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Expression of soluble isoforms of rat CD45. Analysis by electron microscopy and use in epitope mapping of anti-CD45R monoclonal antibodies

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SUMMARY

The CD45 or leucocyte-common antigens are encoded by a single gene but can be found in various forms due to alternative splicing of three exons near the 5′ end of the gene. The CD45 antigens are major glycoproteins of all types of leucocytes. Monoclonal antibodies recognizing restricted epitopes of CD45 have been used to distinguish phenotypic and functional subsets of lymphocytes. To facilitate epitope mapping and biochemical studies, we have expressed the extracellular portions for four different isoforms of rat CD45 in Chinese hamster ovary cells. Constructs were prepared to give four soluble CD45 isoforms, with sequence incorporating either all three alternative exons (sCD45.ABC), the B exon (sCD45.B), the C exon (sCD45.C), or no alternative exons (sCD45.O). These were expressed at approximately 5 mg/l of spent tissue culture supernatant and were antigenically active with monoclonal antibodies (mAb) that recognize all CD45 isoforms. The MRC OX22 and OX32 mAb have been used to split rat $CD4^+$ T cells into functionally distinct subpopulations and the epitopes for these were mapped to the product of exon C. The epitope for MRC OX33, a marker for B cells, requires expression of either the A exon or the A/B exon junction. Electron microscopy showed that the extra segments contributed to an extended structure as has been predicted from the sequence. The shape of the molecule is discussed with regard to other molecules at the leucocyte cell surface.

INTRODUCTION

CD45 or the leucocyte-common antigen (L-CA, CD45, or T200) is a family of heavily glycosylated cell surface glycoproteins with apparent M_r of 180,000–240,000 that are expressed exclusively and abundantly on all lymphoid and myeloid cells (reviewed in ref. 1). The large cytoplasmic segment of CD45, 705 amino acids in the rat, has protein phosphotyrosine phosphatase activity.[2] CD45 shows cell-type specific heterogeneity in apparent M_r, as judged by SDS–PAGE.[1] In the rat, thymocytes express mainly the smallest form with an M_r 180,000, B cells a broad band around M_r 240,000, and T cells exhibit four bands with M_r 180,000, 190,000, 200,000 and 220,000.[3] Variation in the apparent M_r of CD45 arises primarily from the insertion or absence (via differential alternative exon usage) of combinations of up to three heavily O-glycosylated polypeptide segments at a point six amino acids from the NH_2-terminus as illustrated in Fig. 1.[4–7] Six different isoforms of CD45 have been identified from cDNA clones (usage of ABC, AB, BC, B, C and O exons) and another inferred (A exon alone) from hybridization studies.[4–7] Recently the form with no extra exons, CD45.0, has been shown to interact with the B-cell antigen, CD22.[8]

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Anti-CD45 monoclonal antibodies (mAb) that recognize epitopes restricted to some of the CD45 isoforms have been widely used to define functional subsets of $CD4^+$ T cells in human, rat and mouse.[9–15] In the rat, the mAb MRC OX22 and OX32 recognize all B cells, all $CD8^+$ T cells, two-thirds of the $CD4^+$ T cells, and only about 3% of thymocytes,[16] while MRC OX33 recognizes a B-cell specific form of CD45.[3] *In vivo*, $OX22^{high}$ $CD4^+$ T cells have been found to mediate graft-versus-host reaction but not B-cell help, while the converse is true for $OX22^{low}$ $CD4^+$ T cells.[11,16] These functional divisions correlate superficially those between mouse Th1 and Th2 clones.[17] However, it appears that a dichotomy of function based upon the recognition by one mAb is a simplistic view; for example, $OX22^{low}$ $CD4^+$ T cells include some that are capable of B-cell help and some that can inhibit the autoaggressive activity of $OX22^{high}$ $CD4^+$ T cells.[18]

In this report we describe the production of soluble isoforms of CD45, the analysis of the dimensions of the sCD45 isoforms by electron microscopy following low-angle rotary shadowing and epitope mapping of the MRC OX22, MRC OX32 and MRC OX33 mAb.

The nomenclature used throughout this paper for the expressed CD45 proteins is as follows: sCD45.ABC is the soluble form of the CD45 variant utilizing all three alternative exons (A,B, and C); sCD45.B is the soluble form of the CD45

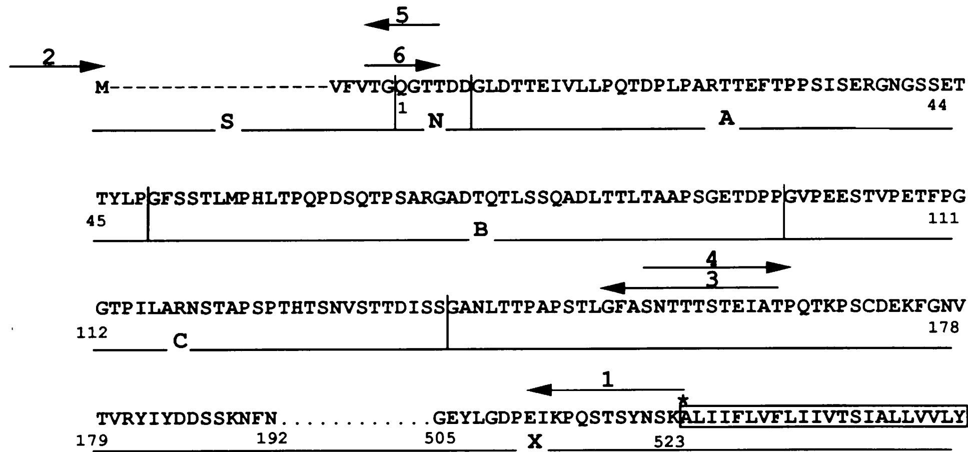

Figure 1. Relationship of the oligonucleotide primers 1–6 to the CD45.ABC extracellular protein sequence. The position and sense of each primer (sequences of which are listed in Materials and Methods) are indicated by an arrow above the protein sequence that it encodes. Segments S, N, A, B, C, and X represent respectively, the CD45 nucleotide sequences for the expected signal peptide (dashes indicate sequence unknown), the NH_2-terminal six amino acids, exon A, exon B, exon C, and the extracellular region that is 3′ of the alternative exon splice site, respectively. The numbering below the amino acid sequence is from ref. 4. The asterisk marks the position of the introduced stop codon with respect to the protein sequence.

variant utilizing the B (and no other) alternative exon sequence present; sCD45.C is the CD45 variant utilizing the C (and no other) alternative exon present; and sCD45.O is the CD45 variant utilizing none of the alternative exons. We have specifically avoided the term sCD45R for the proteins because such terminology is currently used to describe CD45 variants on the basis of their epitope expression. Use of the CD45R terminology here could be misleading in that, for example, a CD45RB antibody will recognize both CD45.ABC and CD45.B, and so a 'CD45RB' protein could be either of these proteins. In our nomenclature, sCD45.B is an exact, detailed term for the protein that utilizes only the B alternative exon.

MATERIALS AND METHODS

Cells and mAb

T cells, B cells, thymocytes and TDL were obtained by standard methods from specific pathogen-free AO or PVG.RT1c rats bred in the CIU (Oxford, U.K.). All mAb used are described in ref. 3 and are termed OX1, OX22, etc. for short.

Oligonucleotide primers

The positions of the oligonucleotide primers with respect to the extracellular CD45.ABC protein sequence are shown schematically in Fig. 1 and are detailed below. Nucleotides matching the CD45 sequence are underlined. Primer 1, used for reverse transcriptase (RT) polymerase chain reaction (PCR) and PCR, is anti-sense and introduces a stop codon before the transmembrane segment of rat CD45 and includes *Sac*I and *Bam*HI sites: 5′ tag tag agc tcc gga tcc tat tta gaa tta taa gat gtt gat tg. Primer 2, the 5′ sense oligonucleotide used for PCR of the signal sequence, incorporates 5′ *Hin*dIII and *Xba*I sites and was designed on the basis of the extensive homology between the 5′ untranslated regions (immediately adjacent to the signal) of human and mouse CD45 cDNA[5,6] with the final ATG matching the putative initiation codon: 5′ tag tag aag ctt cta gag ctg atc tcc aga tat gac cat g. Primer 3, the 3′ oligonucleotide used in PCR for the signal sequence and alternative exon variants, is anti-sense to the region 37–59 base pair (bp) downstream of the splice junction for the alternative exons and introduces a *Sal*I site without changing the translated sequence: 5′ tag tag tta tag acc tgt cgacgt ggt ggt att gct tgc aaa gc. Primer 4 is partly complementary to primer 3 and was used as a 5′ PCR oligonucleotide: 5′ tag tag cta gag tcg aca gaa ata gct acc cct c. Primer 5 (anti-sense) was used to introduce (by oligonucleotide-directed mutagenesis using a mutagenesis kit from Amersham International, Amersham, Bucks, U.K.) a *Kpn*I site into the sequence near the codon for the putative NH_2-terminal amino acid: 5′ c acc atc gtc ggt ggt acc ttg ccc tgt gac. Primer 6 is a sense oligonucleotide designed for PCR and partly complements the sequence surrounding the introduced *Kpn*I site: 5′ tag ggg caa ggt acc acc gac.

RT-PCR and PCR

RT-PCR analysis of total RNA from 10^6 T cells or thymocytes was performed essentially as described in ref. 19. CD45-specific cDNA.mRNA hybrids were prepared using sequence-specific primer 1. One-hundredth of the reaction product was then subjected to PCR in 100 μl with 1 μM primer pairs 2 (or 6) and 3, 200 μM each of dATP, dCTP, dGTP, and dTTP (Pharmacia, Uppsala, Sweden), 50 mM KCl, 10 mM Tris, pH 9·3, 2 mM $MgCl_2$ and 1 U of Ampli-Taq (Perkin-Elmer Cetus, Emeryville, CA). Reactions were in a thermocycler (Perkin-Elmer/Cetus) for 30 cycles (94°, 1 min; 60°, 2 min; 72°, 1 min). Products (10 μl) were analysed on a 3% NuSieve: 1% Seakem (FMC Corp., Rockland, ME) gel and visualized by ethidium bromide staining. PCR (under the same conditions) using pLC-65[4] with primers 4 and 1 produced a 1·1 kb *Sal*I-*Sac*I cDNA product corresponding to that part of the extracellular CD45 sequence not amplified from cDNA.mRNA with primers 2 (or 6) and 3. PCR products were purified from low-melting temperature agarose gels and digested with appropriate restriction enzymes before ligations.

Determinations of CD45 signal sequence

cDNA/mRNA prepared as above from thymocytes and lymph node cells was used as a template for the PCR to obtain the sequence for the signal peptide. Following digestion with

*Hin*dIII and *Sal*I, the products were cloned into M13mp19 and six independent clones gave the same sequence.

Plasma constructs for expression of sCD45 isoforms

(1) A termination signal was introduced adjacent to the transmembrane coding sequence and the majority of the extracellular coding sequence was amplified by PCR from plasmid pLC-65 template[4] using primers 1 and 4, cut with *Sac*I and *Sal*I and ligated into the M13 vector containing the signal sequence.

(2) A *Kpn*I site was introduced into the resulting clone (M13mp 19-OL) using primer 5 by site-directed mutagenesis.

(3) Because PCR amplification with primers 2 and 3 of cDNA containing alternative exon segments was inefficient, primers 6 and 3 were used, the products cut with *Kpn*I and *Sal*I and cloned into the modified clone M13mp19-OL.

(4) Expression vectors for sCD45 isoforms were made by inserting the *Xba*I-*Bam*HI fragment from the M13mp19 constructs into pEE6.HCMV.GS (kindly provided by Celltech Ltd, Slough, U.K.) digested with *Xba*I and *Bcl*I.[20,21] Double-stranded sequencing and restriction digest mapping of these constructs were performed to confirm their identity.

Expression and purification of sCD45 isoforms

The pEE6 vector gives expression from a human cytomegalovirus (HCMV) promoter/enhancer and contains a glutamine synthetase gene that permits transfectants to be selected with the glutamine synthetase inhibitor, methionine sulphoximine, MSX.[20,21] After transfection of CHO cells with the four sCD45 constructs, the supernatants from clones were assayed for the presence of soluble CD45 isoforms by inhibition of an indirect radioactive binding assay using rabbit antisera raised against rat CD45.[22] Positive clones were adapted for large-scale production of supernatant in roller bottles and for maximum yields the cells were grown to exhaustion in the presence of 2 mM sodium butyrate.[21] The sCD45 proteins were purified by affinity chromatography using the anti-CD45 mAb OX1 or OX30.[22]

Epitope mapping by inhibition assay

Purified IgG from OX22, OX30, OX32, and OX33 mAb were radioiodinated by the chloramine T method. Each radiolabelled mAb (25 μl, 0·2 μg/ml, 2×10^7 c.p.m./μg) was incubated at 4° for 1 hr with or without dilutions (25 μl) of the recombinant sCD45 isoforms proteins. Thoracic duct lymphocytes (TDL) [2×10^6 cells in 25 μl phosphate-buffered saline/bovine serum albumin (PBS/BSA)] were added, the mixture vortexed and incubated at 4° for 1 hr. Cells were washed twice with PBS/BSA and the radioactivity associated with the cells was assessed using a LKB-Wallac 1261 Multigamma gamma-counter (LKB, Uppsala, Sweden).

Rotary shadowing and electron microscopy

Purified sCD45.0 and sCD45.ABC were diluted into 70% glycerol 0·1 M NH_4HCO_3 at about 10 μg/ml and immediately sprayed as a mist onto the surfaces of freshly cleaved mica sheets. After evaporation to dryness, rotary evaporation with platinum-carbon and transmission electron microscopy were as described previously.[3] The length of the molecules were estimated by measuring those molecules that were extended as described previously.[3] Eighty molecules were measured for each determination.

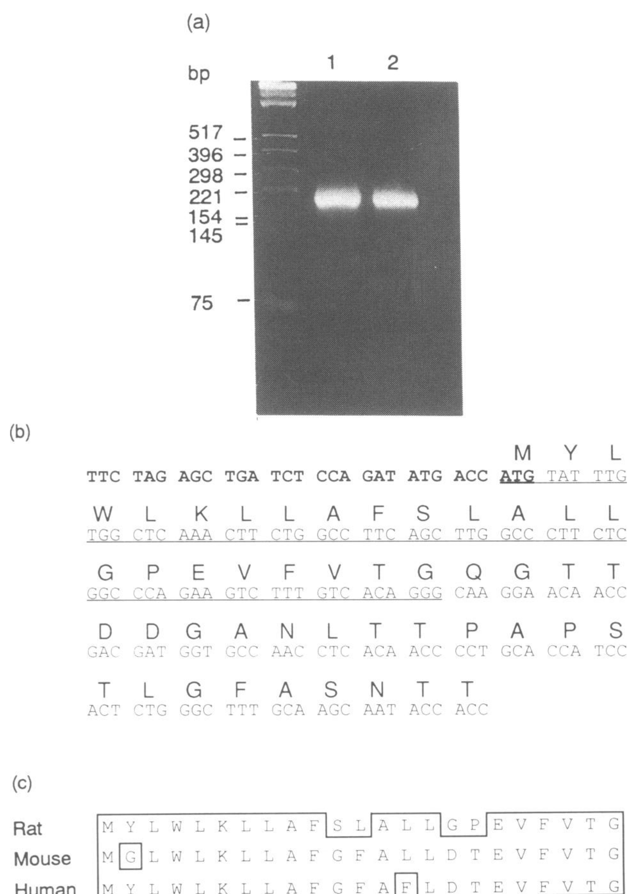

Figure 2. Rat CD45 signal sequence. (a) PCR products amplified from T-cell (1) and thymocyte (2) cDNA.mRNA hybrid templates using primers 2 and 3 were electrophoresed on agarose gels. The size of standard markers in base pairs is given. (b) Nucleotide sequence of the rat CD45 signal peptide and the deduced amino acid sequence for clone M13mp-L.1. The nucleotide sequence for the signal peptide is underlined and that of the 5′ PCR primer is in bold. The one-letter amino acid code is shown above the codon. These sequence data are available from EMBL/GenBank/DDBJ data libraries under accession number X59924. (c) Alignment of the CD45 signal amino acid sequence in human, mouse, and rat. Residues identical in two or three species are boxed.

RESULTS

Determination of the rat CD45 signal sequence

The nucleotide sequence for the signal peptide needed to be determined since none of the cDNA clones isolated previously extended beyond about 12 nucleotides upstream of the codon for the putative NH_2-terminal amino acid.[4] PCR employing primers 2 and 3 (see Materials and Methods and Fig. 1) with cDNA.mRNA templates from T cells and thymocytes resulted in a single major product of ~200 bp (Fig. 2a). After subcloning into M13, all six clones examined gave the same sequence which coded for a 23 amino acid signal sequence and part of the CD45.0 sequence (Fig. 2b). Comparison of the deduced signal

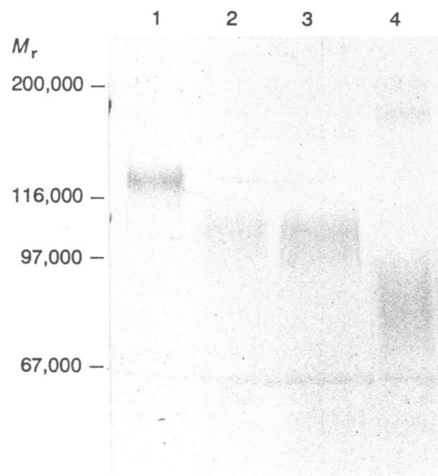

Figure 3. SDS–PAGE of recombinant soluble isoforms of rat CD45 on a 7·5% polyacrylamide gel. The proteins were visualized by Coomassie staining. Lane 1, sCD45.ABC; lane 2, sCD45.B; lane 3, sCD45.C; lane 4, sCD45.O. The positions of marker proteins are indicated.

Table 1. Relative molecular masses of soluble CD45 isoforms

sCD45 isoform	Actual SDS–PAGE M_r value*	Polypeptide M_r value†	Predicted M_r value‡
sCD45.ABC	140,000	57,454 (523)	156,000
sCD45.B	110,000	48,247 (440)	117,000
sCD45.C	105,000	47,404 (432)	107,000
sCD45.O	85,000	43,264 (391)	97,000

* Approximate values (± 5000) estimated from SDS–PAGE by comparison of the migration of sCD45 isoforms with known M_r standards.

† Values calculated from the known amino acid compositions of the extracellular segments of CD45 isoforms. The number in parentheses represents the number of amino acids.

‡ Values estimated by subtraction of the deduced M_r of the cytoplasmic and transmembrane amino acids (84,000) from the M_r of the whole protein, assuming CD45.ABC has a M_r of 240,000, CD45.B has a M_r of 200,000, CD45.C has a M_r of 190,000, and CD45.O has a M_r of 180,000.[3]

sequences for human,[6] mouse[5] and rat CD45 shows that there are four unique amino acids in the rat sequence (Fig. 2c).

Although a number of different PCR products were expected from rat T cells using the above strategy due to combinations of alternative exons, amplification with primers 2 and 3 only produced the fragment corresponding to CD45.O (with no alternative exon sequence). However, PCR with 5′ primer 6 (corresponding to nucleotides encoding the NH_2-terminal common six amino acids) and 3′ primer 3, resulted in a number of PCR products (without signal sequence) representing alternative exon usage (data not shown). This difference may result from secondary structure preventing reverse transcription (particularly with the alternative exons expressed) to include the signal and probably explains the inability to obtain complete cDNA clones in previous attempts.[4]

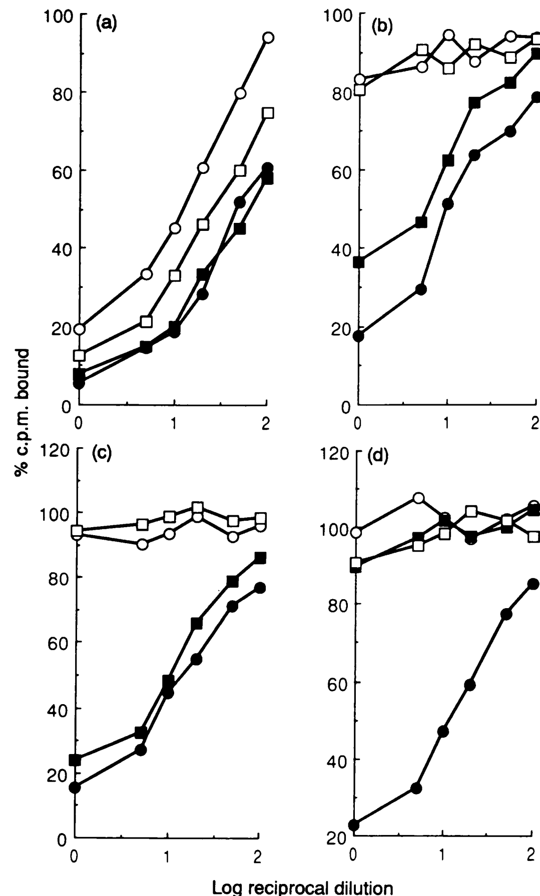

Figure 4. Epitope mapping of OX22, OX32, and OX33. Inhibition assays were performed with radiolabelled OX30 (a), OX22 (b), OX32 (c), and OX33 (d). Dilutions of sCD45.ABC (●), sCD45.B (○), sCD45.C (■), and sCD45.O (□) proteins were made in PBS/BSA. The first dilution in each case is approximately 300 μg/ml. Results are expressed as a percentage of the radioactivity associated with cells incubated with radiolabelled mAb alone. One hundred per cent represents $4{\cdot}0 \times 10^4$ c.p.m. (a), $9{\cdot}0 \times 10^3$ c.p.m. (b), $9{\cdot}2 \times 10^3$ c.p.m. (c) and $1{\cdot}9 \times 10^4$ c.p.m. (d). Typical data from one of five assays performed in duplicate are shown.

Expression of soluble CD45 isoforms

The constructs for the sCD45 isoforms were prepared by PCR amplification cDNA.mRNA hybrid templates from rat lymphocytes with primer pair 6 and 3 to generate alternative exon sequence fragments; these were then subcloned into the vector with the signal sequence and the remaining extracellular part; the whole construct was subcloned into expression vector pEE6.HCMV.GS. Four constructs were expressed; pEE6-sCD45.ABC, pEE6-sCD45.B. pEE6-sCD45.C. and pEE6-sCD45.O comprising the extracellular cDNA (i.e. truncated at the codon immediately 5′ of the transmembrane coding region) for the isoforms containing all three alternative exons, the B exon alone, the C exon alone, and no alternative exon, respectively. The level of expression of all four isoforms in spent tissue culture media was about 5 mg/l based on amino acid analysis after purification of the proteins.

SDS–PAGE analysis (Fig. 3) of the proteins purified by affinity chromatography showed that the isoforms ran as single broad bands whose apparent M_r values corresponded well with

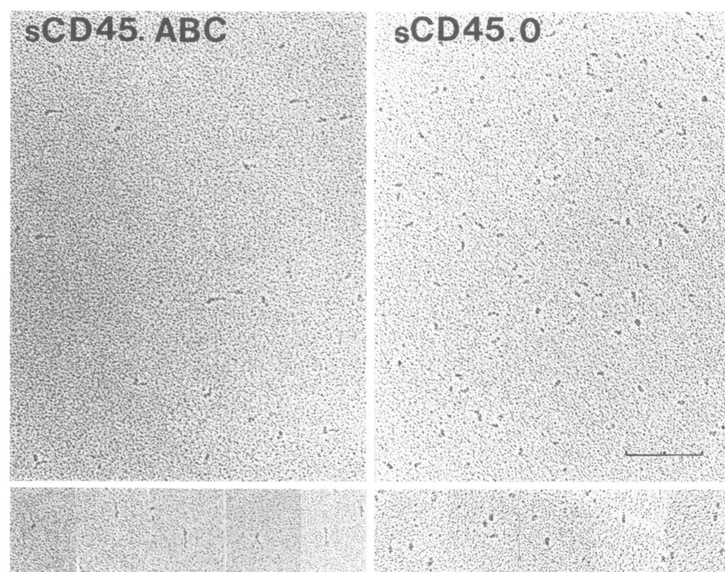

Figure 5. Electron micrographs of sCD45 following low-angle rotary shadowing: sCD45.ABC and sCD45.O. The sCD45.ABC is considerably longer than the sCD45.O form. The bar corresponds to 200 nm.

the values predicted after allowing for glycosylation (Table 1). The broadness of the band for each isoform probably reflects variation in glycosylation.

Epitope mapping of OX22, OX32 and OX33 mAb

The epitopes recognized mAb OX22, OX32 and OX33 were mapped by testing the ability of the purified sCD45 isoforms to inhibit the binding of these mAb to TDL. OX30, which recognizes an epitope common to all CD45 species was included as a positive control. Figure 4 shows that the binding of radiolabelled OX30 to TDL was inhibited by all isoforms. In contrast, only sCD45.ABC and sCD45.C were able to inhibit the binding of radiolabelled OX22 (Fig. 4b) and OX32 (Fig. 4c) to TDL. These data suggest that the expression of alternative exon C is necessary and sufficient for binding of OX22 and OX32. When purified proteins were incubated with radiolabelled OX33, only sCD45.ABC could inhibit its subsequent binding to TDL (Fig. 4d); thus, the OX33 epitope resides within the exon A-coded segment or is coded by the exon A/B junction. (The epitope is unlikely to reside at the B/C boundary because a tryptic peptide comprising this region could not inhibit the binding of OX33 to spleen cells.[3] Similar results were obtained using spleen cells as targets and from a plate assay, where only sCD45.ABC and sCD45.C proteins (adhered to soft plates) could bind radiolabelled OX22 and OX32 and only sCD45.ABC and sCD45.C proteins (in solution) could abrogate such binding (data not shown). OX31, which is competitive with OX22,[3] was able to bind to sCD45.ABC and sCD45.C but not sCD45.O proteins adhered to soft plates as expected.

It is probable that the isoforms expressed in CHO cells are glycosylated differently from leucocyte CD45, and may show variation in reactivity with mAb directed against carbohydrate-dependent epitopes. However all the anti-CD45 mAb reacted with recombinant sCD45 and it is presumed that the glycosylation is either not important or is sufficiently similar not to affect these antigenic sites. In other species restricted anti-CD45 mAb react with murine CD45 expressed on fibroblast cell lines[23] and an anti-human CD45RA reacts with unglycosylated material produced by *in vitro* translation.[24] However some epitopes are dependent on glycosylation.[25]

Electron microscopy of purified sCD45 isoforms

The molecular dimensions of the sCD45.O and sCD45.ABC were clearly distinguished by electron microscopy after low-angle rotary shadowing (Fig. 5). The sCD45.0 forms a rod-like structure with dimensions of about 28 nm in length which is close to that previously estimated by electron microscopy of the tryptic fragment of thymocyte CD45, which consists of the extracellular and transmembrane segments of the smallest CD45 form.[26] The sCD45.ABC contains a similar rod-like segment together with a long thin segment giving an overall length of about 51 nm. The extra segments in sCD45.ABC contain 132 amino acids compared to 391 for the remaining common extracellular part and therefore the extra segments contribute disproportionally to the length. These had been predicted to have an extended structure, based on the amino acid sequence and extensive O-glycosylation.[4,27,28]

DISCUSSION

The finding that the sCD45.ABC and sCD45.C (and not sCD45.B) were able to inhibit the binding of OX22 (and OX32) to TDL indicates that the epitope for OX22 is present within the C exon. Johnson *et al.*[29] purified a tryptic glycopeptide from spleen CD45 using a OX22 affinity column and the sequence data obtained from this material indicated that the NH_2-terminal section corresponded to protein sequence coded by exon B. However, the full sequence of this tryptic glycopeptide was not obtained and this sequence would have continued either to include the start of the C exon up to Arg^{118} (numbering from ref. 4 and Fig. 1; i.e. B and C exons were expressed together) or to exclude exon C (i.e. if B exon was expressed without the C

exon). This clears up some of the confusion concerning the exon-coded segment that is recognized by these mAb, as the peptide data have been misinterpreted at OX22 being dependent on expression of B exon alone.[18,30]

Because a tryptic peptide (presumably spanning the sequence encoded by the B/C exon boundary) did contain the OX22 epitope[4,29] and we have now shown that the C exon alone is sufficient for reactivity, it can be deduced that the OX22 epitope is within amino acid residues Gly^{98} to Arg^{118}. It is possible that glycosylation precludes cleavage after the Arg but this seems unlikley since the OX32 epitope also requires exon C, is trypsin sensitive[3] and probably encompasses a region around Arg^{118} (unless trypsin is cleaving at an unusual site).

Given that OX22 (and OX32) recognize CD45 species containing the C-exon product and that OX22 gives similar labelling to that obtained with exon-B-dependent mAb in mouse and man,[31] it is possible that exons B and C are expressed concordantly. Recently three subsets of human $CD4^+$ T cells have been distinguished on the basis of expression of exons A and B.[31] Caution should be taken in trying to compare data obtained from different species; the presence of an epitope for mAb that recognizes one of the alternatively spliced exons does not preclude the possibility that other alternative exons are also present. Thus only when mAb recognizing all three alternative exons are available for human, mouse and rat CD45 will it be possible to compare properly the functions of different $CD4^+$ CD45-differentiated subsets. At present, there are no mAb that recognize the peptide encoded by exon C on human CD45, so a direct comparison must await these reagents.

The electron microscopy of sCD45.O and sCD45.ABC showed that the extra segments have an extended structure. This is illustrated in Fig. 6 which shows schematically that although the extra segments consist of only 132 amino acids they contribute to almost the same length as the remainder of the extracellular portion (391 amino acids). Not all the molecules in Fig. 6 are expressed on a single cell but are shown to illustrate their relative sizes. Thus molecules like CD45 and more especially the CD45.ABC form found on B cells, because of their extended structures and their abundance, are likely to be amongst the first to intereact with other cell surfaces. There may also need to be considerable reorganization before molecules such as those involved in antigen recognition can approach sufficiently closely to the surface of the target cell to interact specifically.

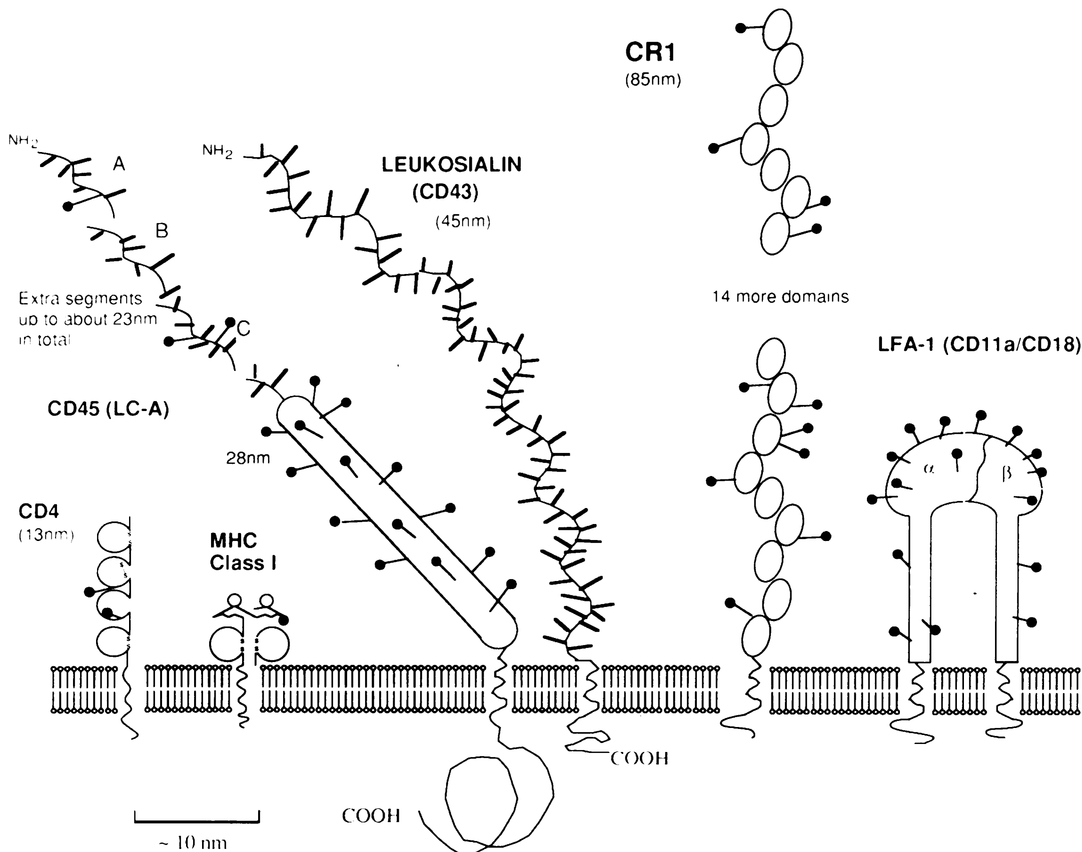

Figure 6. A model for leucocyte surface molecules drawn approximately to scale. The approximate lengths of the extracellular portions are given in nm. The dimensions are based on electron microscopy for rat CD45 (this study and ref. 26) rat CD43[32] and human complement receptor 1.[33] That for the human LFA-1 integrin (CD11a/CD18) is based on the dimensions for the related integrin, the fibronectin receptor.[34] The shape of CD4 is calculated from the dimensions of the first two domains determined from X-ray crystallography,[35] while that of the major histocompatibility complex (MHO) class 1 molecule is from the X-ray crystallography structure.[36] *N*-linked carbohydrate sites (—o); indicates possible O-linked carbohydrate sites (—).

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REFERENCES

1. Thomas M.L. (1989) The leukocyte common antigen family. *Ann. Rev. Immunol.* **7,** 339.
2. Tonks N.K., Charbonneau H., Diltz C.D., Fischer E.H. & Walsh K.A. (1988) Demonstration that the leucocyte common antigen CD45 is a protein tyrosine phosphatase. *Biochemistry*, **27,** 8695.
3. Woollett G.R., Barclay A.N., Puklavec M. & Williams A.F. (1985) Molecular and antigenic heterogeneity of the rat leukocyte-common antigen from thymocytes and T and B lymphocytes. *Eur. J. Immunol.* **15,** 168.
4. Barclay A.N., Jackson D.I., Willis A.C. & Williams A.F. (1987) Lymphocyte specific heterogeneity in the rat leucocyte common antigen (T200) is due to differences in polypeptide sequences near the NH_2-terminus. *EMBO J.* **6,** 1259.
5. Saga Y., Tung J.S., Shen F.W. & Boyse E.A. (1987) Alternative use of 5′ exons in the specification of Ly-5 isoforms distinguishing hematopoietic cell lineages. *Proc. natl. Acad. Sci. U.S.A.* **84,** 5364.
6. Ralph S.J., Thomas M.L., Morton C.C. & Trowbridge I.S. (1987) Structural variants of human T200 glycoprotein (leukocyte-common antigen). *EMBO J.* **6,** 1251.
7. Streuli M., Hall L.R., Saga Y., Schlossman S.F. & Saito H. (1987) Differential usage of three exons generates at least five different mRNAs encoding human leukocyte common antigens. *J. exp. Med.* **166,** 1548.
8. Stamenkovic I., Sgroi D., Aruffo A., Sy M.S. & Anderson T. (1991) The B lymphocyte adhesion molecule CD22 interacts with leukocyte common antigen CD45RO on T cells and α2-6 sialyltransferase, CD75, on B cells. *Cell*, **66,** 1133.
9. Spickett G.P., Brandon M.R., Mason D.W., Williams A.F. & Woollett G.R. (1983) MRC OX-22, a monoclonal antibody that labels a new subset of T lymphocytes and reacts with the high molecular weight form of the leukocyte-common antigen. *J. exp. Med.* **158,** 795.
10. Morimoto C., Letvin N.L., Distaso J.A., Aldrich W.R. & Schlossman S.F. (1985) The isolation and characterization of the human suppressor inducer T cell subset. *J. Immunol.* **134,** 1508.
11. Arthur R.P. & Mason D. (1986) T cells that help B cell responses to soluble antigen are distinguishable from those producing interleukin 2 on mitogenic or allogeneic stimulation. *J. exp. Med.* **163,** 774.
12. Akbar A.N., Terry L., Timms A., Beverley P.C. & Janossy G. (1988) Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. *J. Immunol.* **140,** 2171.
13. Bottomly K., Luqman M., Greenbaum L., Carding S., West J., Pasqualini T. & Murphy D.B. (1989) A monoclonal antibody to murine CD45R distinguishes CD4 T cell populations that produce different cytokines. *Eur. J. Immunol.* **19,** 617.
14. Terry L.A., Brown M.H. & Beverley P.C. (1988) The monoclonal antibody, UCHL1, recognizes a 180,000 MW component of the human leucocyte-common antigen, CD45. *Immunology*, **64,** 331.
15. Birkeland M.L., Johnson P., Trowbridge I.S. & Pure E. (1989) Changes in CD45 isoform expression accompany antigen-induced murine T-cell activation. *Proc. natl. Acad. Sci. U.S.A.* **86,** 6734.
16. Powrie F. & Mason D. (1989) The MRC OX-22$^-$ CD4$^+$ T cells that help B cells in secondary immune response derive from naive precursors with the MRC OX-22$^+$ CD4$^+$ phenotype. *J. exp. Med.* **169,** 653.
17. Mosmann T.R. & Coffman R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann. Rev. Immunol.* **7,** 145.
18. Powrie F. & Mason D. (1990) OX-22^{high} CD4$^+$ T cells induce wasting disease with multiple organ pathology: prevention by the OX-22^{low} subset *J. exp. Med.* **172,** 1701.
19. McKnight A.J., Barclay A.N. & Mason D.W. (1991) Molecular cloning of rat interleukin 4 cDNA and analysis of the cytokine repetoire of subsets of CD4$^+$ T cells. *Eur. J. Immunol.* **21,** 1187.
20. Cockett M.I., Bebbington C.R. & Yarranton G.T. (1990) High level expression of tissue inhibitor of metalloproteinases in Chinese Hamster ovary cells using glutamine synthetase gene amplification. *Biotechnology*, **8,** 662.
21. Davis S.J., Ward H.A., Puklavec M.J., Willis A.C., Williams A.F. & Barclay A.N. (1990) High level expression in Chinese hamster ovary cells of soluble forms of CD4 T lymphocyte glycoprotein including glycosylation variants. *J. biol. Chem.* **265,** 10410.
22. Sunderland C.A., McMaster W.R. & Williams A.F. (1979) Purification with monoclonal antibody of a predominant leukocyte-common antigen and glycoprotein from rat thymocytes. *Eur. J. Immunol.* **9,** 155.
23. Johnson P., Greenbaum L., Bottomly K. & Trowbridge I.S. (1989) Identification of the alternatively spliced exons of murine CD45 (T200) required for reactivity with B220 and other T200-restricted antibodies. *J. exp. Med.* **169,** 1179.
24. Streuli M., Matsuyama T., Morimoto C., Schlossman S.F. & Saito H. (1987) Identification of the sequence required for expression of the 2H4 epitope on the human leucocyte common antigens. *J. exp. Med.* **166,** 1567.
25. Pulido R. & Sanchez-Madrid F. (1990) Glycosylation of CD45: carbohydrate composition and its role in acquisition of CD45RO and CD45RB T cell maturation-related antigen specificities during biosynthesis. *Eur. J. Immunol.* **20,** 2667.
26. Woollett G.R., Williams A.F. & Shotton D.M. (1985) Visualisation by low-angle shadowing of the leucocyte-common antigen. A major cell surface glycoprotein of lymphocytes. *EMBO J.* **4,** 2827.
27. Jackson D.I. & Barclay A.N. (1989) The extra segments of sequence in rat leucocyte common antigen (L-CA) are derived by alternative splicing of only three exons and show extensive O-linked glycosylation. *Immunogenetics*, **29,** 281.
28. Cyster J., Somoza C., Killeen N. & Williams A.F. (1990) Protein sequence and gene structure for mouse leukosialin (CD43), a T lymphocyte mucin without introns in the coding sequence. *Eur. J. Immunol.* **20,** 875.
29. Johnson P., Williams A.F. & Woollett G.R. (1985) Purification of membrane glycoproteins with monoclonal antibody affinity columns. In: *Hybridoma Technology in the Biosciences and Medicine* (ed. T.A. Springer), p. 163. Plenum Press, New York.
30. Sparshott S.M., Bell E.B. & Sarawar S.R. (1991) CD45R CD4 T cells subset-reconstituted nude rats: subset-dependent survival of recipients and bi-directional isoform switching. *Eur. J. Immunol.* **21,** 993.
31. Mason D. & Powrie F. (1990) Memory CD4$^+$ T cells in man form two distinct subpopulations, defined by their expression of isoforms of the leucocyte common antigen. CD45. *Immunology*, **70,** 427.
32. Cyster J.G., Shotton D.M. & Williams A.F. (1991) The dimensions of the T lymphocyte glycoprotein leukosialin and identification of linear protein epitopes that can be modified by glycosylation. *EMBO J.* **10,** 893.
33. Weisman H.F., Bartow T., Leppo M.K., Marsh H.J., Carson G.R., Concino M.F., Boyle M.P., Roux K.H., Weisfeldt M.L. & Fearon D.T. (1990) Soluble human complement receptor type 1: *in*

vivo inhibitor of complement suppressing post-ischemic myocardial inflammation and necrosis. *Science*, **249,** 146.
34. Nermut M.V., Green N.M., Eason P., Yamada S.S. & Yamada K.M. (1988) Electron microscopy and structural model of human fibronectin receptor. *EMBO J.* **7,** 4093.
35. Wang J., Yan Y., Garrett T.P.J., Liu J., Rodgers D.W., Garlick R.L., Tarr G.E., Husain Y., Reinherz E.L. & Harrison S.C. (1990) Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. *Nature*, **348,** 411.
36. Bjorkman P.J., Saper M.A., Samraoui B., Bennett W.S., Strominger J. L. & Wiley D.C. (1987) Structure of the human class I histocompatibility antigen, HLA-A2. *Nature*, **329,** 506.