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Recognition of CD52 allelic gene products by CAMPATH-1H antibodies

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SUMMARY

Cloning of the CD52 from a B-lymphocyte tumour cDNA library revealed two closely related sequences differing only at two amino acids C-terminal to the proposed point of glycosyl-phosphatidylinositol (GPI)-linkage. When transfected into CHO cells only one of these sequences gave high-level expression of the antigen recognized by the prototypic anti-CD52 antibody CAMPATH-1 whereas in JURKAT cells good expression levels were obtained with both sequences. Fusion of the sequence from the second sequence to DNA encoding the extracellular domain of CD4 indicated that this sequence was capable of directing GPI linkage. The possible implications for the function of CD52 and serotherapy with anti-CD52 antibodies are discussed.

INTRODUCTION

The CD52 gene product is the antigen recognized by the CAMPATH-1 series of monoclonal antibodies.[1] This series of antibodies was identified by their ability to give very efficient lysis of human lymphocytes in the presence of human complement. Because of this property the CAMPATH-1 series has been developed for the serotherapy of lymphoid malignancies and autoimmune disease. CAMPATH-1 was the first antibody to be fully humanized and this humanized version (CAMPATH-1H) has been shown to have beneficial effects in non-Hodgkin's lymphoma[2] and rheumatoid arthritis.[3] The antigen recognized by the CAMPATH-1 series is CD52 and the structure of this molecule is now known in some detail. The mature peptide is a mere 12 amino acids in length and it is attached to the cell membrane via a glycosyl-phosphatidyl-inositol (GPI) linkage.[4,5] The polypeptide is *N*-linked glycosylated[6] explaining the apparent mobility on sodium dodecylsulphate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of 20 000–28 000 MW. The cDNA for the CD52 gene has been cloned and sequenced and shown to encode a 37-amino acid prepeptide which is subsequently processed to yield the mature CD52 peptide. It is postulated that the efficiency of anti-CD52 antibodies in lympholysis is partly due to the small size of the molecule and its lateral mobility in the membrane due to its GPI-anchorage. Both features which would allow antibody redirected complement or other effector mechanisms to be more effective.

In this study we report the successful expression of the CD52 in heterologous cells and confirm the presence of a second related sequence in human lymphocytes. Transfection of the second cDNA sequence gave much lower levels of expression of the CD52 antigen than the original sequence in CHO cells whereas in JURKAT cells the levels of expression were much more comparable suggesting differences in the efficiency of processing the gene product of the CD52 alleles in different cell types.

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MATERIALS AND METHODS

Cell culture

Wien 133 cells were cultured in filter-sterilized Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine and 500 IU/ml penicillin/500 μg/ml streptomycin. CHO dihydrofolate reductase $(dhfr)^-$ cells were detached by pretreatment with 0·02% versene containing 1·25% trypsin, washed in medium and cultured in filter sterilized Dulbecco's modified Eagles medium (DMEM) supplemented with 10% (v/v) heat-inactivated FCS, 2 mM L-glutamine, non-essential amino acids, hypoxanthine, thymidine and 500 IU/ml penicillin/500 μg/ml streptomycin. Transfected CHO $dhfr^-$ cells were cultured in DMEM supplemented with 10% (v/v) heat-inactivated *dialysed* FCS, 2 mM L-glutamine, non-essential amino acids and 500 IU/ml penicillin/500 μg/ml streptomycin. As the gene of interest became expressed, methotrexate, an inhibitor of dhfr, was added to the medium at an initial level of 3×10^{-8} M. This procedure selected for random amplification of the resident plasmid DNA associated with higher level gene expression. Once growth of the transfectants was stable at 3×10^{-8} M the methotrexate concentration was increased to 1×10^{-7} M. A repeat of several rounds of amplification led to maximal expression levels for the selected gene. The highest concentration of methotrexate employed was in the order of 1×10^{-6} M.

FACScan analysis and flow cytometry
Cell lines were washed and resuspended in fluorescence activated bell sorter (FACS) buffer (5% (v/v) FCS, 0·1% sodium azide (NaN_3) in phosphate-buffered saline (PBS)) at 1×10^5 cells/ml. The cells were dispensed into 96-well U-bottomed microtitre plates at 100 μl per well and 10 μl fluorescein isothiocyanate (FITC)-labelled antibody was then added. The cells were incubated at 4° for 30 min, to stain, before the plate was centrifuged at 1000 *g* in a Sorvall RT6000B bench centrifuge for 5 min. The excess liquid was removed and the cell pellets were resuspended in 100 μl wash buffer (0·1% sodium azide in PBS) and the process was repeated twice more. Once the cells had been fluorescently labelled they were finally resuspended in 100 μl FACS fix buffer (1% paraformaldehyde in PBS) and analysed on a FACScan (Becton Dickinson, Oxford, UK).

PIPLC treatment
Transfected CHO cells were diluted to 10^6/ml in medium and 100 μl either treated with 5 units of the enzyme phosphatidylinositol-specific phospholipase C (PIPLC, Boehringer Mannheim, Lewis, East Sussex, UK) or medium alone for 1 hr at 37°. The cells were then washed and stained with either FITC-labelled anti-CD4 or FITC-labelled CAMPATH-1H.

cDNA cloning and expression
To isolate the cDNA for the CD52 antigen from Wien 133, two 25-base pair (bp) polymerase chain reaction (PCR) primers were made which corresponded to either the 5′ sequences of the coding strand and which incorporated a *Hin*dIII site or to the 3′ sequences of the gene plus an included *Eco*RI site complementary to the coding strand, both based on the published sequence.[4] The Wien 133 cells were used to prepare total RNA by the guanadinium isothiocyanate method and the mRNA was isolated by magnetic separation on a Dynal column. 'SuperScript', (Gibco BRL, Paisley, UK), was used to produce a first strand cDNA and then a second strand copy was synthesized. A PCR performed on the resulting cDNA utilizing the primers mentioned previously produced an expected band of 206 bp. The band was purified on an agarose gel after cutting with the enzymes *Hin*dIII/*Eco*RI and the insert was cloned into either PUC 18 or PUC 19 cut with *Hin*dIII/*Eco*RI. After checking the sequence utilizing a Sequenase 2 kit (USB), the correct bands were excised from the holding vectors and cloned into an expression vector pRDN-1.

The vector, pRDN-1, contains the strong B-actin promoter and polyadenylation signals either side of a polylinker containing both *Hin*dIII and *Eco*RI sites therefore, it was possible to insert the CD52 antigen directly into the correct orientation. The vector also contains a dhfr gene cassette which permits possible selection and amplification of the correct antigen expressing colonies with methotrexate. After mapping positive colonies by restriction analysis the final selected plasmids pRDN AG and pRDN ΔC1H were grown up in large shake flasks and the plasmid DNA purified by caesium gradients. The CD52 containing DNA, was transfected into the CHO dhfr$^-$ line B11 using Promega Transfectam. Successful transfection with the CD52 constructs was monitored by methotrexate resistance and surface expression of the antigen, as assessed by FACS analysis, with a wide spread of expression levels seen only for the authentic CAMPATH-1 construct. Following amplification of both constructs in 3×10^{-8} M methotrexate, single highly expressing or resistant clones of the transfected cell lines were isolated by dilution cloning.

CD4/CAMPATH antigen chimeric constructs
Full length extracellular CD4 was jointed to the GPI-displaced signal peptide of either the construct pRDN AG or pRDN ΔC1H via PCR utilizing overlap primers designed to correspond to either the proline residue at residue +373 in CD4 or the proline residue +11 in CAMPATH antigen CDw52 whilst retaining the original frame. The resulting constructs named pRDN CD4AG and pRDN CD4ΔC1H were confirmed as correct via sequencing with the Sequenase 2 kit supplied by USB. Both constructs, as well as full length CD4 or CAMPATH antigen in pRDN-1, were transfected into CHO dhfr$^-$ cells as before and amplified to 3×10^{-8} M methotrexate prior to dilution cloning.

RNAase protection assay
CHO cells containing either pRDN AG, pRDN ΔC1H or the parental cells were used as a source of template for RNAase protection assays. A certain number of cells, i.e. 10^7/ml or 10^6/ml, were lysed with the lysis buffer from a USB RNA protection kit and the lysates were mixed with either single-stranded forward or reverse ^{32}P-labelled probes made by RNA runoffs from the whole CAMPATH antigen cloned into a Bluescript vector. The reactions were left overnight at 37° before the products were treated with RNAase to remove excess probe and the products separated on a 6% sequencing gel. The dried gel was autoradiographed.

In vivo *experiments*
Parental or transfected CHO cells to be studied were harvested from flasks, washed and resuspended in PBS at 2×10^7/ml before being mixed with an equal volume of 6 mg/ml matrigel for injection subcutaneously (0·1 ml) into nude mice. After 1 month, approximately, tumours were excised from the animals, teased into single cell suspensions and either analysed by FACS for CD52 expression.

Immunoprecipitations and Western blotting
CHO cells were lysed in 3% nonidet P-40 (NP-40) lysis buffer (containing 20 mM MOPS, 15 mM EGTA, 3% NP-40, 2 mM phenylmethylsulphonyl fluoride, 1 mM Na_2VO_4 (10 μg/ml aprotonin, 2 mM EDTA) for 15 min on ice. After which time the lysate was centrifuged at 10 000 *g* for 5 min at 4°. The clarified supernatant was precleared with Sepharose-4B for 30 min at 4°, and the precleared lysate incubated with Q4120 (anti-CD4, Sigma, St Louis, MO)–coupled Sepharose for 1 hr at 4° with end-over-end rotation. The beads were then washed twice in lysis buffer and resuspended in non-reducing SDS–PAGE sample buffer and boiled for 3 min. The samples were run on 8% SDS–PAGE gels, blotted onto nitrocellulose and the blots blocked with 3% non-fat milk in PBS. The blots were then probed with biotin-Q4120 followed by streptavidin–horseradish peroxidase and developed by enhanced chemiluminescence (ECL, Amersham, Amersham, UK).

RESULTS

Cloning and expression of the CD52 gene

Complementary DNA was prepared from the B-cell lymphoma Wien 133 and used as a template for the PCR amplification

of a 206-bp fragment containing the cDNA for CD52. A cDNA clone corresponding to the reported sequence for CD52[4] was cloned into the mammalian expression vector pRDN-1 and the resulting construct transfected into both dhfr/CHO cells and JURKAT T cells. In the case of CHO cells there was no requirement for co-transfection with plasmid containing a selective marker as pRDN-1 contains the dhfr gene. JURKAT were co-transfected with the CD52 gene in pRDN-1 and the p321 plasmid which confers neomycin resistance. Both cell lines expressed the CD52 gene product as recognized by the anti-CD52 antibody CAMPATH-1H (Fig. 1), indicating that there are apparently no stringent species or cell lineage requirements for the expression of this human leucocyte antigen. Untransfected CHO cells did not stain with CAMPATH-1H (data not shown and Fig. 2). Parental JURKAT cells have low to negligible levels of CD52, Fig. 1 shows three subclones of JURKAT cells expressing different levels of CD52 after transfection, indicating that there is some clonal variation in the expression of CD52, analysis of pooled transfectants was subsequently used to assess efficiency of expression of CD52. The expression of CD52 on CHO cells was reduced by treatment with PIPLC indicating that, as in lymphocytes, the CD52 antigen is linked to the membrane via a GPI-anchor (Fig. 1).

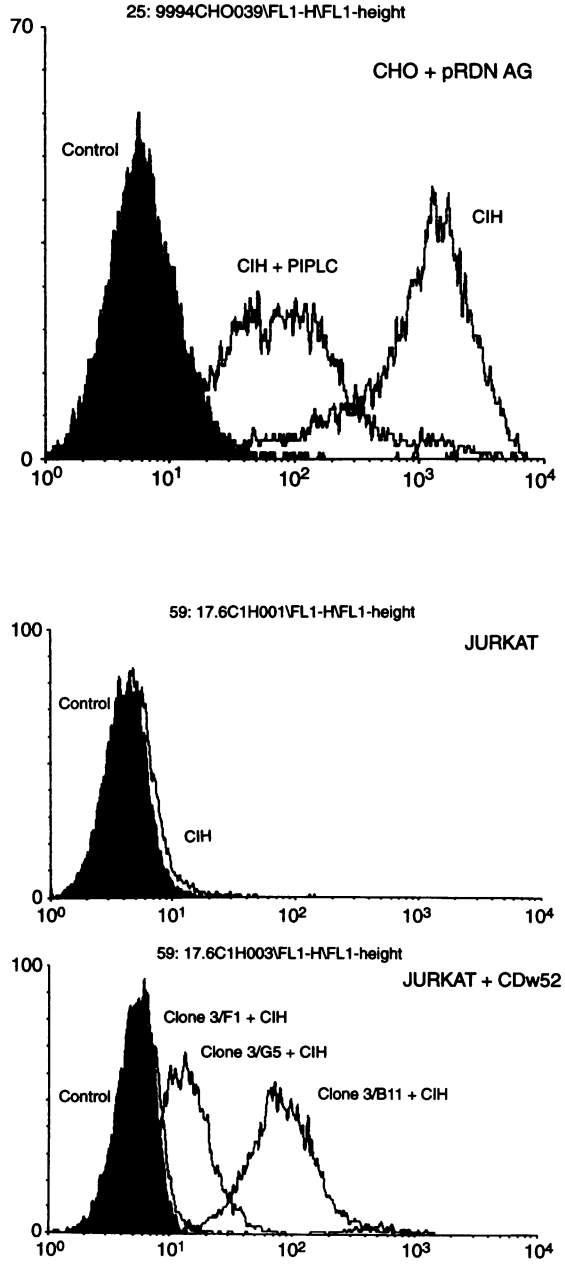

Figure 1. Heterologous expression of CD52. The cDNA for CD52 was transfected into either CHO cells or JURKAT cells. After appropriate selection, cells were stained with FITC-labelled CAMPATH-1H and analysed by flow cytometry. Treatment with PIPLC to remove GPI-anchored proteins was performed as described in the Materials and Methods. (JURKAT, control JURKAT cells; JURKAT + CDw52, JURKAT cells transfected with cDNA encoding CD52).

Sequence analysis of the CD52 gene

During the cloning and sequencing of the PCR products from the Wien 133 cDNA it became apparent that two sequences were represented in the cDNA. These were found in approximately equal proportions amongst independent clones (Table 1). The two sequences were AAC ATA and AGC ATG at positions 151–156 which would result in changes from Asn-Ile to Ser-Met at positions 16 and 17 of the mature peptide, a site

CDw52 sequence

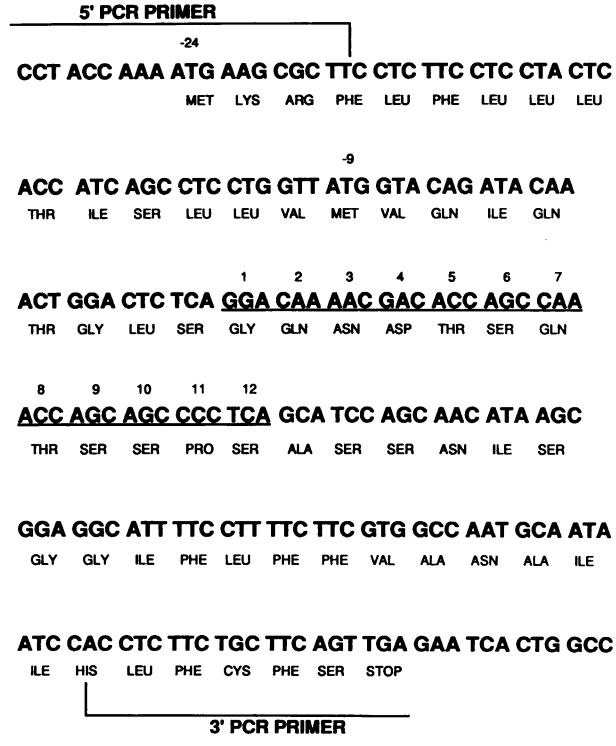

Figure 2. The DNA sequence of the CD52 gene is shown with the primers use for the PCR reactions to amplify the gene.

Table 1. Sequence analysis of PCR inserts

	CDw52	ΔC1H
Exp. 1	1/2*	1/2
Exp. 2	4/8	4/8
Exp. 3	2/3	1/3
Total	7/13	6/13

*Data are expressed as the number with that particular sequence divided by the total number sequenced for each experiment.

on the C-terminal side of the proposed GPI-anchorage site (Fig. 2). This difference was also noted by Xia *et al.*[4] in independently isolated cDNA clones but since only one clone was isolated the authors were unable to conclude whether this sequence represented a second allele or a cloning artefact. Since the observed changes were in the region of the site for the addition of the GPI-anchor (Fig. 3) we decided to clone and express this second variant. Figure 4 shows the result of stable transfection of the two forms into CHO cells. The original version (CD52) led to high-level expression of CD52 as measured by CAMPATH-1H staining. The second form ΔC1H showed only a very low level of expression of the CD52 antigen. Ribonuclease protection assay analysis indicated that both transfectants were making the appropriate mRNA (Fig. 5). The difference in expression levels was maintained throughout the amplification with methotrexate (data not shown). The epitope recognized by the CAMPATH-1H antibody is composed of contributions from both the peptide sequence and the GPI-anchor.[5] Thus it is possible that if the observed change in peptide sequence leads to a change in the position of the attachment of GPI-anchor then the antigen expressed from the alternative sequence will not be recognized by the CAMPATH-1H antibody. In order to test whether the alternative sequence could function as a membrane-anchoring sequence it was therefore necessary to attach another determinant to the potential anchor sequences.

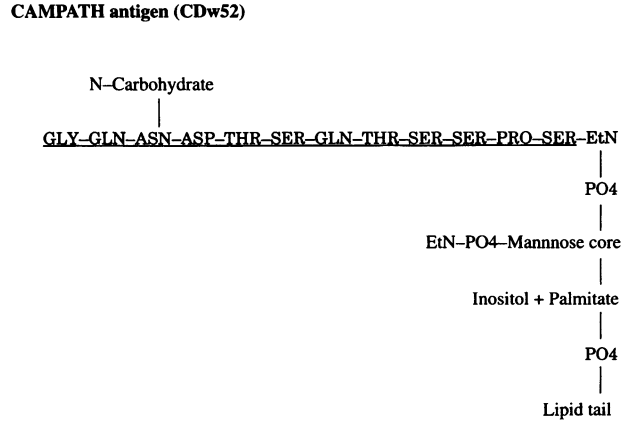

Figure 3. The amino acid sequence of the CD52 gene product with the proposed site of the GPI-anchor is shown.

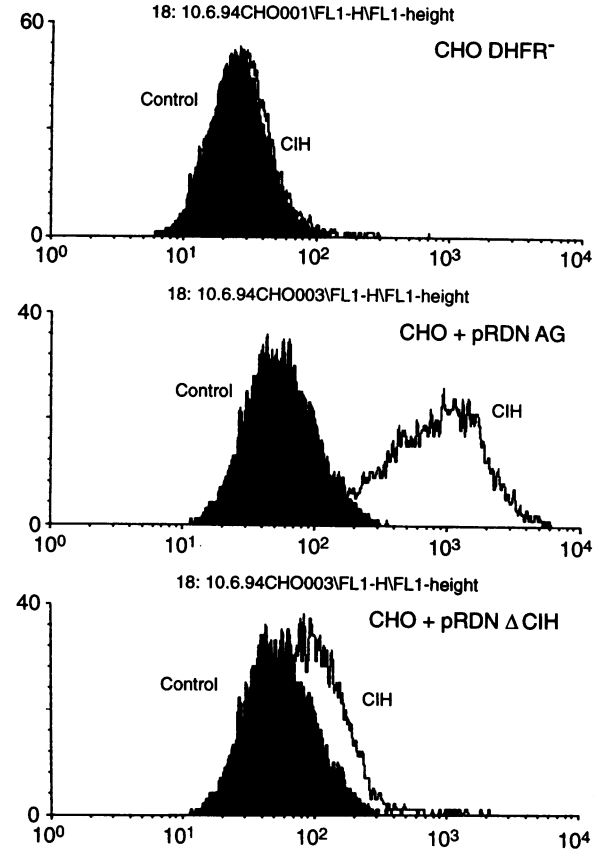

Figure 4. Expression of the two related CD52 genes; cDNA for CD52 (pRDN AG) or the alternative sequence pRDN ΔC1H were transfected into CHO cells and appropriate selection and amplification applied. Untransfected CHO cells (CHO DHFR$^-$) pRDN AG-transfected CHO cells (CHO + pRDN AG), or pRDN ΔC1H-transfected CHO cells were stained with FITC-labelled CAMPATH-1H and analysed by flow cytometry.

Construction of CD4 extracellular domain–CD52 chimerae

In order to determine whether the CD52 sequence could function as a membrane-anchoring sequence, chimeric genes were constructed consisting of the coding regions for extracellular domains of CD4 and the anchoring sequence from CD52 or ΔCIH. These constructs were transfected into CHO cells and monitored for CD4 expression. Both chimeric molecules were expressed as detected by staining with anti-CD4 antibody (Fig. 6). The replacement of the extracellular domain of CD52 with that of CD4 prevented the detection with anti-CD52 antibodies. In fact, transfectants expressing the CD4/ΔCIH chimera showed higher levels of CD4 staining than either the CD4/Ag chimera or the transmembrane CD4 construct. Furthermore, immunoprecipitation analysis indicated that the transfected constructs yielded recombinant proteins of the correct size, those constructs encoding GPI-anchored molecules being smaller than the full-length CD4 molecule (Fig. 7). Thus both structures can direct the extracellular domain of CD4 to the cell membrane. The

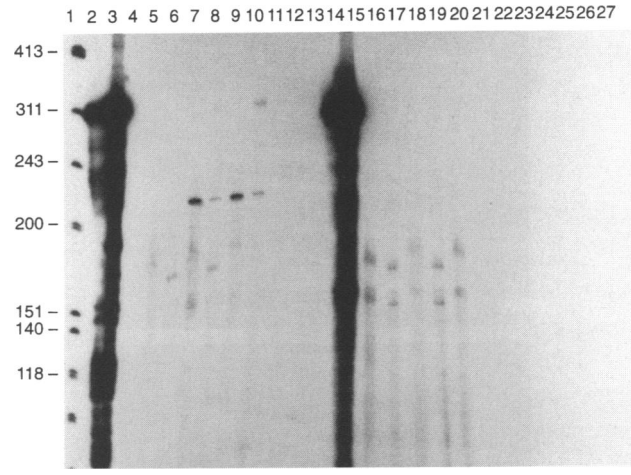

Figure 5. Measurement of mRNA levels by RNAase protection; CHO cells transfected with pRDN AG or CHO cells transfected with pRDN ΔC1H were used as template for RNA protection studies. Either 10^7 cells/ml or 10^6 cells/ml were incubated with CAMPATH antigen probes transcribed in forward or reverse orientation in the Bluescript vector KS$^+$ (10^5 c.p.m. per reaction). Lane 1, ^{32}P end-labelled Ø174 marker cut with *Hinf*; lane 3, 5 μl reverse probe; lanes 5–10, cell lysates plus reverse probe digested with RNAase of which lane 5 10^7/ml CHO cells, lane 6 10^6/ml CHO cells, lane 7 10^7/ml CHO cells plus pRDN AG, lane 8 10^6/ml CHO cells with pRDN AG, lane 9 10^7/ml CHO cells with pRDN ΔC1H, lane 10 10^6/ml CHO cells with pRDN ΔC1H; lanes 2, 4, 11–13 blank; lane 14, 5 μl forward probe; lanes 15–20, cell lysates plus forward probe digested with RNAase of which lane 15 10^7/ml CHO cells, lane 16 10^6/ml CHO cells, lane 17 10^7/ml CHO cells plus pRDN AG, lane 18 10^6/ml CHO cells with pRDN AG, lane 19 10^7/ml CHO cells with pRDN ΔC1H, lane 20 10^6/ml CHO cells with pRDN ΔC1H; lanes 21–27 blank. Lanes 7–10 show the expected band at 206 bp.

transfectants were treated with PIPLC to determine whether the CD4 was linked via a GPI-anchor, in both cases expression levels were diminished by this treatment. By contrast CHO cells transfected with a construct encoding a full-length CD4 cDNA containing a transmembrane and cytoplasmic domain expressed CD4 which was unaffected by PIPLC treatment (Fig. 6).

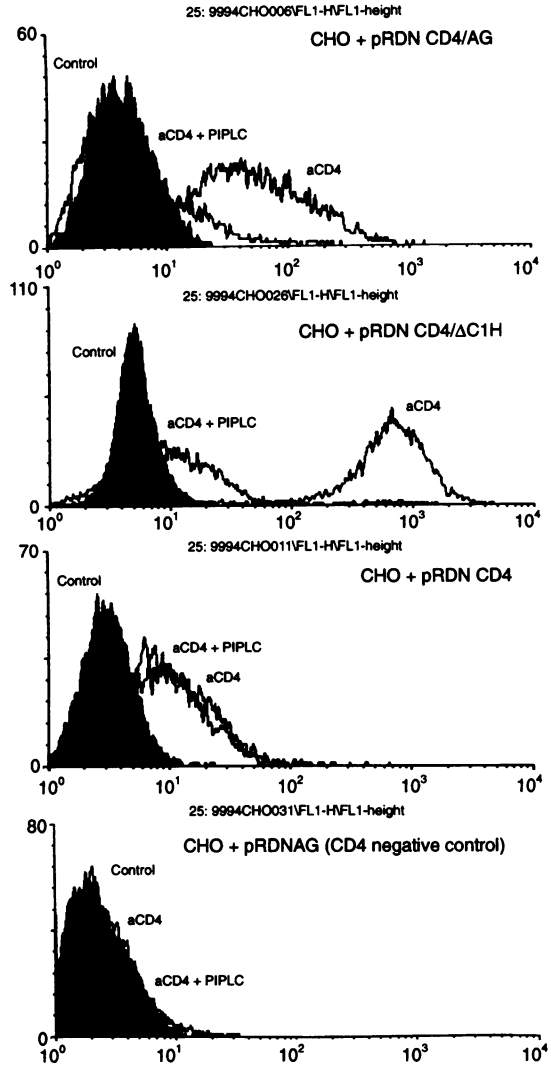

Figure 6. PIPLC treatment of transfectants; pRDN CD4/Ag, pRDN CD4/ΔC1H and CD4 CHO cell transfectants were incubated with either medium alone or PIPLC and then stained with FITC-labelled anti-CD4 and analysed by flow cytometry.

Expression of CD52 alleles in JURKAT cells and normal human peripheral blood mononuclear cells

In order to determine whether the inefficient expression of the alternative CD52 allele in CHO cells was related to cell type, the cDNA for both alleles were transfected into JURKAT T cells and the transfected pools were subjected to flow cytometric analysis. Figure 8 shows that both alleles are efficiently expressed in this lymphocytic cell line. However, even in this cell line the expression of the alternative allele is somewhat lower than the originally described allele, possibly reinforcing the notion that this gene product is less efficiently processed, the originally described allele showing an approximately sevenfold higher peak fluorescence than the alternative allele. The expression of the two alleles in normal peripheral blood mononuclear cells (PBMC) was investigated by sequencing the PCR products amplified from cDNA prepared from 11 normal individuals. In two of 11 individuals only the originally described allele could be found, in six the alternative allele was the only product present and in the other three both products could be isolated. Flow cytometric analysis of the PBMC indicated that all the individuals expressed CD52 at approximately the same level (data not shown).

Morphological and functional characteristics of transfectants

The function of the CD52 gene product is currently unknown. Other GPI-anchored structures such as CD59[7] and CD55[8] have been shown to act to protect cells from complement attack. During the course of experiments in which we expressed the two CDw52 sequences we noticed a major difference in morphology between the two transfected cell lines. Whereas untransfected CHO cells and CHO cells transfected with the ΔCIH gene product were rounded in appearance, cells transfected with the originally described CD52 gene adopted an elongated, spindly morphology (Fig. 9). We wished to determine whether this difference may have any corollary in terms of functional behaviour. To determine whether expression of the different

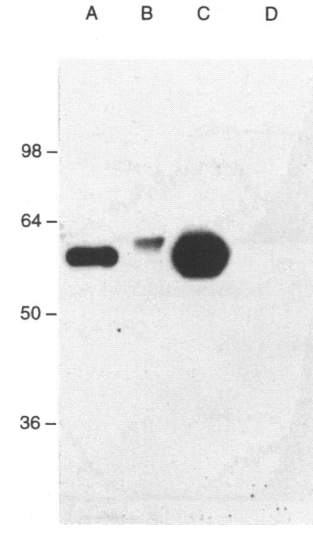

Figure 7. Immunoprecipitation analysis of transfectants; Lysates from CHO pRDN CD4/AG, CHO pRDN CD4/ΔC1H, CHO-CD4 or CHO cells were immunoprecipitated using anti-CD4 Sepharose and the immunoprecipitated proteins were separated by SDS–PAGE, transferred to nitrocellulose by Western blotting and probed with anti-CD4 antibodies. Lane A, pRDN CD4/AG; lane B, pRDN CD4/ΔC1H; lane C, CHO-CD4; lane D, CHO.

genes had an effect on growth *in vivo*, 1×10^6 cells of each cell type were transplanted subcutaneously into nude mice and monitored for tumour growth. Table 2 shows the data from two separate experiments in which the identical results were obtained. Only mice receiving CHO cells expressing the CD52

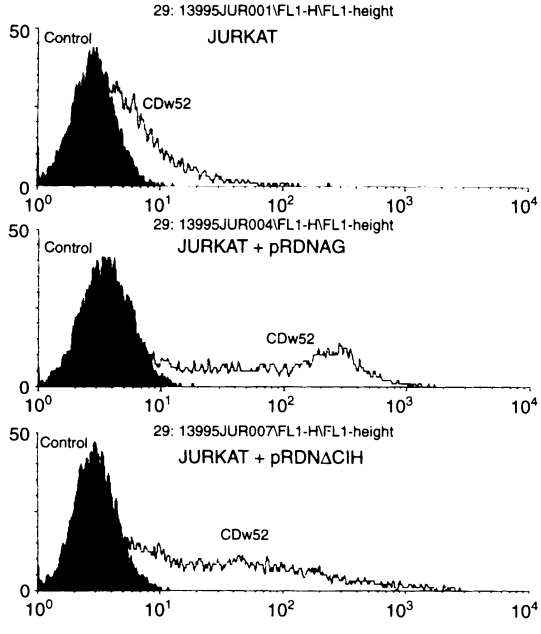

Figure 8. Expression of CD52 alleles in Jurkat T cells; Jurkat T cells were transfected with either pRDN AG or pRDN ΔC1H as described in the Materials and Methods. The transfected T cells were stained after passage in select medium with FITC-labelled CAMPATH-1H antibody as indicated.

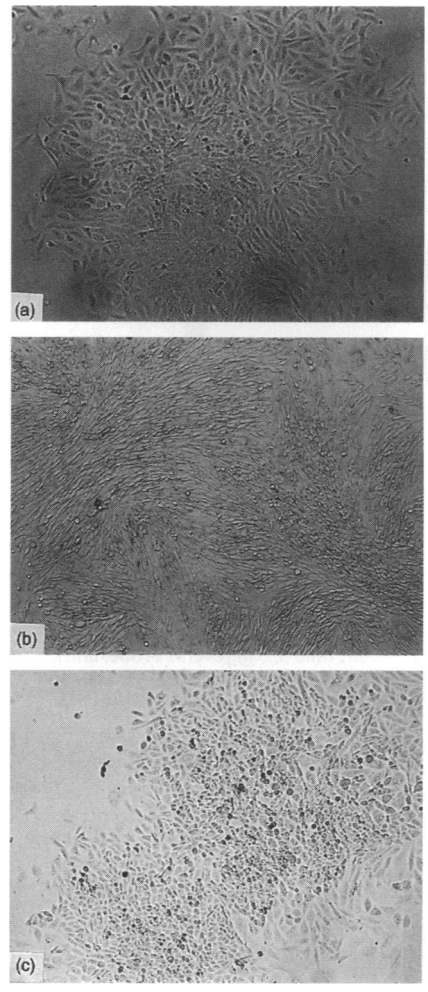

Figure 9. Morphological differences between transfected cell lines; photomicrographs of subconfluent CHO cells. (a) Parental CHO cells, (b) pRDN AG-transfected CHO cells, and (c) pRDN ΔC1H-transfected cells.

(CHO/pRDN AG) gene presented with tumours, mice receiving untransfected CHO cells or CHO cells transfected with the ΔCIH gene (CHO/pRDN ΔCIH) showed no sign of tumour growth. These data may indicate that the CD52 gene product may play a role in either cell–cell adhesion or protection of the

Table 2. Growth of CHO transfectants *in vivo*

	CHO	CHO/pRDN AG	CHO/pRDN ΔC1H
Exp. 1	0/10*	4/10	0/10
Exp. 2	0/10	3/10	0/10
Exp. 3	0/10	9/10	ND†
Total	0/30	16/30‡	0/20

* Data are expressed as the number of mice exhibiting tumour growth divided by the total number of mice inoculated.

† ND, not determined.

‡ $P < 0{\cdot}001$ according to χ^2 analysis.

cell from extracellular influences, both roles would be consistent with enhanced survival of the transfectants *in vivo*.

DISCUSSION

The CD52 antigen was first discovered by screening for antibodies which were effective in fixing human complement.[1] Rat antibodies of the CAMPATH-1 series were the prototype anti-CD52 antibodies and these have been developed for therapeutic use as lymphocyte-depleting agents in the treatment of lymphoid malignancy and autoimmune disease.[2,3] The interest in the potential therapeutic exploitation of the CAMPATH-1 series has been paralleled by research into the nature of the antigen. Xia *et al.* showed that the CD52 antigen is a 12-amino acid-glycosylated polypeptide which is linked to the plasma membrane via GPI-linkage.[5] The determinant recognized by CAMPATH-1 is formed from a contribution from both the peptide backbone and the GPI-anchoring. The requirements for the attachment of a GPI-anchor are to have a small amino acid such as serine—to which the anchor is attached—separated by 10–12 amino acids from a strongly hydrophobic domain.[9,10]

During the cDNA cloning of CD52 Xia *et al.*[4] noted that two closely related sequences were found, differing at two amino acids C-terminal to the GPI-anchoring site. Whereas the CD52 cDNA codes for Asn-Ile in these two positions, the alternative ΔCIH encodes Ser-Met. When transfected into CHO cells the 'wild-type' version of the gene encoding Asn-Ile at positions 16 and 17 led to high-level expression of the antigen recognized by the CAMPATH-1H antibody. Subsequently there has been an independent report in which the two sequences have been noted.[11] We first expressed CD52 in CHO and JURKAT cells and were able to detect the antigen with CAMPATH-1H antibody. The antigen was sensitive to treatment with PIPLC indicating a GPI-linkage to the cell membrane. The expression in both hamster and human cells of differing tissue origin suggests that there is no tissue or species specificity controlling the expression of the originally described CD52 allele. This is similar to other GPI-anchored proteins such as CD55 and CD59 which have also been expressed in heterologous cell lines such as CHO or Hela cells.[12,13] CHO cells transfected with the alternative Ser-Met version of the gene expressed much lower levels of CD52 despite an identical sequence in the coding region for the predicted CD52 peptide backbone. Two possible explanations for this observation are first, that the GPI-linkage differs for this sequence and forms an antigen not recognized by CAMPATH-1H despite being expressed, or second, that the alternative sequence is inefficiently processed in these transfectants. The analysis of the processing of the CD52 GPI-anchor is hampered by the fact that there are a limited number of anti-CD52 antibodies and the majority recognize the same epitope which is at least partially made up of elements of the GPI-anchor. Therefore, to study further the properties of the CD52 sequence we constructed CD4/CD52 chimeric genes and expressed these in CHO cells. Flow cytometry of the transfectants indicated that both the CD52 and the ΔCIH sequences allowed the membrane expression of CD4 and furthermore in both cases the expression was sensitive to treatment with PIPLC, CHO cells expressing full-length transmembrane-anchored CD4 were also treated with PIPLC and in this case CD4 expression was found to be PIPLC-insensitive. Thus, the ΔCIH sequence can be processed to generate a GPI-anchor with the CD4 extracellular domains, in fact expression levels are somewhat higher than with the original CD52 sequence. It is therefore possible that the inability to obtain CAMPATH-1H reactivity with ΔCIH is due to an inefficiency of the entire ΔCIH sequence to be processed and transported to the cell surface. This is not however due to an intrinsic inability of the ΔCIH sequence to form a GPI-linkage. Other possibilities are that the ΔCIH gene product is processed but that the product is not recognized by antibodies of the CAMPATH-1 series possibly due to the attachment of the GPI-anchor at an alternative site or possibly due to more rapid turnover or degradation.

The existence of two forms of the CD52 gene has now been reported by several independent laboratories.[4,11] If the CD52 gene has different alleles it is interesting to speculate what effect this would have on expression of CD52. In the normal population there have been no reports of $CD52^-$ patients suggesting that if people homozygous for the alternative form exist they express the antigen efficiently. Indeed our analysis of PBMC from normal donors indicates that a significant proportion have only the second allele and yet express high levels of CD52 on their lymphocytes. This is consistent with our data obtained with CD4–CD52 chimeric molecules but not with the low-level expression of CD52 in CHO cells transfected with the alternative sequence. A possible explanation is that the alternative gene product is less efficiently processed in CHO cells leading to low expression levels. In lymphocytes the gene may be processed more efficiently leading to higher levels of expression, consistent with this possibility is the fact that the expression of CD52 on normal lymphocytes is very high. The experiment shown in Fig. 8 supports this hypothesis. Both alleles are efficiently expressed in JURKAT T cells, however even in the T-cell line there is better expression in the transfected cell pool with the original allele, with almost a sevenfold difference in the peak fluorescence. This compares to an approximate tenfold difference in the CHO cell transfectants.

The function of the CD52 gene product is not known at present. The data presented here suggest that expression of CD52 in CHO cells gives them a selective growth advantage *in vivo*. Interestingly, the pRDN ΔCIH transfectants were similar to CHO cells both morphologically and in terms of *in vivo* tumorigenicity. This suggests that the relatively inefficient expression of CD52 by these cells does not lead to the changes in morphology and the same selective advantage. Such an observation would be consistent with either a role for CD52 in cell protection from the host environment or with a role in cell–cell adhesion both of which would favour enhanced survival. The transfected cell lines described here may prove to be extremely useful reagents for the elucidation of the function of CD52.

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