Immunology 1997 **91** 1–6

Characterization of the bovine Cα gene

W. R. BROWN,*† H. RABBANI,* J. E. BUTLER† & L. HAMMARSTRÖM* *Department of BioSciences at NOVUM and Department of Clinical Immunology, Huddinge Hospital, Karolinska Institute, Huddinge, Sweden, and †Department of Microbiology, The University of Iowa Medical School, Iowa City, IA, USA

SUMMARY

The complete genomic sequence of a bovine Cα gene is reported here. The genomic sequence was obtained from a Cα phage clone that had been cloned from a genomic EMBL4 phage vector library. The Cα sequence had previously been expressed as a chimeric antibody and identified as IgA using IgA-specific antibodies. Intron/exon boundaries were determined by comparison of the genomic sequence with an expressed bovine Cα sequence obtained from spleen by reverse transcription-polymerase chain reaction (RT-PCR). Analysis of 50 Swedish bovine genomic DNA samples using genomic blots and five different restriction enzymes failed to detect evidence of polymorphism. However, *Pst*I digests of Brown Swiss DNA showed a restriction fragment length polymorphism (RFLP), suggesting that at least two allelic variants of bovine IgA exist. Comparison of the deduced amino acid sequence of bovine IgA with sequences available for other species indicated that the highest homology was with that of swine, another artiodactyl. This was the highest homology observed for all mammalian IgA compared except for that between IgA1 and IgA2 in humans. Bovine IgA shares with rabbit IgA3 and IgA4, an additional N-linked glycosylation site at position 282. However, the collective data indicate that cattle are like swine and rodents and unlike rabbits in having a single locus of the gene encoding IgA of this species.

INTRODUCTION

Bovine IgA was characterized in the late 1960s during a period when many believed that IgG1 was the major 'secretory immunoglobulin' of domesticated ruminants.[1] Although IgG1 had for some time been recognized as the predominant immunoglobulin in both bovine colostrum and milk,[2,3] additional studies showed that bovine lacteal secretions also contained a 11S protein that was associated with an approximately 80 000 MW glycoprotein, subsequently shown to be the bovine homologue of secretory component. Bovine IgA was also shown to be the major immunoglobulin in almost all exocrine body fluids of cattle except lacteal, and to be synthesized by tissues associated with these body fluids.[4]

Cattle, like swine, are group III mammals, the offspring of which obtain their passive maternal IgG only after birth via a lacteal transport mechanism, unlike human infants who are provided maternal IgG via transplacental transport.[5] Thus, the mammary gland of cows is the site of IgG transport, resulting in IgG1 being the major immunoglobulin in the colostrum of this species. This situation contrasts with that of the group I mammals (e.g. primates and rabbits), in which the mammary gland is not used in IgG transport so IgA remains the major colostral immunoglobulin. It is now recognized that this biological difference also explains the historical confusion caused by the failure of investigators to find IgA as the predominant lacteal immunoglobulin in cattle.[1]

The physiochemical properties of bovine immunoglobulins from serum and colostrum have been characterized extensively[6] and the sequences of a number of bovine IgG immunoglobulins have been characterized at the genetic level.[7–9] Although at least three IgG subclasses, IgE and IgA have been expressed as chimeric antibodies,[10,11] the sequence and genetic polymorphism of bovine IgA have not been reported.

IgA is encoded by a single Cα gene in mice and two subclass-encoding genes in humans,[12] while 13 subclasses are present in rabbits.[13] Among the group III mammals, the Cα gene of only the swine has been characterized and, interestingly, the gene occurs in two allelic forms: IgA^a, which has a six-amino acid hinge, and IgA^b, which has a two-amino acid hinge resulting from an apparent G to A mutation in the last nucleotide of intervening sequence 1.[14,15] Such species variation signals the need to characterize IgA fully in each species rather than extrapolating from one species to another. In cattle, it has been reported that the bovine haploid genome contains one Cα gene that may exhibit allelic restriction polymorphism.[11] This observation extended earlier studies in which an IgA allotypic polymorphism was demonstrated serologically.[16]

Received 11 November 1996; revised 17 January 1997; accepted 19 January 1997.

Abbreviations: Cα, IgA constant region gene; GCG, Genetics Computer Group; PAM, percentage accepted mutations; RT-PCR, reverse transcription-polymerase chain reaction; SDS, sodium dodecyl sulphate; SSC, sodium saline citrate; TAE, Tris–acetate–EDTA.

Correspondence: Dr L. Hammarström, Department of Biosciences at Novum, Karolinska Institute, S-14186 Huddinge, Sweden.

In light of the diversity of IgA among different species and in order to achieve our long-term goals of characterizing the immunoglobulin locus of cattle and engineering bovine antibodies with specificities for human gut pathogens, we report here the characterization of the bovine Cα gene.

MATERIALS AND METHODS

Nucleotide and amino acid sequence analysis

A Cα-containing phage clone (clone 25), from a bovine genomic library constructed in the vector EMBL4, was kindly provided by Dr K. Knight (Loyola Medical Centre, Loyola University, Chicago, IL).[10] Restriction fragments containing portions of the bovine Cα gene were subcloned into the phagemid vector pBluescript II (Stratagene, La Jolla, CA). The nucleotide sequence was determined in both directions using the *fmol* DNA Sequencing System (Promega Corp., Madison, WI) with appropriate [γ^{32}P] end-labelled primers (Table 1). Sequence data comparison was done using the BLAST programs (National Center for Biotechnology Information, NIH, Bethesda, MD),[17] the Genetics Computer Group Inc. (GCG, Madison, WI) Sequence Analysis Software Package and the LASERGENE software package (DNASTAR Inc., Madison, WI). Sequence similarity analyses to establish percentage sequence similarities and phylogenetic relationships were done using the LASERGENE software with the clustal method of alignment. Briefly, the clustal method groups sequences into clusters by first aligning them pair-wise, and then as groups using weighted tables to construct multiple alignments. The weighted tables assign values based on evolutionary substitution patterns and physiochemical similarity. The default PAM250 (percentage accepted mutations) residue-weighted table was used; this signifies 2·5 mutations per residue.

Southern blot analysis of genomic DNA

Southern blot analyses were performed as described elsewhere[18] using bovine genomic DNA isolated from peripheral blood leucocyte (PBL) samples (kindly provided by O. Distl, Institut für Tierzucht, Universität München, and L. Björk, Department of Food Science, Ultuna Veterinary School, Uppsala, Sweden). Briefly, approximately 4 μg of enzyme-restricted DNA was electrophoresed through a 0·7% agarose gel in 1 × Tris–acetate–EDTA (TAE) prior to blotting onto a nylon membrane (Hybond-N; Amersham, Arlington Heights, IL). The membrane was hybridized with the ^{32}P-labelled probe consisting of the 4·9 kb *Eco*RI/*Sal*I bovine Cα encoding fragment of phage clone 25. Blots were hybridized in 0·04 M Tris (7·5), 0·9 M NaCl/40% formamide/10% dextran sulphate/1% sodium dodecyl sulphate (SDS)/0·1% sodium pyrophosphate and 100 μg/ml of salmon sperm DNA at 42° for 24 hr. The blots were then washed in 2 × sodium saline citrate (SSC)/0·5% SDS for 2 × 5 min at room temperature, followed by a wash in 0·1 × SSC/0·1% SDS for 2 × 15 min at 65°.

Polymerase chain reaction

Total RNA was extracted from bovine spleen using TRIzol reagent (Gibco BRL, Gaithersburg, MD) following the manufacturer's instructions. First-strand cDNA was synthesized from total RNA using an antisense CH3 primer and Superscript II reverse transcriptase (Gibco BRL). Specific segments of the Cα gene were amplified by reverse transcription-polymerase chain reaction (RT-PCR) from spleen-derived first-strand cDNA, using the sense Cα1 exon primer and the antisense Cα2 exon or the antisense Cα3 exon primers (primers 1, 5 and 6; Table 1). PCR products were subcloned via T/A cloning into a pBluescript plasmid T vector as described previously.[19] Recombinant clones were sequenced as above.

Table 1. Primers used for cloning and sequencing*

Description	5′–3′ sequence
1. Sense Cα1 exon	CAGCGTGTCCGTCAGGAACT
2. Antisense Cα1 exon	GAAGTTCCTGACGGACACGCT
3. Antisense Cα1 intron	TGGTCCACACAGGGTCGGGGT
4. Sense Cα2 exon	GCTGTGCCGATCCCTGGAACA
5. Antisense Cα2 exon	CAGAGCAGGAGAAAGTCTGTC
6. Antisense Cα3 exon	GCGGCGGCAGCAGGTGGACC
7. Sense Cα3 exon	GCCCTCAACGAGCTGGTGACG
8. Antisense 3′ untranslated region	CTTCAGGCGAGCACGGAGTT
9. T3 vector primer	AATTAACCCTCACTAAAGGG
10. T7 vector primer	GTAATACGACTCACTATAGGGC

*Cα primers were based on partial sequence data from the bovine Cα phage clone.

RESULTS

Two restriction fragments spanning the entire Cα gene, subclone B, a 610-bp *Sac*II/*Xho*I fragment, and subclone C, a *Xho*I/*Xba*I fragment of approximately 1·8 kb, were subcloned from the Cα phage clone into pBluescript. Initial nucleotide sequence analysis of subclone C indicated that the 3′ end (*Xba*I end) shared sequence homology with a region downstream of the polyadenylation signal for the secreted form of the gorilla

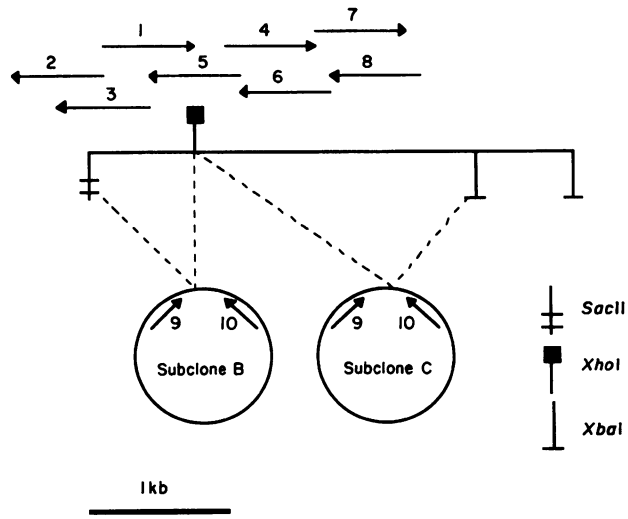

Figure 1. Sequencing strategy for the bovine Cα gene. A partial restriction map is shown with the restricition sites indicated. Subclone B is a *c.* 600-bp *Sac*II/*Xho*I fragment in pBluescript and subclone C is an *c.* 2-kb *Xho*I/*Xba*I fragment in pBluescript. Primers 9 and 10 are vector primers and primers 1–8 are IgA-specific. The sequencing direction is designated by arrows and filled circles denote the approximate start point for each sequence.

```
                GTCCGCGGGGCCGTCAGGGGTCCGGTCAGCAGAGGGCTGCTGTCTCACAGCGCACCCTGTGTTTCA   66

GAG AGT GAA ACC AGC CCC AGC ATC TTC CCG CTG AGC CTT GGG AAC AAC GAC CCA GCC GGG  126
Glu Ser Glu Thr Ser Pro Ser Ile Phe Pro Leu Ser Leu Gly Asn Asn Glu Pro Ala Gly

CAG GTG GTC ATC GGC TGC CTG GTC CAG GGC TTC TTC CCG TCG GCA CCC CTG AGC GTG ACC  186
Gln Val Val Ile Gly Cys Leu Val Gln Gly Phe Phe Pro Ser Ala Pro Leu Ser Val Thr

TGG AAC CAG AAT GGC GAC AGC GTG TCC GTC AGG AAC TTC CCT GCC GTG CTG GCC GGG AGC  246
Trp Asn Gln Asn Gly Asp Ser Val Ser Val Arg Asn Phe Pro Ala Val Leu Ala Gly Ser

CTG TAC ACC ATG AGC AGC CAG CTG ACC TTG CCG GCC AGC CTG TGC CCA AAA GGC CAG TCC  306
Leu Tyr Thr Met Ser Ser Gln Leu Thr Leu Pro Ala Ser Leu Cys Pro Lys Gly Gln Ser

GTG ACC TGC CAA GTG CAG CAC CTC TCC AAA GCC AGC AAG ACC GTG GCC GTG CCC TGC ATA  366
Val Thr Cys Gln Val Gln His Leu Ser Lys Ala Ser Lys Thr Val Ala Val Pro Cys Ile

ATT CAA G GTCAGAGGGCAGGCTGGGGTGAGGCCCCGTCCTCCCACCCCGACCCTGTGTGGACCATTTTCCTCTACT  442
Ile Gln G

CAGACAAGAGGGGGTTGAGCCCCCAGTGGGGGTGCCATGAGGGGCCCGGGCTGGTGCTGAAGGCCAGGGGAGGTGGGCA  521


GAGGGCACTGACCTGCCCCGCCTCTCTTTCAG AC TCA AGT TCA TGT TGC GTG CCG AAC TGC GAG CCC  588
                                 lu Ser Ser Ser Cys Cys Val Pro Asn Cys Glu Pro

AGC CTG TCC GTG CAG CCA CCA GCC CTC GAG GAC CTG CTC CTG GGC TCC AAC GCC AGC CTC  648
Ser Leu Ser Val Gln Pro Pro Ala Leu Glu Asp Leu Leu Leu Gly Ser Asn Ala Ser Leu

ACG TGC ACA CTG AGT GGC CTG AAA AGC GCC GAG GGC GCC AGC TTC ACC TGG AAC CCG ACA  708
Thr Cys Thr Leu Ser Gly Leu Lys Ser Ala Glu Gly Ala Ser Phe Thr Trp Asn Pro Thr

GGT GGG AAG ACC GCC GTC CAG GGG TCG CCC AAG CGT GAC TCC TGT GGC TGC TAC AGC GTG  768
Gly Gly Lys Thr Ala Val Gln Gly Ser Pro Lys Arg Glu Ser Cys Gly Cys Tyr Ser Val

TCC AGC GTC CTG CCG GGC TGT GCC GAT CCC TGG AAC AGT GGA CAG ACT TTC TCC TGC TCT  828
Ser Ser Val Leu Pro Gly Cys Ala Asp Pro Trp Asn Ser Gly Gln Thr Phe Ser Cys Ser

GTC ACC CAC CCC GAG TCC AAG AGT TCA CTG ACC GCC ACC ATC AAG AAA GAC TTA GGTGGGC  889
Val Thr His Pro Glu Ser Lys Ser Ser Leu Thr Ala Thr Ile Lys Lys Glu Leu G

CTGGACCACTGCGTGTGGGCAGCTGGGCACTCCACACACAAGGCCCCCTCCCAAGCCTGCCCCTCCCTCTCCAGCCCTG  968

CCCACTCCTCACAGAGGGAGACTGAGGCATGGGGGGCCCAGGGGACAGGGGTGCTCTCCTCCCTGTGAGACCCCTGACC 1047


CCTGACCCCCCCTCTGCCCTGCAG GG AAC ACG TTC CGG CCT CAG GTC CAC CTG CTG CCG CCG CCG 1112
                         ly Asn Thr Phe Arg Pro Gln Val His Leu Leu Pro Pro Pro

TCG GAG GAG CTG GCC CTC AAC GAG CTG GTG ACG CTG ACG TGC CTG GTG CGG GGA TTC AGC 1172
Ser Glu Glu Leu Ala Leu Asn Glu Leu Val Thr Leu Thr Cys Leu Val Arg Gly Phe Ser

CCC AAG GAG GTG CTG GTG CGT TGG CTG CAG GGC AAT CAA GAG CTG CCC CGC GAG AAG TAT 1232
Pro Lys Glu Val Leu Val Arg Trp Leu Gln Gly Asn Gln Glu Leu Pro Arg Glu Lys Tyr

CTG ACC TGG GGC CCC CTG CCC GAG GCT GGC CAG AGC GTC ACC ACC TTC GCC GTG ACC AGC 1292
Leu Thr Trp Gly Pro Leu Pro Glu Ala Gly Gln Ser Val Thr Thr Phe Ala Val Thr Ser

GTG CTA CGC GTG GAC GCC GAG GTC TGG AAG CAG GGG GAC ACC TTC TCC TGC ATG GTG GGC 1352
Val Leu Arg Val Asp Ala Glu Val Trp Lys Gln Gly Asp Thr Phe Ser Cys Met Val Gly

CAC GAG GCC CTG CCC CTG GCC TTC ACC CAG AAG ACC ATC GAC CGC CTG GCG GGT AAA CCC 1412
His Glu Ala Leu Pro Leu Ala Phe Thr Gln Lys Thr Ile Asp Arg Leu Ala Gly Lys Pro

ACC CAC GTG AAC GTG TCC GTG GTC ATG TCA GAG GTG GAT GGG GTC TGC TAC TGA GTGCCCC 1473
Thr His Val Arg Val Ser Val Val Met Ser Glu Val Asp Gly Val Cys Tyr

ACCCCTAGCTCTTGAATAAACTCCGTGCTCGCCTGAAGCAGCTCCGCACTTCCCTGT                    1530
```

Figure 2. Nucleotide and deduced amino acid sequence of the bovine Cα gene. Nucleotides are numbered at the right. Intronic sequences are italicized and the TGA stop codon is underlined.

Cα1 Domain

```
                120                                             160
Bovine          ESETSPSIFPLSLGNNDPAGQVVIGCLVQGFFPSAPLSVTWNQNGDSVSV
Porcine         V-----K----T--SSE---Y---A---RD----E--T---SPSREG-I-
HuIgA2 A2m(1)   A-P---KV-----DSTPQD-N--VA--------QE------SES-QN-TA
HuIgA2 A2m(2)   A-P---KV-----DSTPQD-N--VA--------QZ------SES-ZN-TA
HuIgA1          A-P---KV-----CSTQ-D-N---A--------QE------SES-QG-TA
Murine          --ARN-T-Y--T-PPALSSDP-I----IHDY---GTMN---GKS-KDITT

                                              200
Bovine          RNFPAVL..AGSLYTMSSQLTLPASLCPKGQSVTCQVQHLSKASKTVAVP
Porcine         ----PAQ..--G-----------VEQ--AD-ILK--------S-QS-N--
HuIgA2 A2m(1)   ----PSQDAS-D---T--------TQ--D-K----H-K-YTNP-QD-T--
HuIgA2 A2m(2)   ----PSQBAS-D---T--------TZ--D-K----H-K-YTNS-QD-T--
HuIgA1          ----PSQDAS-D---T--------TQ-LA-K----H-K-YTNP-QD-T--
Murine          V---PA-.AS-GR----N------VE--E-E--K-S---D-NPVQELD-N

Bovine          CIIQD
Porcine         -KV..
HuIgA2 A2m(1)   -PV..
HuIgA2 A2m(2)   -RV..
HuIgA1          -PV..
Murine          -SG..
```

Cα2 Domain

```
                                240
Bovine          ..........SSSCCVPNCEPSLSVQPPALEDLLLGSNASLTCTLSGLK
Porcine         L.........P-DP-PQC-K----L-----A------------------
HuIgA2 A2m(1)   P............PPP-C-H-R--LHR----------E-N-----T--R
HuIgA2 A2m(2)   P............PPP-C-H-R--LHR----------E-N-----T--R
HuIgA1          PSTPPTPSPSTPPTPSPSC-H-R--LHR----------E-N-----T--R
Murine          P........TPPPPITI-S-Q----L-R----------D--I----N--R

                       280                                      320
Bovine          SAEGASFTWNPTGGKTAVQGSPKRDSCGCYSVSSVLPGCADPWNSGQTFS
Porcine         KS--V----Q-S---D---A--T----------I---------K-E---
HuIgA2 A2m(1)   D-S--T---T-SS--S----P-E--L--------------Q---H-E--T
HuIgA2 A2m(2)   D-S--T---T-SS--S----P-E--L--------------Q---H-E--T
HuIgA1          D-S-VT---T-SS--S----P-E--L--------------E---H-K--T
Murine          NP---V---E-ST--D---KKAVQNS--------------ER----AS-K

Bovine          CSVTHPESKSSLTATIKKDLG
Porcine         -TAA-S-L--A-----T-PKV
HuIgA2 A2m(1)   -TAA---L-TP---N-T-S.-
HuIgA2 A2m(2)   -TAA---L-TP---N-T-S.-
HuIgA1          -TAAY----TP----LS-S.-
Murine          -T------GT.--G--A-VTV
```

Cα3 Domain

```
                                360
Bovine          NTFRPQVHLLPPPSEELALNELVTLTCLVRGFSPKEVLVRWLQGNQELPR
Porcine         ----------------------------------D--------G-----
HuIgA2 A2m(1)   -----E----------------------A------D--------S-----
HuIgA2 A2m(2)   -----E----------------------A------D--------S-----
HuIgA1          -----E----------------------A------D--------S-----
Murine          ---P------------------LS------A-N---------H--E--SP
                       400                                      440
Bovine          EKYLTWGPLPEAGQSVTTFAVTSVLRVDAEVWKQGDTFSCMVGHEALPLAF
Porcine         D---V-ES---P--AIP-Y-----------D--------------------
HuIgA2 A2m(1)   ------ASRQ-PS-GT-------I---A--D--K----------------
HuIgA2 A2m(2)   ------ASRQ-PS-GT-------I---A--D--K-E--------------
HuIgA1          ------ASRQ-PS-GT-------I---A--D--K----------------
Murine          -S--VFE--K-P-EGA--YL-------S--T-----QY----------MN-

Bovine          TQKTIDRLAGKPTHVNVSVVMSEVDGVCY
Porcine         ----------------------A-AE-I--
HuIgA2 A2m(1)   ----------------------A----T--
HuIgA2 A2m(2)   -------M------I------A-A--T--
HuIgA1          ----------------------A----T--
Murine          --------S----N-S---I---G--I--
```

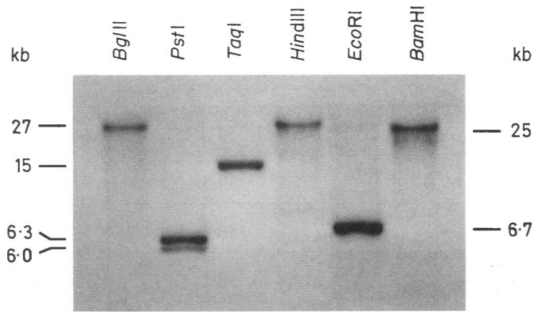

Figure 4. Genomic Southern blot analysis of Brown Swiss DNA obtained from peripheral blood lymphocytes. Four micrograms of DNA was digested with one of six different restriction enzymes, electrophoresed through a 0·7% agarose gel and blotted onto a nylon membrane. The blot was screened with a probe consisting of a 4·9-kb *Eco*RI/*Sal*I bovine Cα-encoding fragment of phage clone 25. Enzymes are indicated at the top and approximate molecular weights (in kilobase pairs) of hybridizing bands are shown on the left and right.

IgA2 constant region gene (data not shown). Therefore sequence analysis of subclone C focused on the 5′ (*Xho*I) end. A partial restriction map and the sequencing strategy for the bovine Cα gene are shown in Fig. 1.

Sequence data of the Cα subclones was aligned to determine the entire sequence of the secreted form of the bovine Cα gene (Fig. 2). Boundaries between exons and introns were confirmed by comparison of the genomic sequence and the nucleotide sequence of a bovine spleen-derived PCR product that extends from the middle of the CH1 domain to the beginning of the CH3 domain (data not shown). The amino acid sequence of bovine Cα was deduced using LASERGENE software and compared with the amino acid sequences of other mammalian alpha chains (Fig. 3).

In order to determine whether polymorphisms are associated with bovine Cα, the 4·9-kb *Eco*RI/*Sal*I fragment containing the bovine Cα gene was utilized as a probe in Southern blots of genomic DNA. Analysis of 50 Swedish bovine genomic DNA samples (data not shown), each independently digested with five restriction enzymes, revealed a single hybridizing band of ≥ 25 kb by digestion with *Bgl*II, *Hin*dIII or *Bam*HI. *Taq*I digestion resulted in a 15-kb hybridizing band, whereas a 6·7-kb band was obtained with *Eco*-RI. A single hybridizing band of 6 kb was observed with *Pst*I-digested DNA for all 50 samples tested of the Swedish strain. However, *Pst*I digestion of a DNA sample from a Brown Swiss animal resulted in two hybridizing bands of 6·0 kb and 6·3 kb (Fig. 4).

DISCUSSION

The nucleotide sequence of the bovine Cα gene (Fig. 2) is typical of mammalian Cα genes in having three exons separated by two introns, the latter of which contains splice junctions that conform to the GT/AG.[20–22] The sequence presented in Fig. 2 is of the secreted form of IgA. Partial sequence analysis of both ends of the *Xba*I fragment at the 3′ end of the map shown in Fig. 1 revealed homology with the intron located 5′ of the human Cα membrane coding region (data not shown). Based on these data, we would predict that the membrane exon for bovine IgA is located downstream of the second *Xba*I site shown in Fig. 1.

Southern blot analysis of 50 Swedish bovine genomic DNA samples resulted in one hybridizing band for each of five restriction enzymes tested (data not shown), indicating that the bovine genome contains a single Cα gene. Southern analysis of *Pst*I-restricted genomic DNA from a Brown Swiss animal revealed the presence of two hybridizing bands with the bovine Cα probe (Fig. 4), suggesting a restriction fragment length polymorphism (RFLP). These data are in general agreement with previous data that indicated the presence of a single Cα gene in the bovine genome of the Brown Swiss breed but the presence of an allelic restriction polymorphism in some breeds, e.g. Holstein.[11]

Comparison of the deduced amino acid sequence of bovine IgA with other mammalian Cα sequences (Fig. 3) indicated typical immunoglobulin domain features, such as conserved Cys and Trp residues, as well as typical IgA features, such as a carboxy terminal tail consisting of 19 amino acids that contains the canonical Cys–Tyr motif as the last two residues of the secreted form. N-linked glycosylation sites (Asn–X–Thr/Ser)[23] are present in all species compared at the Cα2 and Cα3 domains. Bovine IgA also has an additional site at position 282 (Fig. 3) that is not present in humans, swine or mice but is present in the rabbit IgA3 and IgA4 sequences.[13]

The greatest variation among sequences was observed for the hinge region, which for bovine Cα is only five amino acids long and consists of three serine and two cystine residues. This is comparable in size to the porcine IgA^a hinge and longer only than human IgA2 and the IgA^b hinge deletion allelic variant of swine. The species heterogeneity observed in the hinge is also observed among IgG[24] and IgM.[25]

The bovine IgA amino acid sequence is most similar to porcine IgA, the only other artiodactyl mammal for which an IgA sequence is available. The *c.* 75% sequence similarity between the bovine and porcine sequences (Table 2) is the greatest observed between all mammalian IgA genes except the human IgA1 and IgA2 subclasses.

It then becomes important to determine what features of IgA antibodies are essential for their optimal *in vivo* performance. The availability of sequence data on IgA from a number of species, and the current biotechnical skills available for engineering antibodies and the availability of transgenic farm animals, now make this a realistic objective. Obtaining the data provided here for IgA from cattle, the world's largest producer of this antibody class among domesticated species, is a necessary step for studies on the functional activity of IgA administered orally either: (1) naturally administered to the

Figure 3. (Page 4.) Comparison of mammalian Cα amino acid sequences using the Pileup program of GCG. Amino acids are numbered according to the Bur IgA α1 chain.[12] Domain boundaries were determined by comparison with partial cDNA sequence data that linked the Cα1 and Cα2 domains and the Cα2 and Cα3 domains (data not shown). Sequence data for bovine, porcine,[14] human IgA1 (HuIgA1) and human IgA2 $A2m^1$ allotype (HuIgA2 $A2m^1$)[26] and murine IgA[27] are deduced amino acid sequences whereas the human IgA2 $A2m^2$ allotype (HuIgA2 $A2m^2$) sequence is from Torano & Putnam,[28] and Flanagan *et al.*[29]. Dashes indicate identity to bovine Cα and dots indicate gaps inserted to maximize homology. The hinge region is highlighted in bold.

Table 2. Sequence pair distances of mammalian IgA amino acid sequences*

	Bovine IgA	Porcine IgA	Human IgA1	Human IgA2	Murine IgA	
1		74·6	67·3	68·1	59·8	Bovine IgA
2	23·8		68·7	69·0	58·5	Porcine IgA
3	30·2	29·1		94·1	56·1	Human IgA1
4	28·9	28·4	5·0		57·2	Human IgA2
5	39·4	37·8	41·2	40·6		Murine IgA

*Clustal method of alignment with PAM250 residue weight table with results given as percentage similarity.

offspring during passive transfer of maternal immunity, or (2) therapeutically administered to animals or humans.

REFERENCES

1. Sullivan A.L., Prendergast R.A., Antunes L.J., Silverstein A.M. & Tomasi T.B. (1969) Characterization of the serum and secretory immune systems of the cow and sheep. *J Immunol* **103,** 334.
2. Butler J.E., Coulson E.J. & L.G.M. (1968) Identification of glycoprotein-a as a probable fragment of bovine IgA. *Fed Proc* **27,** 617.
3. Mach J.P., Pahud J.J. & Isliker H. (1969) IgA with 'secretory piece' in bovine colostrum and saliva. *Nature* **223,** 952.
4. Butler J.E., Maxwell C.F., Pierce C.S., Hylton M.B., Asofsky R. & Kiddy C.A. (1972) Studies on the relative synthesis and distribution of IgA and IgG1 in various tissues and body fluids of the cow. *J Immunol* **109,** 38.
5. Butler J.E. (1971) Transmission of immunity from mother to young. In: *Fertility and Sterility* (eds T. Masegawa, M. Hayasbi, F.J.B. Ebling & I. Henderson), pp. 92–98. Excerpta Medica, Amsterdam.
6. Butler J.E. (1986) Biochemistry and biology of ruminant immunoglobulins. *Prog Vet Microbiol Immunol* **2,** 1.
7. Symons D.B., Clarkson C.A., Milstein C.P., Brown N.R. & Beale D. (1987) DNA sequence analysis of two bovine immunoglobulin CH gamma pseudogenes. *J Immunogen* **14,** 273.
8. Symons D.B., Clarkson C.A. & Beale D. (1989) Structure of bovine immunoglobulin constant region heavy chain gamma 1 and gamma 2 genes. *Mol Immunol* **26,** 841.
9. Kacskovics I. & Butler J.E. (1996) The heterogeneity of bovine IgG2. VIII. The complete cDNA sequence of bovine IgG2a (A2) and an IgG1. *Mol Immunol* **33,** 189.
10. Knight K.L. & Becker R.S. (1987) Isolation of genes encoding bovine IgM IgG, IgA and IgE chains. *Vet Immunol Immunopathol* **17,** 17.
11. Knight K.L., Suter M. & Becker R.S. (1988) Genetic engineering of bovine Ig. Construction and characterization of hapten binding bovine/murine chimeric IgE IgA, IgG1, IgG2, and IgG3 molecules. *J Immunol* **140,** 3654.
12. Putnam F.W. (1977) In: *The Plasma Proteins* (ed. F. W. Putnam), p. 1. Academic Press, NY.
13. Burnett R.C., Hanly W.C., Zhai S.K. & Knight K.L. (1989) The IgA heavy chain gene family in rabbit: cloning and sequence analysis of 13 C alpha genes. *EMBO J* **8,** 4041.
14. Brown W.R. & Butler J.E. (1994) Characterization of a C alpha gene of swine. *Mol Immunol* **31,** 633.
15. Brown W.R., Kacskovics I., Amendt B.A. *et al.* (1995) The hinge deletion allelic variant of porcine IgA results from a mutation at the splice acceptor site in the first C alpha intron. *J Immunol* **154,** 3836.
16. De Benedictis G., Capalbo P. & Dragone A. (1984) Identification of an allotypic IgA in cattle serum. *Comp Immunol Microbiol Infect Dis* **7,** 35.
17. Altschul S.F., Gish W., Miller W., Myers E.W. & Lipman D.J. (1990) Basic local alignment search tool. *J Mol Biol* **215,** 403.
18. Smith C.I. & Hammarstrom L. (1986) Detection of immunoglobulin genes in individuals with immunoglobulin class or subclass deficiency. Evidence for a pretranslational defect. *Mono Allergy* **20,** 18.
19. Marchuk D., Drumm M., Saulino A. & Collins F.S. (1991) Construction of T vectors, a rapid and general system for direct cloning of unmodified PCR products. *Nucleic Acids Res* **19,** 1154.
20. Breathnach R., Benoist C.K.O.H., Gannon F. & Chambon P. (1978) Ovalbumin gene: evidence for a leader sequence in mRNA and DNA sequences at the exon intron boundaries. *Proc Natl Acad Sci USA* **75,** 4853.
21. Mount S.M. (1982) A catalogue of splice junction sequences. *Nucleic Acids Res* **20,** 459.
22. Seif I., Khoury G. & Dhar R. (1979) BKV splice sequences based on analysis of preferred donor and acceptor sites. *Nucleic Acids Res* **6,** 3387.
23. Marshal R.D. (1972) Glycoproteins. *Annu Rev Biochem* **41,** 673.
24. Day E.D. (1990) In: *Advanced Immunochemistry* (ed. E.D. Day), pp. 1–693. Wiley-Liss, NY.
25. Sun J. & Butler J.E. (1996) The complete sequence of swine switch μ and Cμ. *Immunogenetics,* in press.
26. Flanagan J.G., Lefranc M.P. & Rabbitts T.H. (1984) Mechanisms of divergence and convergence of the human immunoglobulin alpha 1 and alpha 2 constant region gene sequences. *Cell* **36,** 681.
27. Auffray C., Nageotte R., Sikorav J.L., Heidmann O. & Rougeon F. (1981) Mouse immunoglobulin A: nucleotide sequence of the structural gene for the alpha heavy chain derived from cloned cDNAs. *Gene* **13,** 365.
28. Torano A. & Putnam F.W. (1978) Complete amino acid sequence of the alpha 2 heavy chain of a human IgA2 immunoglobulin of the A2m (2) allotype. *Proc Natl Acad Sci USA*, **75,** 966
29. Flanagan J.G. & Rabbitts T.H. (1982) Arrangement of human immunoglobulin heavy chain constant region genes implies evolutionary duplication of a segment containing gamma, epsilon and alpha genes. *Nature*, **300,** 709.