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Chemical composition and tissue distribution of the human CDw44 glycoprotein

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SUMMARY

The CDw44 glycoprotein was purified from $2{\cdot}3 \times 10^{11}$ $CD3^+$ $CD4^+$ $CD8^-$ T-chronic lymphocytic leukaemia (CLL) cells using F10-44-2 monoclonal antibody affinity chromatography, DEAE–Sepharose anion-exchange chromatography, passage down carboxymethyl (CM)–Sepharose cation-exchange columns, wheat germ lectin affinity chromatography and gel-permeation chromatography. On elution in non-ionic detergents from the DEAE column, two distinct peaks of antigen activity were obtained. The CDw44 glycoprotein in each peak was a glycoprotein of 85,000 MW, but the amino acid composition of the peaks was noticeably different. Carbohydrate compositions showed that each peak contained approximately 30% (w/w) carbohydrate, the composition suggesting both 0-linked and complex N-linked glycans. Modulation studies with the F10-44-2 antibody on normal peripheral blood mononuclear cells (PBMC) demonstrated that the CDw44 glycoprotein of T cells consisted of one fraction that was readily modulated, and the other which was resistant to modulation. Detailed tissue distribution studies for CDw44 were performed using the F10-44-2 antibody on frozen sections of human tissues. CDw44 has a restricted tissue distribution, but is found on many highly diverse cell types (e.g. T lymphocytes, smooth muscle cells, some secretory glands, skin epithelial cells).

INTRODUCTION

There has been a great deal of interest in recent years in membrane glycoproteins specific to particular lineages of cellular differentiation, because these molecules are most likely to be involved in the specialized functions of the cells on which they are expressed. However, in most instances, the cell surface markers have not been truly specific to individual lineages of differentiation. Among T lymphocytes, for example, the Thy-1 molecule is expressed in the central nervous system (Reif & Allen, 1964) and the CD4 and CD8 molecules on macrophage- and dendritic-type cells (e.g. Crocker *et al.*, 1987). The CD5 molecule is expressed on some B cells (Caligaris-Cappio, Gobbi & Janossy, 1982). In the B-lymphocyte system, many cell surface markers are shared with kidney tissue (Jones *et al.*, 1988).

Many cell types might share the same functional requirements mediated by a particular molecule of restricted tissue distribution. However, the same molecule might mediate different functions in different tissues, the specificity being consequent on the uniqueness of the individual microenvironments in which the molecule functions (e.g. IL-1 in leucocytes and brain; Breder, Dinarells & Saper 1988). In addition, molecular heterogeneity within what initially appears to be a homogeneous population of molecules might offer subtle opportunities for functional differentiation within families of related molecules.

Some years ago, we described a T-lymphocyte membrane glycoprotein of MW around 100,000, now designated CDw44, which has an interesting and relatively restricted tissue distribution (Dalchau, Kirkley & Fabre, 1980a) and is coded for by the short arm of chromosome 11 (Goodfellow *et al.*, 1982). On the basis of similarity of molecular weight and presence of on brain and leucocytes, it was originally thought that this glycoprotein might be homologous to the W3/13 antigen (leukosialin) of the rat (Standring *et al.*, 1978), but this is now known not to be the case.

During T-lymphocyte differentiation, CDw44 appears late, being found only in the thymus medulla and on peripheral T cells (Dalchau *et al.*, 1980a). During myelopoiesis, however, it is present in highest amounts in the earlier stages of differentiation (Morstyn *et al.*, 1981). In the colon, the antigen is found in colonic epithelial cells in the crypts of Lieberkuhn, but is lost as these cells migrate out of the crypts on to the villi (Daar & Fabre, 1983). In the central nervous system, the antigen has a slightly lower molecular weight and is found exclusively in the white

Abbreviations: BSA, bovine serum albumin; CLL, chronic lymphocytic leukaemia; DOC, sodium deoxycholate; PAGE, polyacrylamide gel electrophoresis; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; RAM, immunoadsorbent purified rabbit $F(ab')_2$ anti-mouse $F(ab')_2$; RBC, red blood cell; SDS, sodium dodecyl sulphate; TBS, Tris-buffered saline.

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matter, being completely absent from the grey matter (McKenzie, Dalchau & Fabre, 1982). Letarte and co-workers (Letarte, Iturba & Quackenbush, 1985; Letarte, 1986; Quackenbush & Letarte, 1985) and Haynes and co-workers (Telen, Eisenbarth & Haynes, 1983; Telen, Palker & Haynes, 1984; Haynes *et al.*, 1983) have described monoclonal antibodies which, on the basis of reactivity of exchanged antibodies on purified molecules, all react with the same glycoprotein. Interestingly, CDw44 might be identical to the pgp-1 glycoprotein of phagocytic cells (Omary *et al.*, 1988) and might also be involved in endothelial cell recognition and lymphocyte homing (Jalkanen *et al.*, 1986).

Letarte and co-workers have demonstrated that the MW of CDw44 in B lymphocytes and kidney is 85,000, while in brain and erythrocytes it is 80,000. Such small differences, as previously demonstrated between peripheral blood T cells and brain (McKenzie *et al.*, 1982), are consistent with minor glycosylation differences. However, Omary *et al.* (1988) have recently demonstrated that CDw44-reactive antibodies are able to precipitate an additional major protein of 145,000 MW from some tissues. A colon epithelial cell line carried only the 145,000 MW protein, while a cutaneous T-cell lymphoma cell line had both 85,000 and 145,000 MW proteins.

In this report we have purified the CDw44 glycoprotein from $CD3^+$ $CD4^+$ $CD8^-$ T-chronic lymphocytic leukaemia cells, primarily to determine the composition of the carbohydrates. During the purification and analyses, suggestive evidence was obtained for the existence of two forms of the 85,000 MW CDw44 glycoprotein. Moreover, we demonstrate that normal T cells have two forms of CDw44 on their surface, one which is readily modulated and the other which does not modulate at all. We also present a detailed analysis of the distribution of CDw44 in human tissues, as this might help future work defining heterogeneity of CDw44 glycoproteins in different cell types.

MATERIALS AND METHODS

Source of CDw44 glycoproteins

The source of cells was a patient with T-chronic lymphocytic leukaemia (CLL) who was undergoing therapeutic leucophoresis. The cells were kindly made available to us by Dr Edna Dewer, Department of Pathology, University of Edinburgh.

The T CLL cells were washed three times in phosphate-buffered saline (PBS) and viability assessed by trypan blue exclusion. Viability was always greater than 90%. The cells were centrifuged once again and resuspended in Tris-buffered saline (TBS; 0·15 M NaCl, 0·02% Na azide, 0·025 M Tris, pH 7·4 at 4°) at 2×10^8 cells/ml. An equal volume of 10% Brij 96:Brij 99 (Sigma, Poole, Dorset) detergents (1:2) in TBS was then added, with the proteolytic inhibitors phenylmethylsulphonylfluoride and iodoacetamide at a final concentration of 2 mM and 2·5 mM, respectively. After incubation for 1 hr on ice, the suspension was centrifuged at 80,000 *g* for 75 min to remove nuclei and other non-solubilized material. The supernatant was stored at −40° prior to purification of the CDw44 glycoprotein. All of the above procedures and all subsequent steps of the purification were performed at 4° or on ice.

A total of 2·3 litres of extract (from $2{\cdot}3 \times 10^{11}$ T CLL cells) was used.

Monoclonal antibody affinity chromatography

The monoclonal antibody F10-44-2 (Dalchau *et al.*, 1980a) in the form of immune ascites partially purified by ion-exchange chromatography (Dalchau & Fabre, 1982) was coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) at a ratio of 5 mg of protein per ml of swollen gel, essentially as described by the manufacturers. Two 10-ml affinity columns were used, and 1·15 litres of extract was passed through each column at 20 ml/hr. The columns were washed with 100 ml of 0·5% Brij in TBS and eluted with 100 ml of 0·5% Brij, 0·05 M diethylamine in TBS, pH 11·5. Fractions of 10 ml were collected and neutralized immediately with approximately 50 mg of solid glycine.

Plate binding assay

The presence of CDw44 glycoprotein in eluted fractions during the purification procedure was detected by transferring 25 μl of each fraction in duplicate to wells of PVC microtitre plates (Dynatech, Billingshurst, Sussex) and detecting bound CDw44 glycoprotein by radioimmunoassays, as previously described (Dalchau & Fabre, 1982).

The preparation of the affinity-purified rabbit $F(ab')_2$ anti-mouse $F(ab')_2$ (RAM), and iodination using the chloramine T method, were as previously described (Dalchau & Fabre, 1979).

DEAE ion-exchange chromatography

Antigen-containing fractions from the affinity columns were pooled and dialysed against 0·5% Brij in TBS. The final volume of 136 ml was passed at 10 ml/hr down a 25-ml column containing DEAE-CL6B Sepharose (Pharmacia, Uppsala, Sweden) previously equilibrated with 0·5% Brij in TBS. The column was washed with 100 ml of 0·5% Brij in TBS at 20 ml/hr and eluted at 10 ml/hr using a 100-ml linear NaCl gradient from 0·15 M to 1 M, with 0·25 M Tris, 0·02% Na azide, 0·5% Brij in the buffers. Fractions of 3 ml were collected and tested for CDw44 glycoprotein using the plate-binding assay. Two antigen-containing peaks were obtained (see the Results) and each was treated separately in the subsequent purifications.

Wheat-germ lectin affinity and CM–Sepharose ion-exchange chromatography

Preliminary experiments demonstrated that the CDw44 glycoprotein did *not* bind to the cation exchanger CM–Sepharose (Pharmacia) at pH 4·5 in acetate buffer. It was decided to carry out the wheat-germ lectin-affinity step at pH 4·5 and include a CM–Sepharose precolumn as this could remove some contaminating proteins.

The CDw44 glycoprotein peaks were dialysed against 0·5% Brij, 0·05 M sodium acetate, pH 4·5, and passed sequentially down a 5-ml CM–Sepharose column and a 5-ml wheat-germ lectin column (Pharmacia). After washing with 50 ml of 0·5% Brij in the acetate buffer, the wheat-germ column was washed with 25 ml of 0·5% Brij in 0·01 M Tris, 0·02% Na azide, pH 8·4 at 4° (Tris buffer). This was a preliminary step to changing the detergent from Brij to sodium deoxycholate (DOC), which requires low salt and pH > 8·0. The column was then washed with 25 ml of 0·5% DOC in Tris buffer, and eluted with 0·5 M *N*-acetylglucosamine in 0·5% DOC in Tris buffer. Fractions containing antigen were pooled, dialysed against 0·5% DOC in

Tris buffer and concentrated to 5 ml using Amicon diaflo-positive pressure apparatus and a YM10 membrane (Amicon, Woking, Surrey).

Sepharose–CL6B gel permeation chromatography
The CDw44 glycoproteins were applied to upward flowing 1·6 cm × 80 cm Sepharose–CL6B (Pharmacia) columns equilibrated in 0·5% DOC in the Tris buffer, and eluted with this buffer at 12 ml per hour. Fractions of 2 ml were collected and assayed for CDw44 glycoprotein. Each fraction containing antigen was tested by PAGE in SDS, and only fractions containing the CDw44 glycoprotein were pooled and concentrated for subsequent analyses.

Amino acid and carbohydrate analyses
Amino acids and amino sugars were determined on a Locarte mini-amino acid analyser fitted with an automatic loading attatchment and a ninhydrin colorimetric system for detection. The standard system of analysis was that described by Allen, Desai & Neuberger (1976). Samples of glycoprotein were hydrolysed for 24, 48, and 72 hr in 3 M toluene-4-sulphonic acid at 110° under N_2 for determination of amino acids (Lui, 1972). Amino sugars were quantified after hydrolysis in 3 M toluene sulphonic acid at 100° for 24 hr (Allen & Neuberger, 1975). Determinations of half-cystine residues were obtained by estimation of cysteic acid residues resulting from performate oxidation and subsequent hydrolysis of the protein for 24 hr at 110° in constantly boiling HCl before analysis (Hirs, 1956). Corrections for destruction of serine and threonine residues were obtained by extrapolating 24-, 48-, and 72-hr determinations to zero time. The tryptophan content of toluene sulphonic acid hydrolysates could not be reliably determined because of its destruction in the presence of large amounts of carbohydrate. Neutral sugars and sialic acids were determined as pertrimethylsilyl ethers by gas–liquid chromatography (GLC) (Chambers & Clamp, 1971) (after methanolysis and trimethylsilylation of glycoprotein samples) on a Perkin Elmer F33 gas chromatograph, with columns packed with 3·5% SE30 on Diatomite C.

Amino acid and carbohydrate determinations were related by comparing the internal standards used in amino acid analysis (norleucine or p-fluorophenylalanine) to those used in GLC (mannitol or perseitol). Internal standards were added to samples taken from the same stock solution of glycoprotein.

SDS–PAGE
Slab gels of 1·5 mm thickness were used, essentially as described by Laemmli (1970).

Protein estimations
These were done by the method of Lowry *et al.* (1951) using bovine serum albumin (BSA) as the standard and SDS in the copper reagent, as described by Markewell *et al.* (1978). Appropriate concentrations of Brij or DOC were included in the standards.

Flow cytometry
Peripheral blood mononuclear cells (PBMC) were prepared from defibrinated blood by centrifuging over Ficoll–Isopaque of specific gravity 1·077 (Boyum, 1974) followed by incubation in Tris-buffered NH_4Cl to lyse red blood cells (RBC) (Boyle, 1968). T CLL cells from the same patient used as the source of cells for CDw44 purification were prepared from the leucophoresis packs as for PBMC.

One million cells were incubated with 50 μl of saturating concentrations of monoclonal antibodies in 0·5% BSA/PBS. The following antibodies were used: F10-44-2 and F10-16-1 (anti-CDw44; Dalchau *et al.*, 1980a); F10-89-4 (anti-leucocyte common antigen, CD45; Dalchau, Kirkley & Fabre, 1980b); F8-11-13 (anti-high molecular weight form of leucocyte common antigen, CD45R; Dalchau & Fabre, 1981); OKT3; OKT4; OKT11; MF7-14-5 (anti-CD5); W6/32 (anti-MHC class I); NFK-1 (anti-MHC class II); and F15-42-1 (anti-human Thy-1; McKenzie & Fabre, 1981). The OKT antibodies were from Ortho Pharmaceuticals, Raritan, NJ. The W6/32 cell line was from the ATTC, Rockville, MD. The NFK-1 cell line was a kind gift of Dr S. V. Fuggle, Nuffield Dept. Surgery, University of Oxford. The F10-16-1 antibody is an IgG1 mouse monoclonal antibody isolated from the same fusion as F10-44-2. It binds to pure CDw44 glycoprotein, and by summation studies on flow cytometry has been shown to react against an epitope distinct from that recognized by F10-44-2 (R. Dalchau and J. W. Fabre, unpublished observations). After incubation for 1 hr on ice, the cells were washed twice in 0·5% BSA/PBS and incubated with 50 μl of fluorescein-labelled affinity-purified rabbit $F(ab')_2$ anti-mouse $F(ab')_2$ at 25 μg/ml in 20% pooled human AB serum. After a further two washes, the cells were resuspended in 1 ml of 0·5% BSA/PBS and analysed immediately on a cytofluorograph system (Ortho Instruments, Westwood, MA). For the detection of surface immunoglobulin-positive cells, the cells were incubated with fluorescein-labelled, affinity-purified rabbit $F(ab')_2$ anti-human $F(ab')_2$ (RAH). All procedures were at 4° or on ice.

Modulation studies
PBMC were prepared under sterile conditions and incubated in RPMI-1640 medium containing 10% human AB serum, 4 mM L-glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin, and saturating concentrations of immune ascites partially purified by ion-exchange or gel-permeation chromatography. Incubations were at 37° in 5% CO_2 in air.

Immunohistology
Tissues were obtained within 6 hr of death from a cadaver organ donor. These were stored in liquid nitrogen prior to use.

Cryostat sections of 5 μm were cut, air-dried, and fixed in acetone for 5 min at room temperature. They were then either processed immediately or stored at −40° prior to staining. To stain the sections, the F10-44-2 monoclonal antibody was put on the sections at saturating concentrations. This was incubated at room temperature for 30 min and washed twice in TBS. Horseradish peroxidase-coupled rabbit anti-mouse immunoglobulin (Dako, Copenhagen Denmark) was used at a 1:20 dilution in 20% normal human AB serum to block antibodies cross-reacting with human immunoglobulins. The second incubation was at 37° for 30 min, and the slides were then washed twice. The coloration was developed with diaminobenzidine (Sigma Chemical Co., London) at 6 mg/10 ml containing 3 μl of 100 vol. hydrogen peroxide. Sections were counterstained with Harris's haematoxylin.

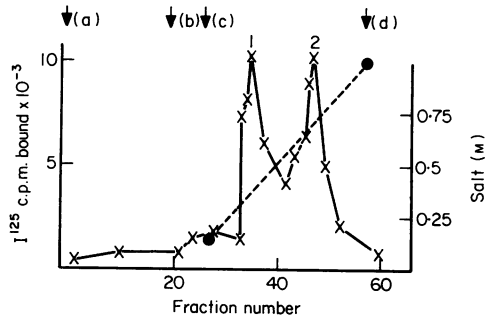

Figure 1. DEAE ion-exchange chromatography of CDw44 glycoprotein. $2{\cdot}3 \times 10^{11}$ T CLL cells were solubilized in the non-ionic detergent Brij, and the CDw44 glycoprotein purified from it by F10-44-2 monoclonal antibody affinity chromatography. The CDw44 antigen in 136 ml was dialysed against 0·5%Brij in 0·15 M NaCl, 0·025 M Tris, 0·02% Na azide pH 7·4 at 4° and applied to a 25 ml DEAE column (a) equilibrated in this buffer. The column was washed in this buffer (b). At (c), a gradient to 1 M NaCl in 0·25 M Tris, 0·02% Na azide, pH 7·4 at 4° (d), was applied. CDw44 antigen activity (——) was measured using a plate binding assay, the ^{125}I c.p.m. bound referring to ^{125}I-labelled RAM. The salt gradient (– – –) is indicated between (c) and (d). Pools 1 and 2 indicate the pools of CDw44 antigen subsequently separately processed.

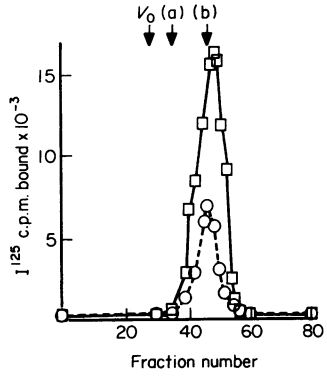

Figure 2. Sepharose CL6B gel permeation chromatography of CDw44 glycoproteins. Pool 1 (□) and Pool 2 (○) of CDw44 antigen in 0·5% Na deoxycholate, 0·01 M Tris, 0·02% Na azide, pH 8·4 at 4° were chromatographed separately on an upward flowing 1·6 cm × 80 cm Sepharose CL6B column in the same buffer. CDw44 antigen activity was measured using the plate binding assay, and ^{125}I c.p.m. bound refer to ^{125}I-labelled RAM. *Vo* is the void volume, determined with blue dextran; (a) is the elution position of thyroglobulin (MW 669,000) and (b), catalase (MW 232,000).

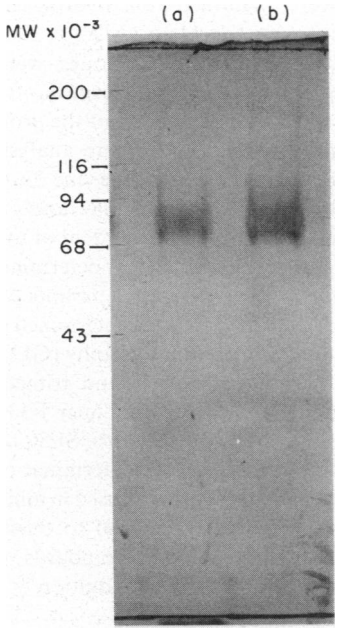

Figure 3. SDS-PAGE of purified CDw44 glycoproteins. 10 μg of Pool 1 (a) and 10 μg of Pool 2 (b) CDw44 glycoprotein were electrophoresed on 10% polyacrylamide slab gels under reducing conditions, and visualized by silver staining. The molecular weight standards were myosin (200,000), β galactosidase (116,000) phosphorylase b (94,000), bovine serum albumin (68,000) and ovalbumin (43,000).

RESULTS

Phenotype of Chronic T CLL cells

An initial sample of the T CLL cells was used for assessment of the CDw44 glycoprotein using the F10-44-2 antibody. Flow cytometry profiles showed that peak fluorescence with the T CLL cells was only a little lower than with normal PBMC (data not shown).

A subsequent comparison of binding of the F10-44-2 antibody to the T CLL cells and several cell lines (Daudi, HSB-2, MOLT-4, MOLT-3 and RPM1-1788) showed that all were positive (Daudi being only very weakly positive). However, the T CLL cells were more strongly positive than any of the above cell lines, and were therefore used for the subsequent purification of the CDw44 glycoprotein.

The phenotype of this T CLL, with the percentage of positive cells as analysed by flow cytometry given in brackets, was $CD3^+$ (99%), $CD4^+$ (95%), $CD2^+$ (95%), $CD5^+$ (98%), $CD45^+$ (98%), MHC class I^+ (93%), $CD8^-$ (1·4%), MHC class II^- (3·5%), $CD45R^-$ (3·8%), surface immunoglobulin$^-$ (2·9%), Thy-1^- (1·6%).

Purification of CDw44 glycoproteins

The purification was from $2{\cdot}3 \times 10^{11}$ T CLL cells and involved F10-44-2 monoclonal antibody affinity chromatography followed by DEAE anion-exchange chromatography, passage through a CM cation-exchange column, wheat-germ lectin-affinity chromatography and finally one or two rounds of Sepharose CL6B gel-permeation chromatography. Details are given in the Materials and Methods. It is important to note that two distinct peaks of CDw44 antigens were eluted from the anion-exchange step, as shown in Fig. 1. These were designated Pool 1 and Pool 2 as indicated, and were processed separately in the subsequent purifications. All CDw44 antigen activity in both pools bound to the wheat-germ lectin columns (data not shown).

The detergent was changed from Brij to DOC on the wheat-germ lectin column, because of the much smaller micelle size of DOC (Tanford & Reynolds, 1976), an advantage for the gel-permeation chromatography step. On Sepharose CL6B gel-permeation chromatography, both Pool 1 and Pool 2 gave a single peak eluting just after catalase (232,000 MW), as shown in

Table 1. Amino acid and carbohydrate composition of the two pools of CDw44 glycoprotein from T CLL cells*

Residue	Pool 1	Pool 2
Asx	10·9	9·9
Thr	8·0	5·9
Ser	11·4	14·9
Glx	9·7	13·0
Pro	7·6	7·4
Gly	12·8	14·5
Ala	9·4	9·6
½ Cys	2·2	1·4
Val	3·5	4·1
Met	1·6	2·1
Ile	4·6	3·4
Leu	5·0	4·6
Tyr	3·1	1·8
Phe	2·9	3·1
His	1·2	0·5
Lys	3·0	2·8
Trp	ND	ND
Arg	2·9	1·2
Glc NAc	4·6	2·9
Gal NAc	3·9	3·5
Fucose	0	0
Gal	6·9	6·0
Man	3·6	3·5
Neu NAc	4·9	5.3
% CHO (w/w)	32·0%	30·4%

* The results are given as residues per 100 amino acid residues.
ND, not done.

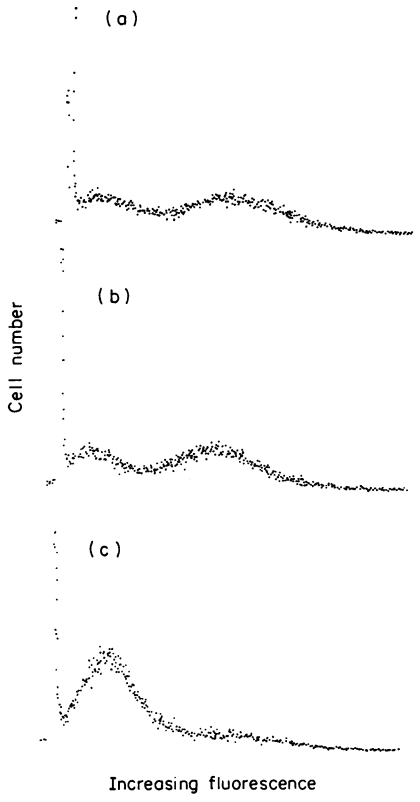

Figure 4. Modulation of the CDw44 glycoprotein. PBMC were incubated at 37° in culture medium containing the F10-16-1 monoclonal antibody for periods of up to 24 hr, and then stained with fluorescein labelled RAM. (a) Control PBMC, before incubation. (b) PBMC incubated for 1 hr. (c) PBMC incubated for 24 hr. PBMC incubated in medium alone for periods of up to 24 hr and then stained for CDw44 gave profiles as in (a).

Fig. 2. Each fraction was assessed for purity using SDS–PAGE and fractions containing only the 85,000 MW protein were pooled. Pool 2 was subjected to a second round of gel filtration. The final samples were analysed by SDS–PAGE and the results are given in Fig. 3. Pool 1 and Pool 2 both gave broad bands at around 85,000 MW, and were indistinguishable from one another.

The 2·3 litres of solubilized T CLL extract, following ultracentrifugation, contained 4832 mg of protein. After the final gel-permeation step, Pool 1 had 312 μg of CDw44 protein, representing 15·9% of the original antigen activity and a 2462-fold purification relative to protein content. Pool 2 contained 116 μg of CDw44 protein, representing 3·1% of the antigen activity and a 1291-fold purification relative to protein.

Amino acid and carbohydrate composition

The results are given in Table 1. There are several points to note. Firstly, the CDw44 glycoproteins are heavily glycosylated, consisting of 32·0% (Pool 1) and 30·4% (Pool 2) by weight of carbohydrate. Secondly, the presence of N-acetyl galactosamine and galactose strongly suggests the presence of O-linked oligosaccharides. The presence of N-acetyl glucosamine with low mannose and high galactose suggests the presence of complex-type N-linked oligosaccharides. Thirdly, the amino acid compositions for Pools 1 and 2 are similar, with many identities in the composition. However, there are differences for several amino acids. This is consistent with the CDw44 polypeptides being homologous but not identical. We could not see protein impurities in our preparations of Pools 1 or 2, but the possibility of minor protein contamination must be borne in mind when interpreting this type of data. Fourthly, both Pools 1 and 2 have substantial numbers of cystine residues, probably about 11 and seven per polypeptide, chain, respectively.

N-terminal sequence analysis

An excellent way of telling if Pools 1 and 2 contained homologous but distinct polypeptides was to compare the N-terminal amino acid sequence of both proteins. Thirty micrograms (approximately 0·5 nM) were analysed on a gas-phase sequencer by Dr David B. Teplow (Division of Biology, CalTech, Pasadena, CA) but no sequence was obtained. The N-termini thus appeared to be blocked.

Modulation studies

PBMC were incubated for periods of 1, 2, 4 and 24 hr at 37° with saturating concentrations of F10-44-2 or F10-16-1 monoclonal antibody, as outlined in the Materials and Methods. At the end

of the incubation period the cells were washed and labelled with fluorescein-labelled RAM for flow cytometry, all procedures being at 4°. The results are given in Fig. 4. Normal PBMC show two peaks of fluorescence (Fig. 4a), the duller peak representing surface immunoglobulin-positive cells, and the brighter peak the T cells, as described previously (Dalchau *et al.*, 1980a). After 1 hr at 37° (Fig. 4b), the surface expression of CDw44 is only slightly changed, with a small shift to the left in both peaks of fluorescence. However, by 4 hr the picture is as in Fig. 4c, and remains unchanged at 24 hr. The number of CDw44-positive cells does not change with the incubation, but virtually all of the cells now shift to a much duller peak of fluorescence. The picture suggests that the T lymphocyte peak of fluorescence contains one set of CDw44 glycoproteins which can be modulated and another which is resistant to modulation. It also suggests that the CDw44 glycoproteins on the duller B-lymphocyte peak consists of molecules resistant to modulation.

Control cultures of PBMC cultured at 37° for 1, 2, 4 and 24 hr in the absence of antibody and then processed for flow cytometry all showed a picture indistinguishable from Fig. 4a, i.e. culture alone did not influence CDw44 expression.

Another control consisted of modulation of CD5 expression with the MF7-14-5 antibody, using culture conditions as above on the same preparation of PBMC. Cell surface expression of the CD5 antigen was much reduced at 1 hr and had completely disappeared by 2 hr.

Tissue distribution studies

The interesting report from Omary *et al.* (1988) suggests that the F10-44-2 and other anti-CDw44 antibodies react with two glycoproteins of quite distinct molecular weight (85,000 and 145,000), and that these two glycoproteins are differentially expressed on different cell types. The 85,000 and 145,000 MW glycoproteins are almost certainly related molecules, because antibodies to two distinct determinants on the 85,000 MW glycoprotein also react with the 145,000 MW glycoprotein (Omary *et al.*, 1988). The expression of CDw44 by various cell types in the body is therefore of interest, since positive cell types could be screened for expression of the 85,000 and 145,000 MW forms. We have therefore made a detailed analysis of the tissue distribution of CDw44, using the F10-44-2 monoclonal antibody. A summary of this data is given in Table 2 and selected photomicrographs in Fig. 5.

As previously reported, CDw44 is widely distributed in the haemopoietic system, and the determinant is carried by a glycoprotein of approximately 85,000 MW on mature T cells (Dalchau *et al.* 1980a; Morstyn *et al.*, 1981). In the brain, CDw44 is found exclusively in the white matter, and here the glycoprotein is of similar, but slightly smaller, molecular weight (McKenzie *et al.*, 1982).

As regards expression on epithelial cells, we have previously reported the expression of CDw44 by colonic epithelial cells (Daar & Fabre, 1983) and alveolar epithelial cells of breast tissue (Daar & Fabre, 1981). Perhaps the most strongly positive epithelial cells are those of the pseudostratified respiratory epithelium of the trachea and major bronchi (Fig. 5a), but it is interesting, that within the lung, this epithelium becomes CDw44 negative in the bronchioles (Fig. 5b). The alveolar epithelium of the lung is CDw44-positive (Fig. 5b). Strongly positive epithelium is also seen in the stratified squamous

Table 2. Detailed distribution of CDw44 antigen in normal human tissues

	Staining with F10-44-2 antibody*
Haemopoietic system	
Thymus cortex	−
Thymus medulla	++
T and B lymphocytes, granulocytes	++
Erythrocytes	+/−
Nervous system	
CNS grey matter	−
CNS white matter	++
Peripheral nerves	++
Epidermis	++/−
Gastrointestinal epithelium	
Tongue, oesophagus	++/−†
Stomach, small and large intestine	+/−‡
Liver	
Hepatocytes	−
Biliary epithelium	+
Urogenital epithelium	
All kidney tubules and glomeruli	−
Epithelium of ureter, bladder, prostate and urethra	++
Detrusor muscle of bladder	++
Testis: germ cell line, Sertoli and Leydig cells	−
Epididymis: epithelium, spermatozoa	−
Respiratory system	
Trachea, pseudostratified epithelium	++
Bronchioles, pseudostratified epithelium	−
Alveolar epithelium	−
Mixed glandular tissue of trachea	+
Parotid glands	
Acinar epithelium	++
Ductal epithelium	++
Pancreas	
Acinar epithelium	−
Ductal epithelium	−
Islets of Langerhans	−
Muscle	
Smooth	−
Skeletal	++
Endocrine tissue	
Thyroid follicular cells	−
Parathyroid	−
Pituitary	−
Adrenal cortex	+/−
Adrenal medulla	−
Miscellaneous tissue	
Connective tissue	++/+§
Cultured fibroblasts	++
Vascular endothelium	−
Villous trophoblast	+
Breast glandular epithelium	+/−
Breast ductal epithelium	++

* Intensity of staining: −, no staining; +, weak staining; ++, strong staining; +/−, some cells positive and some negative.

† More basal layers only of stratified epithelium are positive.

‡ Only cells in deeper parts of crypts. Surface epithelium negative.

§ Density of staining varies at different sites.

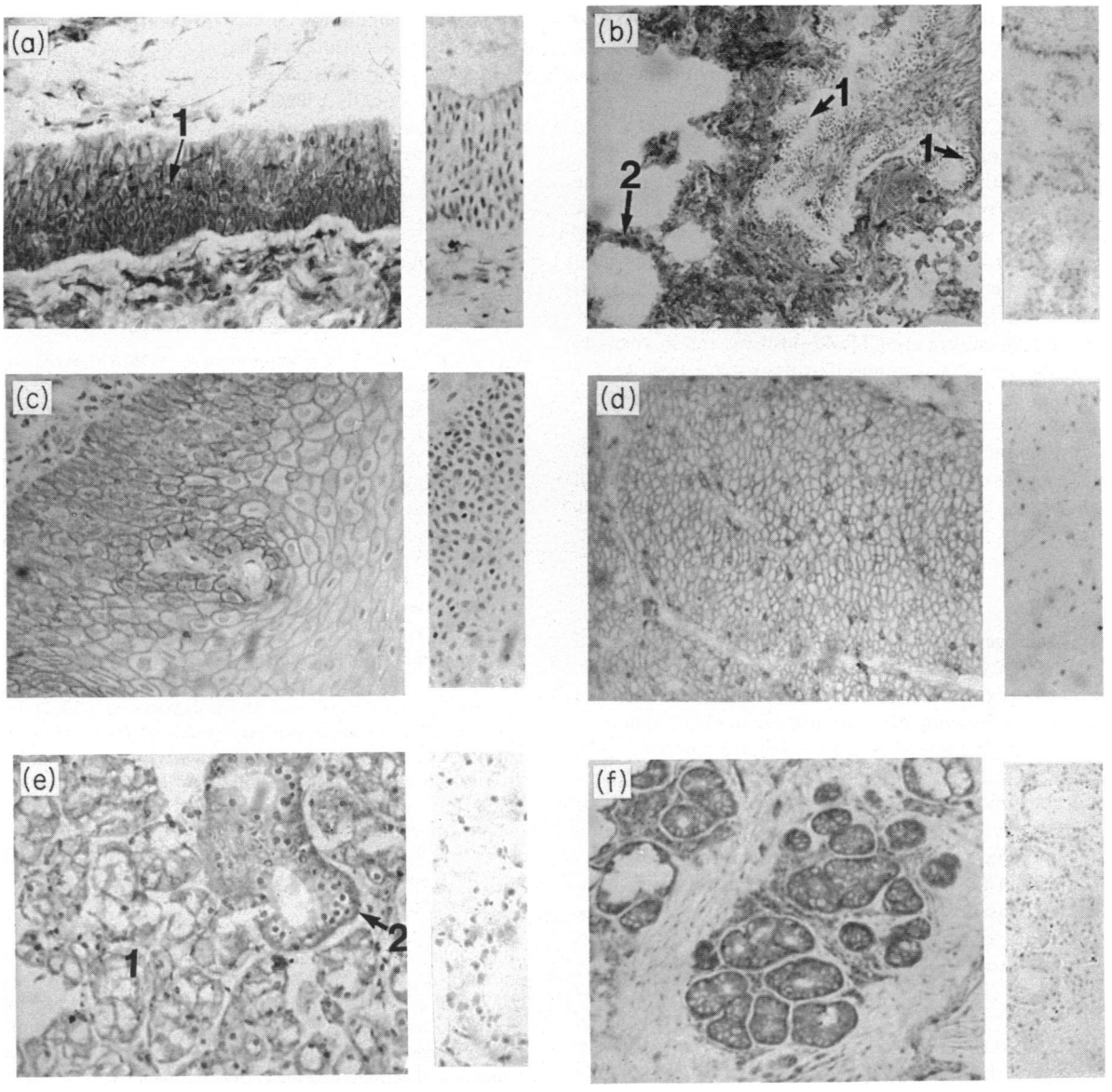

Figure 5. Immunological studies on the tissue distribution of CDw44. Frozen sections of various tissues were stained for CDw44 using the F10-44-2 monoclonal antibody and peroxidase-labelled rabbit anti-mouse immunoglobulin. Sections stained with control IgG1 monoclonal antibody are shown to the right of each photomicrograph. (a) Trachea, ×280 (1) strongly positive pseudostratified epithelium; (b) lung, ×175, note the negative epithelium of bronchi (1) and the positive alveolar epithelium (2), (c) oesophagus, ×280, note the strongly staining basal cells and the negative peripheral cells; (d) bladder, ×280, the positive smooth muscle cells of the detrusor muscle are shown; (e) parotid gland, ×280, the positive glandular cells (1) and ducts (2) are shown; (f) glandular cells of the epiglottis, ×280, strongly positive glandular cells are shown.

epithelium of the oesophagus (Fig. 5c) and skin. Both in the oesophagus and skin, only the deeper cells are positive. As the cells migrate outwards, they become CDw44-negative. The less stratified epithelium of the cornea is CDw44-positive in cell layers. Throughout the gastrointestinal tract (stomach, duodenum, jejunum, ileum and colon), CDw44-positive epithelial cells are seen, but only in the deeper parts of the crypts, as previously reported for the colon (Daar & Fabre, 1983). The epithelial cells on the luminal surface are CDw44-negative. The transitional epithelial cells of ureter and bladder are weakly CDw44-positive. The male urethra has pockets of CDw44-positive epithelial cells, while many cells are CDw44-negative.

Skeletal muscle cells are CDw44-negative. However, smooth muscle is CDw44-positive, as seen in the bronchioles (Fig. 5b) and the detrusor muscle of the bladder (Fig. 5d). The smooth muscle of blood vessels and of the gastrointestinal tract is only weakly CDw44 positive, but that of the bronchi is intensely CDw44-positive.

Some glandular epithelial cells, such as those of the exocrine pancreas, are CDw44-negative, but the exocrine cells of the

parotid (Fig. 5e), the mixed glandular cells in the trachea and epiglottis (Fig. 5f), and the glands of Brunner in the duodenum are all strongly positive for CDw44. All renal tubular cells, basement membranes and glomeruli are CDw44-negative. Hepatocytes are CDw44-negative, but some sinusoidal lining cells (possibly Kupffer cells) are CDw44-positive.

Vascular endothelium is CDw44-negative. Most cells of the endocrine system (parathyroid, anterior and posterior pituitary, adrenal medulla, islets of Langerhans) are CDw44 negative. However, the thyroid follicular cells and some cells in the zona glomerulara of the adrenal cortex are CDw44-positive, the rest of the adrenal cortex being CDw44-negative.

Fibroblasts in culture are CDw44-positive, and in virtually all tissues, the interstitial connective tissues are positive for CDw44, (e.g. Fig. 5c). However, the intensity of staining varies from tissue to tissue.

The epithelium of the epididymis, and the germ-line cells, spermatozoa, cells of Leydig and Sertoli cells of the testis are all CDw44-negative. However, the epithelial cells of the prostate gland are CDw44-positive.

DISCUSSION

The CDw44 antigen is a membrane glycoprotein of restricted tissue distribution, but found on cells of many diverse cell types. In addition to its expression on T lymphocytes and other cells of the haemopoietic system, we demonstrate in this paper that CDw44 is found on some glandular cells, skin epithelial cells, smooth muscle cells and various other cell types. Whether a single molecule subserves a single function in all of these cells, or whether there are several functions mediated by CDw44 is an interesting and unanswered question. Functional heterogeneity could result from the uniqueness of individual microenvironments or be consequent on structural heterogeneity of the CDw44 glycoprotein, probably as a result of an evolving gene family or alternative mRNA splicing.

Omary *et al.* (1988) have demonstrated that anti-CDw44 monoclonal antibodies precipitate two distinct proteins of 85,000 and 145,000 MW. This very interesting observation strongly suggests the presence of structural heterogeneity within the CDw44 glycoprotein. In this paper, in the course of purifying the CDw44 antigen from $CD3^+$ $CD4^+$ $CD8^-$ T CLL cells, we found two distinct peaks of elution of CDw44 antigen from ion-exchange columns. Although both peaks contained only 85,000 MW CDw44 glycoprotein, on amino acid and carbohydrate analysis the compositions were noticeably different. The differential elution from ion-exchange columns could be due to sialic acid differences, and the differences in composition could be due to unrecognized impurities in the samples. However, the samples appeared pure by SDS–PAGE, and the results are consistent with more than one form of CDw44 glycoprotein within the 85,000 MW peak. From the carbohydrate composition, both glycoproteins are heavily (approximately 30% w/w) glycosylated, and probably contain O-linked and complex N-linked glycans.

At a functional level, we found that the CDw44 antigen on T cells exists in two forms, one of which can be modulated from the cell surface using monoclonal antibody alone, and the other which could not be so modulated. Whether or not this difference in capacity for modulation correlates with structural differences is of course unknown.

CDw44 is likely to represent a complex family of related glycoproteins. Our data and that of Omary *et al.* (1988) suggest that CDw44 is likely to prove genetically complicated either at the genomic or cDNA level. The function or functions of these molecules on the many cell types on which they are found is likely to prove very interesting.

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