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A novel multi-gene family of sheep γδ T cells

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SUMMARY

The WC1 protein is a cell surface constituent of bovine γδ T cells and is absent from most or all $CD4^+$, $CD8^+$ T cells and from B cells. It is a single polypeptide chain of 1413 amino acids consisting of 11 non-identical repeats of a 110 amino acid consensus sequence, homologous to the macrophage scavenger receptor cysteine rich (SRCR) domain. A 1059 nucleotide segment of the bovine WC1 cDNA sequence was used as a probe to molecularly clone homologous DNA segments from a sheep genomic library in which the presence of numerous positive plaques was documented. The high representation of such recombinants (1–2/1000 clones) within the library suggested the existence of multiple genes for WC1 (called T19 in sheep) and supported Southern blotting data which revealed an unexpectedly high number of WC1/T19 restriction fragments in sheep genomic DNA. Restriction digests of 27 samples of T19 genomic recombinants were examined by electrophoresis and Southern blotting. All but two pairs of recombinants exhibited non-overlapping restriction digest patterns. Four recombinant DNA samples were partially sequenced and in all cases putative exons were identified and exhibited high homology to appropriate segments of the WC1 cDNA at the levels of both nucleotide and amino acid sequence. Furthermore, multiple nucleotide and amino acid differences occurred between all sequences compared, establishing the existence of a repertoire of non-identical T19 genes, each with the potential to encode a different protein.

INTRODUCTION

T cells expressing γδ T-cell receptors generally express neither CD4 nor CD8 coreceptors and arise earlier in ontogeny than αβ T cells. In the mouse at least, they constitute a relatively minor population of adult T cells overall. The highly selective localization of certain γδ T cells to epithelial tissues in the mouse has been documented as has the limited diversity of the V, D and J regions of both γ and δ chains, in sites such as skin, uterus and other tissues (refs 1–5; for reviews, see refs 6 and 7). Notably, the unusual homing properties of skin-specific or uterine-specific γδ subsets cannot be accounted for by the specificities of their respective T-cell receptors as experiments with mice transgenic for functional, rearranged, 'tissue-specific' γ and δ genes amply demonstrate.[8] Homing potential is a property both of the thymus in which T-cell precursors differentiate and of the stem cells which seed the thymus[6,7] although the identities of neither the putative ligands of 'tissue-specific' γδ T cells nor of their tissue receptors have yet been elucidated.

The γδ T cells of artiodactyls (cows and sheep) are more abundant relative to their αβ counterparts than is true for mice and humans, and there are other unique features of the ruminant immune system which allow its molecular and cellular dissection in ways not possible in rodents. For example, Mackay *et al.*[9] described the antigen (SBU T19) on a population of sheep $CD4^-$ $CD8^-$ $immunoglobulin^-$ lymphocytes. These were later shown to be T lymphocytes expressing γδ T-cell receptors.[10] Parallel findings were later made in the cow.[11] In both sheep and cows these molecules were high molecular weight (> 200 000) proteins.[9,11,12] A number of antibodies is now available to the proteins of this family which is called T19 in sheep and WC1 in cow. To date, no comparable proteins are known in man or mouse.

Wijngaard *et al.*[13] molecularly cloned bovine WC1 from a cDNA library and showed that it consisted of a single polypeptide chain of 1413 amino acids appended by a 27 amino acid signal sequence. The extracellular domain of WC1 consists of 11 essentially contiguous, mutually homologous units, each of about 110 amino acids, all of which exhibit substantial homology to the macrophage scavenger receptor cysteine rich (SRCR) domain.[13,14] The repeats are followed by a short, non-repetitious (13 residue) extracellular segment, a 36

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Abbreviations: BSA, bovine serum albumin; kbp, kilobase pairs; SDS, sodium dodecyl sulphate; SRCR, scavenger receptor, cysteine rich (domain).

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amino acid transmembrane segment and a 131 amino acid cytoplasmic tail. Notably Wijngaard *et al.*[13] accumulated convincing evidence within a single bovine T-cell line that multiple, related WC1 mRNA existed and this observation supported earlier serological studies which implied the existence of different WC1 molecules upon $\gamma\delta$ T cells. These latter studies did not specify how WC1 genes differed from one another and the genes were not accurately enumerated in the previous studies. It was of specific interest to know if a T19 gene repertoire existed in sheep and if it did, to estimate its size. In this work we present evidence for at least 50 and possibly more T19 genes within the genome of sheep, the species in which the molecule was originally described, and show that four T19 recombinant clones arbitrarily selected from 27 cloned isolates derive from potentially functional genes encoding similar but non-identical amino acid sequences.

MATERIALS AND METHODS

Molecular probes

To facilitate the conduct of polymerase chain reaction (PCR) for the generation of T19-specific molecular probes, several oligonucleotide primers were assembled, either identical to or complementary to segments of the published WC1 sequence.[13] Primer C12 corresponded to WC1 nucleotides (nt) 22–45, primer 5161 to nt 2719–2742, primer 5046 to nt 3277–3299 and primer T15 to nt 3829–3849. Three additional primers were the complements of WC1 nucleotides 1041–1065 (primer T11), nt 3453–3476 (primer 5162) and nt 4311–4332 (primer 5047). The nucleotide numbering system is from Wijngaard *et al.*[13] A 1056 base pair (bp) fragment was amplified by PCR from bovine WC1.1 plasmid DNA using primers 5046 and 5047. The amplified fragment included the coding regions of the two membrane proximal SRCR domains and the transmembrane and cytoplasmic domains. PCR mixtures were set up using commercially available kit reagents (Perkin-Elmer Cetus, Norwalk, CT) according to the manufacturer's recommendations; the DNA polymerase used was from *Thermus aquaticus*. PCR mixtures were denatured by heating to 94° for 5 min followed by 30 rounds of amplication using the following conditions: 94° for 30 seconds, 56° for 20 seconds and 72° for 1 min; a Hybaid Omni-gene thermal cycler was used for this purpose. A final extension was carried out at 72° for 10 min. The amplified DNA was gel purified and contaminants removed using GeneClean (BIO 101, La Jolla, CA).

To prepare a homologous sheep T19 probe, single-stranded cDNA was first synthesized from total RNA isolated from sheep lymphocytes, enriched for $\gamma\delta$ T cells using immunospecific panning procedures described elsewhere.[15] This was used as a template for PCR amplification using the same combination of primers and reaction conditions described above and yielded DNA fragments of ~1 kb, as expected. Amplified sheep DNA was cloned into Bluescript plasmid (Stratagene, La Jolla, CA) and sequenced along 2–300 bases from each terminus. Over this region, the sheep clone had around 94% DNA identity to the bovine WC1.1 sequence (data not shown). A further bovine probe (503 bp) was assembled as described above, but using oligonucleotides T15 and 5047 as convergent primers and a sample of WC1.1 DNA as template. This probe was a shortened variant of the 1·06 kilobase pair (kbp) probe above and omitted the DNA sequences corresponding to the SRCR domains.

Sheep genomic library construction

Buffy coat cells from a blood sample, taken from a single ram, were used as a source of genomic DNA which was partially digested with *Mbo*I and then size fractionated by ultracentrifugation on a 10–40% glycerol gradient. The fractions containing fragments of 15–20 kb in length were ligated into the *Bam*H1 site of digested λ EMBL 3 DNA (Promega Corporation, Rozelle, New South Wales, Australia) and packaged into bacteriophage coats using a packaging kit obtained from Amersham International (Amersham, UK). Viable recombinants were plated using the *mcr* A^-/B^- *Escherichia coli* host strain NW2.[16] The resultant library, consisting of $1{\cdot}2 \times 10^6$ independent clonal inserts, was amplified once.

Southern transfer of sheep genomic DNA

Samples of sheep genomic DNA were digested overnight at 37°. The restriction enzymes used were *Eco*R1 (10 U/μl; Boehringer Mannheim, Mannheim, Germany), *Hin*dIII (15 U/μl; Pharmacia, Uppsala, Sweden), *Pst*1 (11 U/μl; Boehringer Mannheim), *Sal*1 (10 U/μl; Boehringer Mannheim) and *Bam*H1 (11 U/μl; Boehringer Mannheim). In all cases, the digestion buffer used was that supplied by the manufacturer. The restriction digests were then electrophoresed overnight on 0·7% agarose gels followed by transfer onto Hybond-N membrane (Amersham, Melbourne, Australia) using a Blot Transfer apparatus (Bethesda Research Laboratories, Bethesda, MD) following the manufacturer's recommendations. Both the sheep and bovine WC1 probes were labelled by random priming using a Boehringer Mannheim DNA labelling kit and α-labelled ^{32}P-dATP (Amersham, UK) according to the manufacturer's instructions. Filter hybridization was carried out at 65° overnight in Church buffer [7% sodium dodecyl sulphate (SDS), 1% bovine serum albumin (BSA), 0·5 M Na_2HPO_4, 1 mM EDTA, pH 7·2) supplemented with 100 μg/ml salmon sperm DNA. The washed membranes were autoradiographed using preflashed XAR film (Kodak, Rochester, NY) and developed after 1 week (bovine probe) or 5 hr (sheep probe).

Screening of the sheep genomic library

Approximately $3–5 \times 10^5$ recombinant λ plaques were lifted onto nylon filters (Amersham, UK), which were then prehybridized in Church buffer at 65° for 2 hr, and hybridized with the ^{32}P-labelled bovine PCR fragment in the same buffer at 65° (16 hr). The filters were washed in 0·1 × SSC, 0·1% SDS at room temperature for 30 min, then once at 65° for 1 hr. Phage DNA was purified using selective precipitation by cetyl trimethylammonium bromide (CTAB) as described previously.[17]

DNA sequencing

DNA from selected λ clones were sequenced directly by the dideoxynucleotide chain termination method using Multi-Pol DNA sequencing system (Clontech, Palo Alto, CA). Template DNA (5 μg) was prepared for sequencing by adding 0·1 μg of the appropriate bovine sequence-specific primer (either 5046 or 5047), boiling for 4 min, snap freezing in an ethanol/dry ice bath then thawing on ice.[17]

RESULTS

Southern transfer of sheep genomic DNA

Samples of sheep DNA were digested with restriction enzymes as indicated in Fig. 1. *Eco*R1 digests of DNA samples from three unrelated animals were examined and one of the DNA samples was also digested with four additional restriction enzymes. T19-related restriction fragments were detected with a ^{32}P-labelled, 1059 bp probe corresponding to nucleotides 3273–4332 of bovine WC1 or its sheep equivalent. This probe encompasses the regions corresponding to the final (carboxyl-terminal) two SRCR repeats, the transmembrane region, the cytoplasmic tail and the stop codon.[13] The data in Fig. 1 clearly establish that multiple genomic fragments corresponding to this region occur in all restriction digests. Eight or more *Eco*RI fragments were detectable with the sheep PCR probe and similar numbers using the restriction enzymes *Hin*dIII, *Bam*HI, and *Pst*I. The *Sal*I digest produced no low molecular weight fragments. In all successful digests, considerable variation in the intensities of individual bands was apparent and several bands seemed broad and heterogeneous.

The channels corresponding to *Eco*RI digested DNA from three unrelated, outbred sheep reveal that most or all of the T19-like restriction fragments are the same, suggesting that allelic polymorphism does not contribute extensively to the complexity of the restriction patterns. Collectively, the data in Fig. 1 were interpreted to suggest that multiple T19-like genes may be present in the sheep genome but owing to a lack of knowledge of the exon–intron structure of the T19-like gene(s) no more precise interpretation of the restriction patterns of Fig. 1 was possible. The great similarity between the species detected by the ovine and bovine probes established that the sequences to which they hybridized within the sheep genome were overlapping or identical.

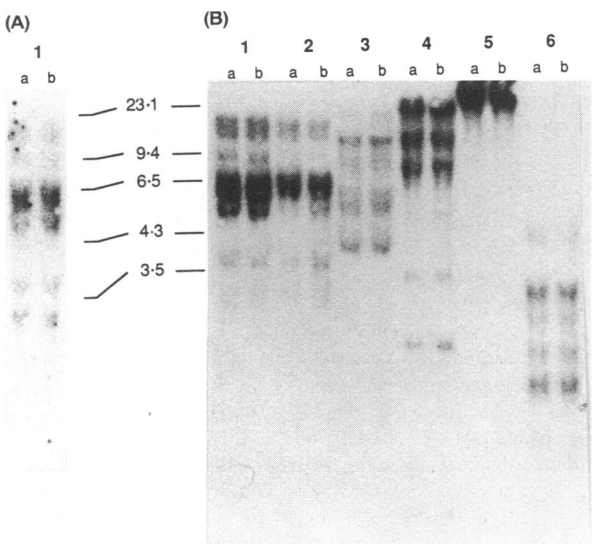

Figure 1. Southern transfer analysis of sheep T19-related restriction fragments. (A) *Eco*RI digests of DNA samples (5 μg) from one sheep were subjected to electrophoresis and blotting as described in the Materials and Methods after digestion with 1 μg/ml of restriction enzyme (a) and 2 μg/ml of enzyme (b). Detection was conducted using the labelled 1·06 kbp bovine WC1 probe followed by autoradiography (1 week). (B) Restriction enzymes were used at two concentrations, 1 μg/ml (tracks a) or 2 μg/ml (tracks b). *Eco*RI was used for tracks 1 and 2, *Hin*dIII in tracks 3, *Bam*H1 for tracks 4, *Sal*I in tracks 5 and *Pst*I in tracks 6. Each track contained 5 μg of digested DNA and the labelled probe was a sheep equivalent to that used in (A). DNA samples used in this experiment were from three unrelated outbred sheep; (A) DNA from one sheep were used; (B) tracks 1a and b were from a second sheep and the remainder of tracks were from a third animal.

Molecular cloning of sheep T19 genes

A sheep genomic library was constructed by inserting partially *Mbo*1 cut sheep DNA into *Bam*H1 digested EMBL3 cloning vector. The library was then amplified and filter replicas of plates containing $3–5 \times 10^4$ plaques were obtained and hybridized to the bovine PCR probe followed by washing under conditions of high stringency. Multiple intense plaques hybridized to the probe used: conservatively, 1–2/1000 plaques exhibited probe affinity. This frequency is 100–1000 times higher than anticipated for a single copy gene. Twenty-seven recombinants were obtained as clonally pure stocks and DNA samples from all recombinants were subjected to hybridization analysis using a range of oligonucleotide probes constructed

Table 1. WC1-oligonucleotide hybridization patterns of T19 genomic clones*

Clone no.	C12	T11	5161	5046	5162	5047
1	–	+	++	–	+/–	–
2	–	–	++	–	–	–
3	–	–	+	–	–	–
4	–	–	+	–	+/–	–
5	–	–	++	–	+/–	–
6	–	–	+	+/–	+/–	–
7	–	–	+++	+++	+/–	+/–
8	+/–	–	+++	++	+/–	–
9	+/–	–	++	++	+/–	–
10	–	–	++	++	+/–	–
11	–	–	++	–	–	–
12	+/–	+/–	+	++	–	–
13	–	–	+	++	+/–	–
14	–	–	+/–	++	+/–	–
15	ND	ND	ND	+++	ND	–
16	ND	ND	ND	+++	ND	–
17	–	–	–	+	–	–
18	ND	ND	ND	+++	ND	+++
19	+/–	–	–	–	+/–	+++
20	+/–	–	–	–	+/–	+++
21	ND	ND	ND	–	ND	+++
22	+/–	–	–	–	–	–
23	–	–	+/–	–	–	–
24	–	–	–	–	–	–
25	–	–	–	–	–	–
26	–	–	–	–	–	–
27	–	–	–	–	–	–

*Oligonucleotide sequences taken from bovine cDNA clone, WC1(13), encompassing the following nucleotides: C12 (22–45), T11 (1041–1065), 5161 (2719–2742), 5046 (3277–3299), 5162 (3453–3476), 5047 (4311–4332). ND, no determination.

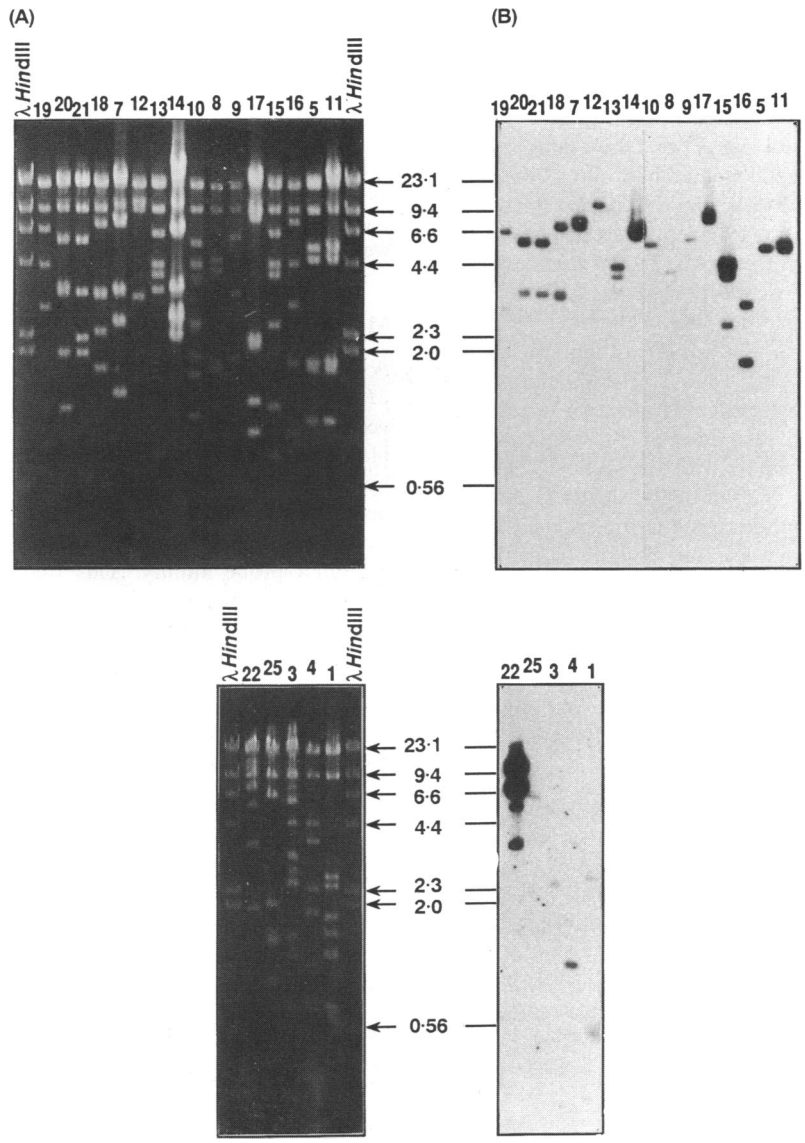

Figure 2. Restriction enzyme analysis of 21 T19-related recombinant phage clones. Small DNA samples (5 μg) of *Eco*RI–*Sal*I double digests from 21 T19-related phage recombinants were subjected to electrophoresis and bands were visualized with ethidium bromide (A, upper and lower panels). Southern blots were then prepared and probed with the sheep 1·06 kbp T19 probe (B, upper and lower panels). Samples of a commercially available *Hin*dIII digest of λ bacteriophage DNA (λ *Hin*dIII) was used to calibrate the gels (molecular weights in kb shown by arrowheads). The uppermost two ethidium bromide bands in the patterns on the left are fragments of the EMBL3 cloning vector.

from the published bovine WC1 sequence and described in the Materials and Methods. The hybridization patterns of Table 1 establish that multiple recombinants hybridize to one or more WC1 oligonucleotides as well as to the 1·06 kbp bovine PCR probe.

Restriction fragment length analysis of T19 genomic clones

Samples of 21 of the T19-like recombinants were digested with *Sal*I and *Eco*RI and the double digests were analysed by gel electrophoresis (Fig. 2). Of the 21 samples depicted in Fig. 2, only two (5 and 11) exhibited patterns which may be identical: another two patterns may derive from overlapping clones (20 and 21). Several other independent DNA samples failed to digest to completion in this experiment and were excluded from consideration. Southern blot analysis using the bovine PCR probe to detect complementary fragments confirmed that all DNA samples examined possessed one or more restriction fragments complementary to the 1 kbp probe used (Fig. 2). The size of these fragments varied between individual recombinants and there was little evidence of conservation at this level. The data in Fig. 2 establish that of the 21 recombinants for which adequate restriction digests were obtained, 19 probably correspond to unique and non-overlapping DNA segments. A PCR reaction was used to assemble a new probe (503 bp) corresponding to WCI nucleotides (3829–4332). The filters of Fig. 2 were treated to detach bound probe and then hybridized with the 503 bp probe

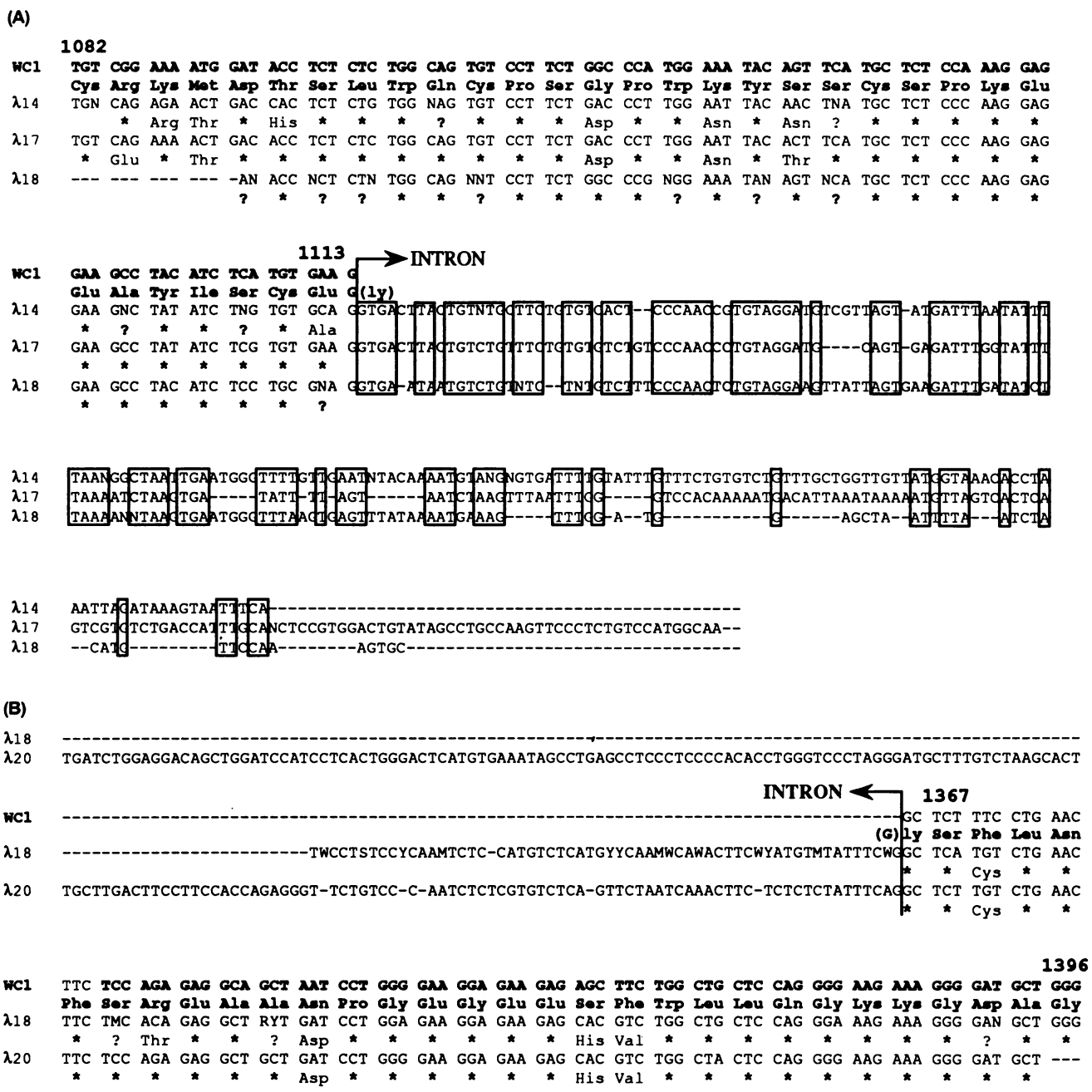

Figure 3. Partial DNA sequences of four T-19 related genomic inserts. DNA samples from clones 14, 17 and 18 were subjected to DNA sequence analysis as described in the Materials and Methods using oligonucleotide 5046 as an extension primer (A) whereas oligonucleotide 5047 was used as a primer for clones 18 and 20 (B). Nucleotide and amino acid sequences are aligned to one another and to the indicated segments of bovine WC1 cDNA (amino acids numbered as in Wijngaard *et al.*[13]). Exon–intron boundaries are indicated by arrowheads and agree with the consensus rules of Breathnach & Chambon.[18] Predicted amino acid identities between the T19 sequences and WC1 are asterisked and differences are indicated under their respective DNA sequences. Nucleotide identities between all three intron sequences in (A) are boxed to emphasize identities after computer-assisted alignment of the sequences to maximize parsimony. In the case of uncertain nucleotide assignments, the following IUB symbols have been used:[19] **W**, A or T; **S**, G or C; **R**, A or G; **Y**, C or T; **M**, A or C; **K**, G or T.

which lacked sequences corresponding to either of the SRCR domains present on the original probe. The shortened probe which corresponded to the non-repetitious, cytoplasmic segment of WCI hybridized to at least one fragment in all of the recombinant digests of Fig. 2 (data not shown).

DNA sequence analysis of T19-like genes

To confirm that the recombinant DNA samples described above indeed corresponded to T19/WC1-like genes, direct sequence analysis was undertaken on four independent clones

(Fig. 3, clones 14, 17, 18 and 20). Three clones, 14, 17 and 18, were sequenced using oligonucleotide 5046 as a primer and the two clones, 18 and 20, were sequenced using oligonucleotide 5047. The following observations are apparent from the data in Fig. 3. Firstly, clones 14, 17 and 18 have the capacity to encode proteins homologous in sequence to part of the 10th SRCR repeat of bovine WC1. The presence of an intron splitting the codon for gly 1114, the last amino acid of the 10th SRCR repeat of WC1 is apparent in all three sequences. Likewise, clones 18 and 20 contain sequences homologous to a segment of the putative cytoplasmic tail of WC1. Secondly, clones 14, 17 and 18 clearly derive from different genes. The sequence differences between them are particularly pronounced when intron sequences are compared (identities between all three sequences are boxed in Fig. 3) but differences which differentiate the amino acid sequences of the respective products are also apparent in all three cases. Likewise, clones 18 and 20, although closely related, are differentiated from one another in both exon and intron sequences.

DISCUSSION

In the present study, the existence of a family of sheep genes related in sequence to the bovine WC1 cDNA is established. The first evidence that such a gene family might exist was derived from studies in which Wijngaard *et al.*[13] demonstrated the existence of three distinct but related bovine WC1 cDNA molecules, all of which differed by restriction mapping. The evidence in the present work for multiple WC1/T19 genes in sheep derives from several observations. Southern blot analysis of restricted sheep DNA using as probes 1·06 kbp segments amplified from bovine and sheep cDNA, revealed multiple DNA species under high stringency hybridization conditions. Regardless of which restriction enzyme was used, multiple probe-reactive bands were obtained. These results establish either that the genomic region which the probe encompasses contains multiple introns and/or that multiple T19-like genes exist. To distinguish these two possibilities, genomic clones were obtained and then characterized. The frequency with which such clones were observed (1–2/1000) was highly consistent with the existence of multiple T19 genes. Two possibilities were considered to discount the significance of this observation.

(1) The probe used for detection, by chance, contained within it a sequence similar to a highly repeated satellite segment.

(2) Selective amplification of one or two T19-like segments had occurred for unknown reasons after the construction of the 'primary' genomic library.

To examine these possibilities, the presence of sequences complementary to a number of oligonucleotides based upon the bovine WC1 sequence within T19-like genomic recombinant DNA samples was evaluated. Most clonal isolates were complementary to one or more of the WC1 oligonucleotides (Table 1). This result would not have prevailed had possibility (1) been correct. In addition, this observation strengthened the belief that most or all of recombinants isolated contained sequences homologous to multiple segments of WC1 cDNA. The second possibility was discounted by the restriction digest patterns of Fig. 2. One or two selectively amplified sequences should have resulted in only one or two restriction patterns rather than the diverse patterns apparent in Fig. 2. The data from this figure in addition to a number of other experiments (not shown) have to date revealed that only two of 27 clonal isolates have identical restriction patterns (clones 5 and 11; Fig. 2). Clearly, if the high multiplicity of T19-like recombinants were owing to selective amplification of a single recombinant, then extensive clone-specific alterations would also need to be invoked in progeny recombinants to account for the data of Fig. 2. Whereas there is some precedent for events of this type in other systems, it seems most unlikely that both selective amplification and subsequent high frequency clone-specific alteration could account for the salient observations of this paper.

Partial sequence analysis of four recombinant inserts (14, 17, 18 and 20; Fig. 3) establish that the proteins which these three recombinants would encode are indeed WC1-like in amino acid sequence (Fig. 3). It is clear that the differences between the four sheep genomic isolates occur in both exons and introns and there is no doubt that all four recombinants correspond to different genes. In fact, the sequences of clones 14 and 17, but not clone 18, in Fig. 3 align better with a segment of the fifth SRCR domain repeat of WC1 than with the corresponding region of the 10th repeat depicted in Fig. 3. The exact location of the aligned residues within the corresponding T19 mRNA and protein sequences of clones 14 and 17 requires additional sequence analysis.

It should be emphasized that clones 14, 17, 18 and 20 were arbitrarily selected for DNA sequence analysis. They should be viewed as random choices from a larger population of clonal isolates. Indeed, the original choice of plaques for clonal purification involved the random sampling of individual members from a much larger population still. Assuming that most or all of the plaques reactive with the WC1 probe indeed contain unique DNA variants of the sheep T19 gene, as is highly consistent with the sequence data of Fig. 3, then a clonal frequency of, say, one in 10^3 is not unreasonable. The sheep genome (approximately 3×10^9 bp) should contain approximately 10^5 genes: and assuming $\leqslant 1$ gene per recombinant insert, the number of T19-like genes in the entire sheep genome can be estimated at $1–2 \times 10^2$. Per haploid genome this would correspond to between 50 and 100 genes.

A technical caveat to this estimate of T19-like gene number was finally considered in this work, namely that the SRCR segments contained within the 1·06 kb cow probe used to obtain recombinant clones, detected multiple, non-overlapping fragments of a smaller number of cloned T19-like genes, i.e. that the SRCR sequences within the probe cross-hybridized with several SRCR sequences within the same gene. Each T19 gene within the sheep genome could then have been represented by several recombinants. This caveat was dismissed by probing the Southern blots of Fig. 2 with a labelled probe which lacked any SRCR sequence. This probe consisted of the WC1 cDNA sequence corresponding to the -COOH terminal (cytoplasmic) segment only and specifically, lacked sequences corresponding to the 10th and 11th SRCR domains which might have facilitated cross-hybridization to other SRCR-coding segments. This probe hybridized to at least one fragment in all of the recombinant clones of Fig. 2 thereby establishing the presence of a non-repetitious cytoplasmic segment in all of these clones. This observation must be interpreted to support

the earlier estimate of 50–100 T19-like genes within the sheep haploid genome.

Two of us (H.C.C. and P.L.J.W.) have estimated that there are about 10 genes encoding WC1 in the cow genome and this estimate is somewhat lower than the corresponding minimal estimate for sheep in this paper. Even so these numbers considerably exceed the multiplicity of genes in man or mouse for other well-understood cell-surface proteins such as CD4 (one per haploid genome), CD8α (one) or CDβ (two). The probable existence of such a large repertoire of variants within the sheep T19 family is compatible with a role whereby individual members of the family might subserve a related but distinct function. We have recently demonstrated within lymph node homing lymphocytes, enriched for γδ T cells, that many T19-like mRNA molecules are present (M. A. O'Keeffe, S. A. Metcalfe, M. D. Glew *et al.*, in press). It is now important to investigate the expression of T19-like genes within single clones. Although all existing serological evidence points to the exclusive expression of T19 on γδ T cells, it has not been possible to date to rule out the expression of related but serologically undetectable gene products on αβ T cells. The findings of this paper should facilitate such an investigation in the future. In addition it will be of considerable interest to establish whether single γδ T cells express a restricted number of T19-like genes as would be compatible with a role for them in lymphocyte homing behaviour. Specifically such a role would predict that a population of 'resident' γδ T cells from one epithelial tissue[1–7] might express only one or a few genes from the T19 repertoire and that γδ T cells from another epithelial tissue would express a different non-overlapping set. Studies to examine the expression of T19 genes in a variety of cell populations in adult animals and during fetal development are now under way.

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