Crry, and studied the effects of functional suppression of rat Crry/p65 with 5I2 *in vivo*. We found that systemic injection of rats with 5I2 caused symptoms resembling endotoxin shock,[17] isolated perfusion of rat kidney with 5I2 caused interstitial nephritis[18] and intradermal injection with 5I2 elicited marked plasma protein leakage and cellular infiltration at the inoculation site.[19] These results suggested that Crry was of great importance in maintaining normal integrity in rats. Intracutaneous administration of MCA44, the mAb against guinea-pig DAF, induced acute inflammation as determined by exudation of Evans Blue dye in the bloodstream.[14]

Recently, we isolated several clones of guinea-pig DAF cDNA and demonstrated the existence of various isoforms of the protein.[20] They included transmembrane and secreted forms in addition to the GPI-anchored form. Analysis with reverse transcription–polymerase chain reaction (RT–PCR) on several tissues and cells indicated that the isotypes are ubiquitously expressed. Although we expected that some tissues might preferentially express the GPI-anchored form of DAF, we could not find any tissues that lost DAF expression following treatment with PIPLC. As we had no other mAb to use against a guinea-pig GPI-anchored membrane protein, release of human DAF from lymphocytes and Thy-1 from rat kidney sections was examined to confirm PIPLC activity. These controls did indeed confirm the activity of the PIPLC used in this study. Therefore, the fact that many guinea-pig DAF molecules are of transmembrane form should be considered when using guinea-pigs as models to study the *in vivo* role of human DAF molecules, as DAF in humans is expressed only as the GPI-anchored form on the membranes. This interpretation was substantiated by the finding that more than one-half of the DAF mRNA from several guinea-pig tissues was of the transmembrane form, as determined by RT–PCR.[35]

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