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MOLECULAR CHARACTERIZATION OF THE WC1 ANTIGEN EXPRESSED SPECIFICALLY ON BOVINE CD4$^-$CD8$^-$ $\gamma\delta$ T LYMPHOCYTES

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Although $\gamma\delta$ T lymphocytes were identified several years ago, the functional importance of these cells remains to be established. $\gamma\delta$ T cells of ruminants are unique in two respects. First, they are present at much higher levels compared to man and rodents. Second, ruminant CD4$^-$CD8$^-$ $\gamma\delta$ T cells uniquely express a 220 kD surface Ag recognized by a panel of mAb, recently clustered as WC1. WC1 has been most extensively studied in sheep with the use of the mAb T19. Here, we report on the isolation of a full length cDNA clone, encoding the WC1 Ag, from a COS cell cDNA expression library prepared from a bovine $\gamma\delta$ T cell line. The protein encoded by the pWC1 cDNA clone was reactive with the bovine mAb CC15 and IL.A29, and with T19. The cDNA clone consisted of 4475 bp and contained a single long open reading frame of 1436 amino acids. The pWC1 cDNA clone encoded a type 1 integral membrane protein with an extracellular domain consisting of 11 scavenger receptor cysteine-rich-repeats with homology to CD5 and CD6. Southern blotting suggested that the bovine genome contained multiple sequences highly related to the isolated WC1 cDNA. Furthermore, WC1-like sequences were present in the genomes of all mammals tested including mouse and man. The molecular characterization of the WC1 Ag as reported here provides a starting point for the definition of its role in $\gamma\delta$ T cell biology.

Despite numerous reports on the immunobiology of $\gamma\delta$ T cells, several key questions remain unresolved. First, there is no general agreement on the nature of Ag and restriction elements "seen" by the $\gamma\delta$ heterodimer (1). Second, although the overall structure and function of TCR/CD3 complexes in $\alpha\beta$ and in $\gamma\delta$ T cells appear to be very similar (2, 3), no functional homologues of the important accessory molecules CD4 and CD8 have been described in CD4$^-$CD8$^-$ $\gamma\delta$ T cells. Third, the molecular basis for the well documented epithelial homing pattern of $\gamma\delta$ T cells is not understood (4). The study of these issues has been hampered in part by the apparent lack of unique surface molecules on $\gamma\delta$ T cells in rodents and man.

The presence of large numbers of $\gamma\delta$ T cells is a particularly prominent feature of the ruminant immune system (5). These cells may represent up to 30 to 50% of the T cells in the blood of young animals and are abundantly present in lymph and at epithelial surfaces. Thus, ruminant $\gamma\delta$ T cells provide an excellent model for studies on $\gamma\delta$ T cell function. An additional advantage of this model is the availability of mAb, clustered as WC1 (6). The WC1 Ag is a 220-kDa surface glycoprotein with a protein backbone of approximately 170 kDa, which has been shown to be unrelated to CD45 (7). Immunohistochemical studies have revealed that the $\gamma\delta$ T lymphocyte is the only cell type in the bovine organism expressing the WC1 Ag. Bovine WC1$^+$ $\gamma\delta$ T cells are negative for the CD2, CD4 and CD8 T cell markers but weakly express the CD5 marker (8).

At present no function is known for the WC1 Ag on these cells. To learn more about its possible function we pursued the isolation of a full length cDNA clone encoding the bovine WC1 Ag using the eukaryotic expression cloning technique originally developed by Aruffo and Seed (9).

MATERIALS AND METHODS

Preparation of cDNA sublibraries. Poly(A)$^+$ RNA was isolated from the bovine CD4$^-$CD8$^-$ $\gamma\delta$ T cell line D409/N2, which was derived from WC1$^+$ peripheral blood lymphocytes immortalized by infection with the intralymphocytic parasite *Theileria parva* (10). A size-fractionated (>2000 bp) oligo dT-primed cDNA library was constructed in the pCDM8 vector using non-self complementary *Bst*XI linkers (11). High voltage electroporation (25 μF, 200 ohm, 12.5 kV/cm) of the library into the *Escherichia coli* strain mc1061/p3 yielded approximately 50,000 primary colonies. These were divided in five sublibraries of 10,000 colonies each.

Isolation of the pWC1 cDNA clone. Trypsinized COS cells were transfected in 4 ml RPMI 1640/2% FCS/100 μm chloroquine containing 400 μg/ml DEAE-dextran and 1 μg/ml of purified plasmid DNA from the sublibraries. After incubation for 2 h at 37°C, the cells were washed in RPMI 1640 and cultured in Dulbecco's modified Eagle's medium/10% FCS for 48 h. Thereafter, cells were fixed with methanol and stained with the WC1 mAb IL.A29 and CC15 mAb (ascites 1/1,000 diluted in PBS/5% FCS for 60 min). As second antibody a peroxidase-conjugated rabbit-anti-mouse antibody was applied (Dako, Glostrup, Danmark, 1/40 diluted in PBS/5% FCS for 60 min). Positive cells were visualized with 0.02% 9-amino-3-ethylcarbazol/0.1% hydrogen peroxide in 0.1 M sodium acetate, pH 4.8 (12). The pWC1 cDNA clone was isolated from one of the sublibraries by screening progressively smaller pools of bacterial colonies.

Surface expression of WC1 Ag on transfected mouse L cells. Thymidine kinase deficient mouse L cells were transfected with pWC1 cDNA clone along with a chicken thymidine kinase gene by calcium phosphate precipitations as previously described (13). Following selective growth in HAT medium, transfectants expressing the WC1 genes were isolated by repeated FACS. Cell-surface staining was performed at 4°C using appropriate dilutions of the mAb followed by isotype-specific anti-mouse Ig conjugated to FITC. The

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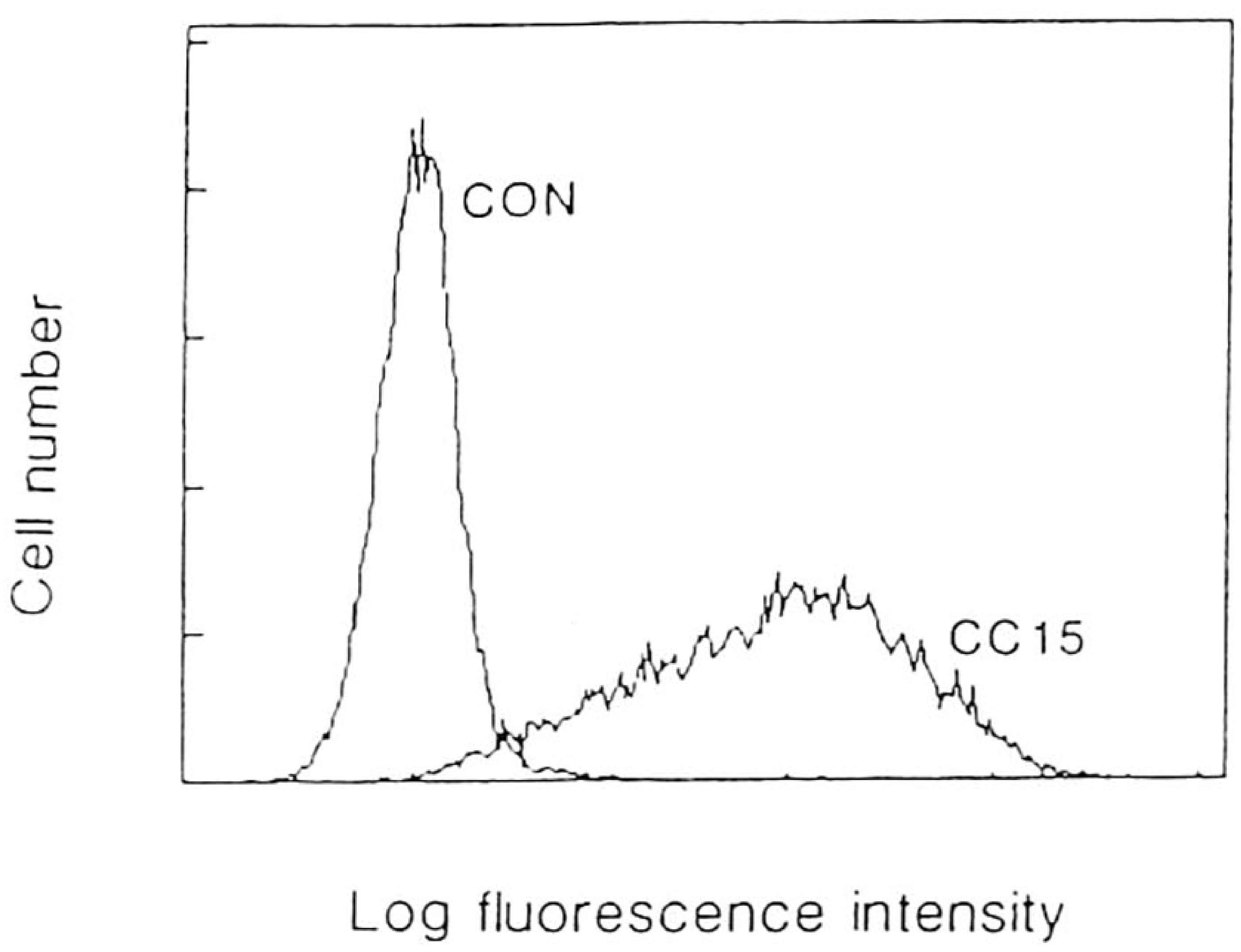

Figure 1. Surface expression of the WC1 Ag on transfected mouse L cells. Trypsinized cells were stained with the WC1 mAb CC15 or with an isotype-matched irrelevant control antibody.

control consisted of cells incubated with isotype-matched irrelevant mAb.

DNA sequencing. Restriction enzyme fragments of the pWC1 cDNA clone were purified and subcloned in the pBlueScript vector. dsDNA sequencing of the full length insert and subcloned fragments were performed according to the dideoxynucleotide method according to Sanger et al. (14), using T7 polymerase (Pharmacia, Piscataway, NJ). 16-Mer universal M13, reverse M13, and internal oligonucleotide primers were synthesized on a 381A oligosynthesizer (Applied Biosystems, Foster City, CA). The sequence of the pWC1 cDNA clone is available at the EMBL database under accession number X63723.

Southern and Northern blot analysis. For Southern blots, genomic DNA was isolated from bovine PBMC and digested with restriction enzymes, for 18 h at 37°C. Ten micrograms of digested DNA was fractionated on a 1% agarose gel. Subsequently, the gel was exposed to UV light for 10 min to nick the DNA, and denatured in 0.5 M NaOH, 0.6 M NaCl for 1 h, neutralized in 1 M Tris-HCl, 1.5 M NaCl, pH 7.4, for 1 h and transferred to nitrocellulose filters. The transferred DNA was baked for 2 h at 80°C, prehybridized in 50% formamide, 5× Denhardt's, 0.5 M NaCl, 0.05 M NaH_2PO_4, 5 mM EDTA, 0.1% SDS containing 150 μg salmon sperm DNA/ml, for 2 h at 42°C, and subsequently hybridized with a full length or transmembrane region pWC1 probe ^{32}P-labeled by random oligo priming. After washing with 0.2× SSC, 0.1% SDS at 65°C for bovine genomic DNA and 2× SSC, 0.1% SDS at 55°C for mouse and human genomic DNA autoradiography was performed. Northern blotting was carried out as described with some minor modifications (15). In short, total RNA (10 μg/lane) was fractionated by electrophoresis on a 1% agarose/formaldehyde gel and transferred to nitrocellulose (Hybond-C, Amersham). Inspection of the ethidium bromide-stained gel revealed the presence of equal amounts of RNA in each sample. The transferred RNA was baked for 2 h at 80°C, prehybridized in 50% formamide, 5× Denhardt's, 0.5 M NaCl, 0.05 M NaH_2PO_4, 5 mM EDTA, 0.1% SDS containing 150 μg salmon sperm DNA/ml, for 2 h at 42°C, and hybridized with a full length pWC1 probe ^{32}P-labeled by random oligo priming. After washing with 0.2× SSC, 0.1% SDS at 65°C autoradiography was performed. The Northern blotting was performed on four *Theileria parva*-infected bovine T lymphocytic cell lines expressing the WC1 Ag, D409/N2, D409/T1, G177/3 and G177/4, and on bovine endothelial cells isolated from aorta and the bovine fibroblasts cell line DCRIVM.

RESULTS

To learn more about the possible function of the WC1 Ag and its restricted expression on $\gamma\delta$ T cells, we applied an adaptation of the eukaryotic expression cloning technique of Aruffo and Seed (9) for the isolation of a cDNA clone encoding the bovine WC1 Ag. The bovine CD4$^-$CD8$^-$ $\gamma\delta$ T cell line D409/N2 was selected as a source of mRNA based on its reactivity with the WC1 mAb CC15, IL.A29

```
-23                                           +1      I
M A L G R H L S L R G L C V L L L G T M V G G Q A L E L R L K D G V H R C E G R V E V K H Q G S W G  27
T V D G Y R W T L K D A S V V C R Q L G C G A A I G F P G G A Y F G P G L G P I W L L Y T S C E G T  77
                                                                  II
E S T V S D C E H S N I K D Y R N D G Y N H G R D A G V V C S G P V R L A G G D G P C S G R V E V H  127
                      *
S G E A W I P V S D G N F T L A T A Q I I C A E L G C G K A V S V L G H E L F R E S S A Q V W A E E  177
                                                                           III        *
F R C E G E E P E L W V C P R V P C P G G T C H H S G S A Q V V C S A Y S E V R L M T N G S S Q C E  227
          *
G Q V E M N I S G Q W R A L C A S H W S L A N A N V I C R Q L G C G V A I S T P G G P H L V E S G D  277
Q I L T A R F H C S G A E S P L W S C P V T A L G G P D C S H G N T A S V I C S G N Q I Q V L P Q C  327
*                                                 IV
N D S V S Q P T G S A A S E D S A P Y C S D S R Q L R L V D G G G P C A G R V E I L D Q G S W G T I  377
                                              *                                       *
C D D G W D L D D A R V V C R Q L G C G E A L N A T G S A H F G A G S G P I W L D N L N C T G K E S  427
                                                            V                                   *
H V W R C P S R G W G Q H N C R H K Q D A G V I C S E F L A L R M V S E D Q Q C A G W L E V P Y N G  477
                                                            *
T W G S V C R N P M E D I T V S T I C R Q L G C G D S G T L N S S V A L R E G F R P Q W V D R I Q C  527
                                                                      VI
R K T D T S L W Q C P S D P W N Y N S C S P K E E A Y I W C A D S R Q I R L V D G G G R C S G R V E  577
I L D Q G S W G T I C D D R W D L D D A R V V C K Q L G C G E A L D A T V S S F F G T G S G P I W L  627
                                                                            VII
D E V N C R G E E S Q V W R C P S W G W R Q H N C N H Q E D A G V I C S G F V R L A G G D G P C S G  677
                                *
R V E V H S G E A W T P V S D G N F T L P T A Q V I C A E L G C G K A V S V L G H M P F R E S D G Q  727
                                                                                    VIII        *
V W A E E F R C D G G E P E L W S C P R V P C P G G T C L H S G A A Q V V C S V Y T E V Q L M K N G  777
T S Q C E G Q V E M K I S G R W R A L C A S H W S L A N A N V V C R Q L G C G V A I S T P R G P H L  827
                                                                                            *
V E G G D Q I S T A Q P H C S G A E S P L W S C P V T A L G G P D C S H G N T A S V I C S G N H T Q  877
                                                            IX
V L P Q C N D F L S Q P A G S A A S E E S S P Y C S D S R Q L R L V D G G G P C G G R V E I L D Q G  927
                                                        *                                       *
S W G T I C D D D W D L D D A R V V C R Q L G C G E A L N A T G S A H F G A G S G P I W L D D L N C  977
                                                                                X
T G K E S H V W R C P S R G W G R H D C R H K E D A G V I C S E F L A L R M V S E D Q Q C A G W L E 1027
                                                                                          *
V P Y N G T W G S V C R S P M E D I T V S V I C R Q L G C G D S G S L N T S V G L R E G S R P R W V 1077
D L I Q C R K M D T S L W Q C P S G P W K Y S S C S P K E E A Y I S C E G R R P K S C P T A A A C T 1127
        XI                                  *
D R E K L R L R G G D S E C S G R V E V W H N G S W G T V C D D S W S L A E A E V V C Q Q L G C G Q 1177
                          *
A L E A V R S A A F G P G N G S I W L D E V Q C G G R E S S L W D C V A E P W G Q S D C K H E E D A 1227
G V R C S G V R T T L P T T T A G T R T T S N S L P G I F S L P G V L C L I L G S L L F L V L V I L 1277
V T Q L L R W R A E R R A L S S Y E D A L A E A V Y E E L D Y L L T Q K E G L G S P D Q M T D V P D 1327
E N Y D D A E E V P V P G T P S P S Q G N E E E V P P E K E D G V R S S Q T G S F L N P S R E A A N 1377
                                                                    1413
P G E G E E S F W L L Q G K K G D A G Y D D V E L S A L G T S P V T F S End
```

Figure 2. Complete deduced amino acid sequence of the pWC1 cDNA clone. Amino acids are numbered on the *right*. The signal sequence and the putative transmembrane region are *underlined*. The putative glycosylation sites are indicated by *asterisks*. The first amino acid of each of the 11 SRCR domains is indicated by *Roman numerals above the sequence*. The nucleotide sequence of the pWC1 cDNA clone is available at the EMBL database under accession number X63723.

```
SRCR consensus   -RLV---G---CEG-VE------WGTV--CD--W------
WC1 consensus    LRL--- G---C-G-VE----G-WG-V  CD--W-L--A-

domain  1:       LRL--- G---C-G-VE----G-WG-V  ----W-L--A-
domain  2:       -RL--- G---C-G-VE------W--V  -D----L--A-
domain  3:       -RL--- G---C-G-VE----G-W---  C---W-L--A-
domain  4:       LRL--- G---C-G-VE----G-WG--  CD--W-L--A-
domain  5:       LR---- ----C-G--E----G-WG-V  C----------
domain  6:       -RL--- G---C-G-VE----G-WG--  CD--W-L--A-
domain  7:       -RL--- G---C-G-VE------W--V  -D----L--A-
domain  8:       --L--- G---C-G-VE----G-W---  C---W-L--A-
domain  9:       LRL--- G---C-G-VE----G-WG--  CD--W-L--A-
domain 10:       LR---- ----C-G--E----G-WG-V  C----------
domain 11:       LRL--- G---C-G-VE----G-WG-V  CD--W-L--A-

SRCR consensus   VVCR-LGCG-G--------F----------V-C-G-E-S
WC1 consensus    VVCRQLGCG-A----GS--F-EG-GPIW-D---C-G-ES-

domain  1:       VVCRQLGCG-A----G---F--G-GPIW-----C-G-ES-
domain  2:       --C--LGCG-A----G---F-------W-----C-G-E--
domain  3:       V-CRQLGCG-A----G-----E----I------C-G-ES-
domain  4:       VVCRQLGCG-A----GS--F--G-GPIW-D---C-G-ES-
domain  5:       --CRQLGCG-------S----EG--P-W-D---C------
domain  6:       VVC-QLGCG-A-----S--F--G-GPIW-D---C-G-ES-
domain  7:       V-C--LGCG-A----G---F-E--G--W-----C-G-E--
domain  8:       VVCRQLGCG-A----------EG---I------C-G-ES-
domain  9:       VVCRQLGCG-A----GS--F--G-GPIW-D---C-G-ES-
domain 10:       V-CRQLGCG-------S----EG--P-W-D---C------
domain 11:       VVC-QLGCG-A-----S--F--G-G-IW-D---C-G-ES-

SRCR consensus   L--C---------C---------GV-C---
WC1 consensus    LW-CP---W----C----H---A-V-CSG-

domain  1:       ---C--------------H---A-V-CSG-
domain  2:       LW-CP--------C----H---A-V-CS--
domain  3:       LW-CP--------C----H---A-V-CSG-
domain  4:       -W-CP---W----C----H---A-V-CS--
domain  5:       LW-CP---W----C--------A---C---
domain  6:       -W-CP---W----C----H---A-V-CSG-
domain  7:       LW-CP--------C----H---A-V-CS--
domain  8:       LW-CP--------C----H---A-V-CSG-
domain  9:       -W-CP---W----C----H---A-V-CS--
domain 10:       LW-CP---W----C--------A---C-G-
domain 11:       LW-CV---W----C----H---A-V-CSG-
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Figure 3. Alignment of the 11 SCRC protein domains in the predicted protein encoded by the pWC1 cDNA. The *top two rows* show the consensus sequence of the 11 WC1 domains (indicated residues occur at least six times), aligned with the consensus sequence of the SRCR domain family (18).

and T19. Size-fractionated (>2.0 kb) cDNA was inserted in the pCDM8 vector (11) and after transformation in *E. coli* mc1061/p3 five sublibraries of 10,000 primary colonies each were obtained. Plasmid DNA was isolated from individual sublibraries and transiently transfected into COS cells using DEAE-dextran. WC1 expression was detected in one of the sublibraries with the use of an immunoperoxidase technique (12) after staining with a mixture of the mAb CC15 and IL.A29. A single full length cDNA clone, termed pWC1, was subsequently isolated by screening progressively smaller pools of bacterial colonies from this sublibrary.

To definitively establish the identity of the isolated cDNA clone, we stably transfected mouse Ltk$^-$-cells with pWC1. FACS analysis of the transfectants revealed that the encoded Ag was expressed on the membrane of transfected cells and was recognized by the bovine WC1 mAb CC15 (Fig. 1) and IL.A29, and the sheep WC1 mAb T19 (not shown).

Sequence analysis (Fig. 2) revealed that the 4475 bp insert contained a single open reading frame of 1436 amino acids with a consensus translation start site at bp 22 (16) with an in-frame translation stop codon at bp 4. The predicted N terminus displayed the structural features of a leader peptide (17). A putative hydrophobic transmembrane region was present near the C terminus of the protein. The extracellular region contained 17 consensus *N*-linked glycosylation sites. The predicted molecular mass of the mature protein backbone was 152 kDa.

The extracellular region consisted of 11 homologous domains with an approximate length of 110 amino acids each (Fig. 3). Database searches of the NBRF and Swiss protein sequence data bases revealed that these domains belonged to the SRCR[2] domain family (18), which includes among others CD5 (19, 20) and CD6 (21). Figure 3 compares the 11 WC1 domains with the reported consensus sequence of the SRCR domain family (18). Inspection of the sequence encoding the extracellular portion of the protein indicated the presence of an internal repeat of approximately 1400 bp (bp 394 to 1776 and bp 2059 to 3441). The homology between these two stretches of DNA

[2] Abbreviations used in this paper: SRCR, scavenger receptor cysteine rich.

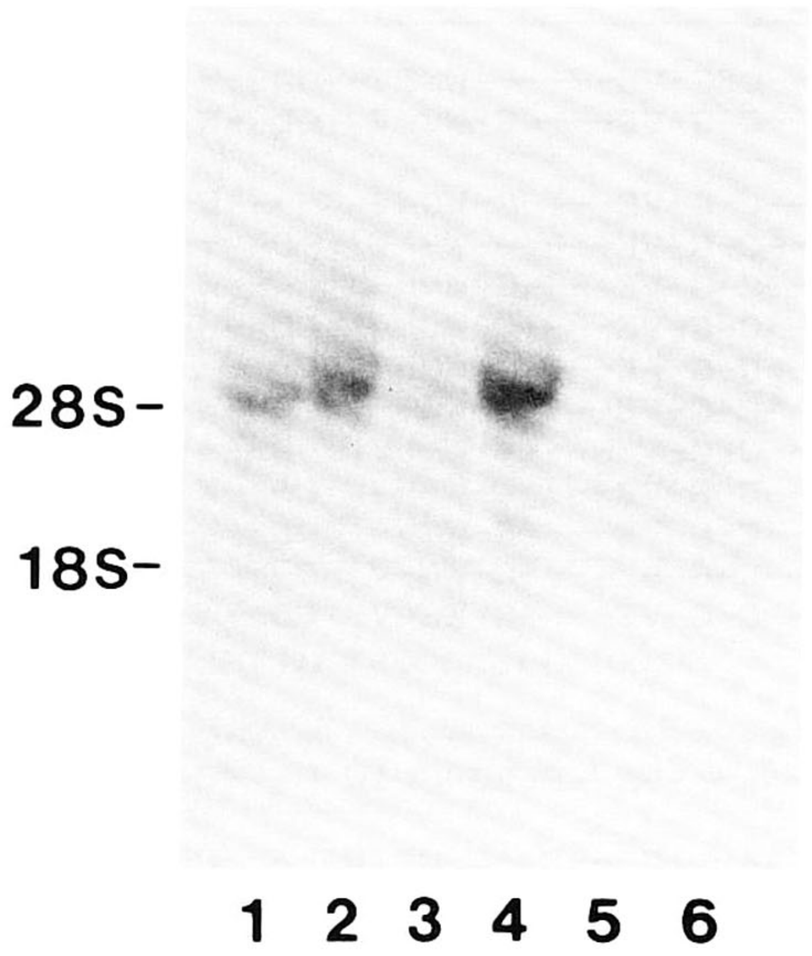

Figure 4. Detection of WC1 mRNA is restricted to T lymphocytic cell lines. Poly(A)$^+$ RNA was purified and hybridized with a full length pWC1 probe. *Lane 1*, bovine γδ T cell line D409/N2; *lane 2*, bovine αβ T cell line; *lane 3*, bovine γδ T cell line G177/3; *lane 4*, bovine γδ T cell line G177/4; *lane 5*, bovine endothelial cells isolated from umbilical vein; *lane 6*, bovine fibroblastic cell line DCRIVM.

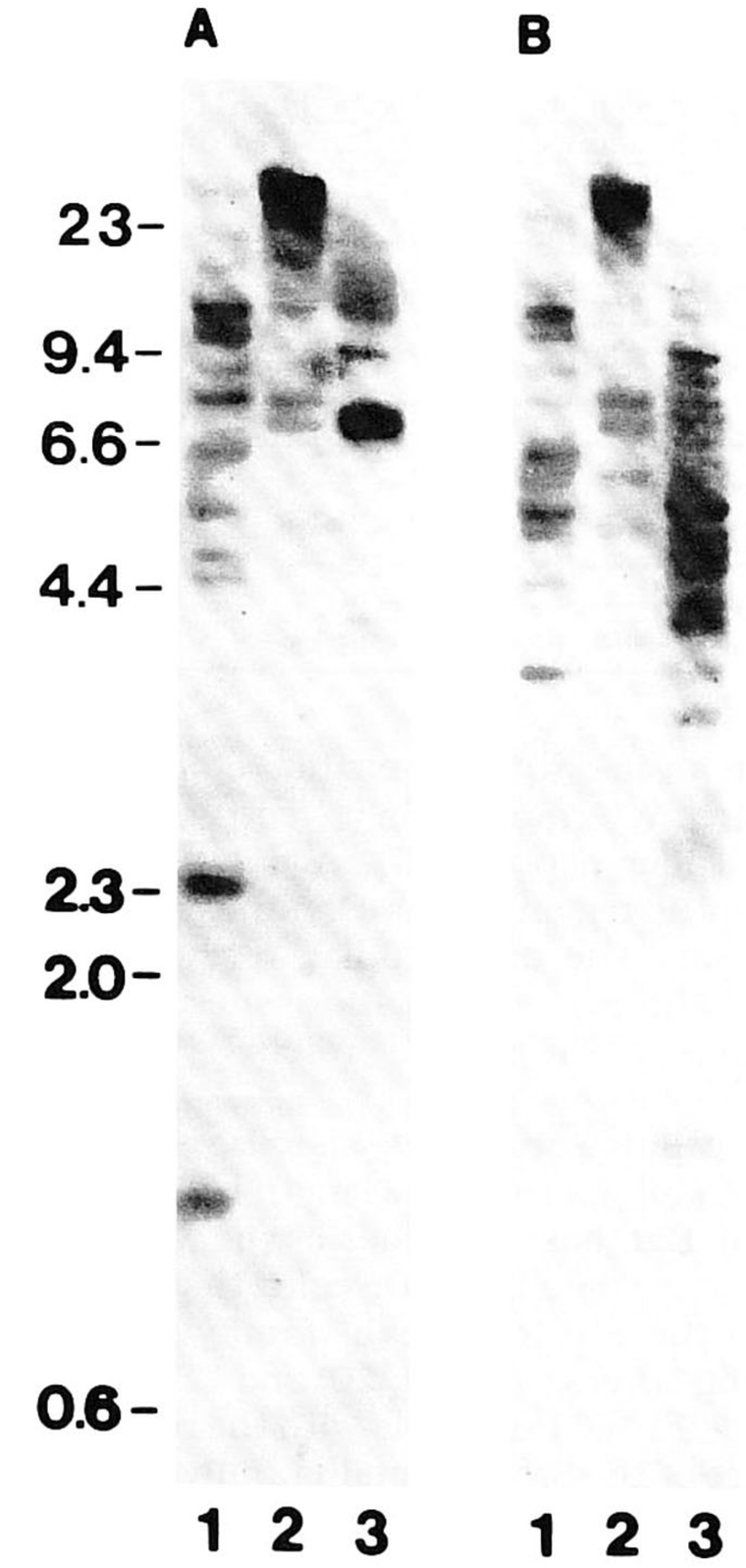

Figure 5. High-stringency probing of bovine genomic DNA digested with restriction enzymes *Bam*HI (*lane 1*), *Kpn*I (*lane 2*), or *Hin*dIII (*lane 3*) using a pWC1 full length probe (*A*) or a pWC1 probe spanning the transmembrane region (*B*). The sizes of λ *Hin*dIII markers are indicated on the *left*.

was 85%. The first version of the repeat started halfway domain 1 and extended to domain 5; the repeated stretch started halfway domain 6 extending to domain 10. Homologies between DNA sequences encoding individual domains were in the order of 60%.

Northern blot analysis resulted in a single strong band of approximately 4.5 kb, which was detected only in the bovine CD4$^-$CD8$^-$ WC1 expressing T cell lines D409/N2, D409/T1, G177/3, and G177/4 (Fig. 4). No signal was detected in bovine endothelial cells and fibroblasts. High stringency Southern blot analysis of *Hin*dIII-digested DNA suggested the existence of several related sequences in the bovine genome. Probing with the full length pWC1 insert revealed >20 bands (Fig. 5*A*), whereas 8 to 10 bands were detected by a probe spanning the transmembrane region (Fig. 5*B*). This suggested the existence of a large family of WC1-like genes. Similarly complex WC1 gene families appear to exist in sheep, goats, pigs, and horses (A. Bensaid, personal communication). In rodents and man, we have observed 2 to 5 hybridizing bands upon low stringency probing with the full length pWC1 insert. Preliminary cloning data indicate that these hybridizing human genomic DNA fragments contain exons encoding SRCR-protein domains highly homologous to those of bovine WC1, and more distantly related to CD5 and CD6 (M. Metzelaar, P. L. J. Wyngaard, and J. C. Clevers, unpublished observations).

DISCUSSION

Bovine and sheep CD4$^-$CD8$^-$ T lymphocytes are characterized by the presence of a 220-kDa glycoprotein on their surface. The WC1 Ag is presently the only known marker specifically expressed by CD4$^-$CD8$^-$ γδ T lymphocytes other than the TCR proper. To learn more about the possible function of the WC1 Ag and the molecular basis of its restricted expression, we applied an adapted form of the eukaryotic expression cloning technique of Aruffo and Seed (9) for the isolation of a full length cDNA clone encoding this Ag. The adaptation of this cloning technique was based on the application of a highly sensitive immunoperoxidase staining procedure. This method allows the unambiguous detection of 1 in 10^6 COS cells (12). We had previously been unsuccessful in cloning WC1 using the "classical" panning technique of Aruffo and Seed (9). In the experience of us and others (22), replicating plasmids undergo rapid internal deletions in COS cells; large plasmids appear particularly prone to this process. As we anticipated that the size of WC1 mRNA would exceed 4 kb, we decided to avoid shuttling through COS cells, and only apply COS cell expression as a read-out system.

We thus isolated the pWC1 cDNA clone, which contained an insert of 4475 bp, which to our knowledge is the largest cDNA clone isolated by expression in COS cells. The cDNA clone closely corresponds to the complete mRNA molecule, because Northern blot hybridization identified an approximately 4.5-kb band. The pWC1 sequence predicts a classical type I membrane protein with an N-terminal signal peptide and a cytoplasmic tail of 131 amino acids; the putative WC1 protein contains 17 consensus *N*-linked glycosylation sites. In the extracellular part of the deduced protein we identified 11 domains which show remarkable homology to each other: 53 amino acid positions are identical in at least 6 out of the

11 domains and 13 of these positions are conserved between all 11 domains. Data base searches revealed that the domains belong to the recently identified SRCR domain family. This family includes the prototypic human, mouse, and bovine macrophage scavenger type I receptors (18), human and mouse CD5 (19, 20), human CD6 (21), human C factor I and the sea urchin speract receptor (23). The consensus SRCR-domain sequence consists of approximately 110 amino acids, of which high sequence conservation occurs at 37 positions (18). The eight conserved cysteine residues are most likely involved in intra-domain disulfide bridging. The level of homology within the SRCR-domain family is particularly striking given the evolutionary distance between sea urchins and mammals. The consensus sequence derived from the domains in pWC1 (Fig. 3), coincided at 30 positions with that defined by Krieger and colleagues (see Reference 18). Strikingly, the member of the SRCR-domain family most closely related to WC1 was the sea urchin speract receptor. A total of 5 of the 11 SRCR domains of the WC1 protein result from an apparent internal duplication event.

Southern blot hybridization of genomic DNA of cattle and other species indicated the presence of a number of related sequences, suggesting the existence of a WC1 gene family. Continued cloning of cDNA and genomic sequences from cattle and other species should disclose the complexity of this putative WC1 gene family.

The WC1 gene appears to be unique in its $CD4^-CD8^-$ γδ T cell-restricted expression pattern. However, no function has been assigned to WC1 yet (5). Recently, it was demonstrated that CD72 is the natural ligand for the SRCR family member CD5 (24). For both CD5 and WC1, the extracellular portion of the protein is almost entirely composed of SRCR domains. By analogy with CD5, it is tempting to speculate that WC1 interacts with a membrane-bound ligand through one or more of its SRCR domains. Given the exquisite expression pattern, the WC1 protein might represent a functional homologue of the CD4/CD8 accessory molecules, interacting with an as yet undefined Ag-presenting restriction element (1). Alternatively, the WC1 protein might control the homing behavior of γδ T cells (25). Ovine γδ T cells are differentially distributed within the gut epithelium. In the lamina propria most of the γδ T cells express the T19 epitope whereas within the epithelium only a few γδ T cells express T19 (7). Identification of the ligands for WC1 might disclose the function of this Ag in the immunobiology of γδ T cells.

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