Available online at www.sciencedirect.com
ScienceDirect
Veterinary Immunology and Immunopathology 124 (2008) 284–294
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Characterization of the bovine immunoglobulin lambda light chain constant IGLC genes

LiMei Chen, Min Li, Qing Li, XingYuan Yang, XiaoRong An *, YongFu Chen

State Key Laboratory for Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing 100094, China

Received 17 October 2007; received in revised form 27 March 2008; accepted 9 April 2008

Abstract

To characterize the bovine immunoglobulin lambda light chain constant region (IGLC) genes, we have isolated a bacterial artificial chromosome (BAC) clone by a PCR based approach from a bovine genomic DNA library, constructed using a genital ridge cell line derived from a male Holstein fetus. The positive BAC clone, containing the bovine IGLC genes, was fully sequenced and had a 138 kb insert. Sequence analysis revealed that the bovine immunoglobulin lambda light chain locus consisted of four joining-constant gene recombination units spanning approximately 20 kb DNA in length. A detailed examination of the recombination signal sequences, RNA splicing sites and coding sequences of the four joining-constant gene recombination units suggested that only two IGLC genes (IGLC2 and IGLC3) were functional while the IGLC1 and IGLC4 appeared to be pseudogenes. This conclusion was further confirmed by a series of RT-PCR amplifications, which also showed that among these four genes the IGLC3 was preferentially expressed in cattle. Phylogenetic analysis indicated that the bovine IGLC genes were more closely related to their equivalents in sheep and goats than that to other mammals.

Keywords: Cattle; Gene rearrangement; Immunoglobulin; Immunoglobulin lambda light chain constant region

1. Introduction

The immunoglobulin light chain plays an important role in maintaining antibody conformation, immunoglobulin repertoire diversification and antigenic moiety recognition. Two distinct immunoglobulin light chain isotypes, kappa and lambda, have been described in mammals, and these are expressed in varying ratios in different species. In humans, 60% of expressed light chains are of kappa origin and in mice this figure rises to 95% (Hood et al., 1967). Mouse and human immunoglobulin light chain loci share certain structural similarities, but also differ in the number of gene segments, subfamilies and arrangements that all determine the light chain repertoire (Dariavach et al., 1987; Lefranc and Lefranc, 2001, 2004; Schroeder, 2006). And these differences have been well characterized (Amrani et al., 2002; Malcolm et al., 1982; Vasicek and Leder, 1990). The human immunoglobulin lambda light chain (IGL) locus is localized on chromosome 22 and contains from seven to eleven immunoglobulin lambda light chain constant region (IGLC) genes, of which 4–5 are functional and each IGLC gene is preceded by its own IGLJ gene (Ghanem et al., 1988).

Abbreviations: IGLC, immunoglobulin lambda light chain constant region; BAC, bacterial artificial chromosome; RSS, recombination signal sequence; PFGE, pulsed field gel electrophoresis; EST, expressed sequence tag.

* Corresponding author at: State Key Laboratory for Agrobiotechnology, College of Biological Sciences, China Agricultural University, Beijing 100094, China. Tel.: +86 10 62733463; fax: +86 10 62734640.
E-mail address: xra@cau.edu.cn (X. An).

0165-2427/$ – see front matter
doi:10.1016/j.vetimm.2008.04.012

Thus, together with approximately 9–33 active IGLV genes, about 116–165 V–J combinatorial combinations could be produced (Lefranc and Lefranc, 2001). The mouse IGL locus is located on chromosome 16 and shows an organization of two clusters containing three functional IGLC genes and one pseudogene (Lefranc, 2003; Lefranc and Lefranc, 2004). As there are only three active IGLV genes within the mouse IGL locus, the number of combinatorial combinations for generating the germline repertoire is reduced and this is reflected in the reduced prevalence of lambda-bearing immunoglobulin in the serum of mice compared with that of humans (Schroeder, 2006).

On the other hand, our current understanding of the IGLC genes in domestic animals such as cattle, sheep and horses, is still limited (Aitken et al., 1999; Jenne et al., 2006; Wagner, 2006; Zhao et al., 2006). The light chain repertoires in these animals are dominated by the lambda chains (Butler, 1997; Gibson, 1974; Griebel and Ferrari, 1994), and kappa chains are expressed at a very low level (Arun et al., 1996; Ford et al., 1994). Two IGLC genes have only recently been cloned and sequenced in sheep (Jenne et al., 2006). Southern blot analyses of genomic DNA identified four distinct IGLC genes in horses, three of which were found to be expressed (Wagner, 2006). The first characterization of a bovine IGL chain at the nucleotide level was done in 1988 (Ivanov et al., 1988), and the sequence was subsequently exploited as a probe for studies of light chain variable sequences (Jackson et al., 1992; Parng et al., 1996; Sinclair et al., 1995). Studies on the bovine light chain repertoire suggest that only a single IGLJ (or a very limited number of IGLJ segments) is (are) expressed, although there may be more present in the IGL locus (Parng et al., 1996; Sinclair et al., 1995). It was also suggested that there were at least four IGLC genes in cattle of which one was preferentially utilized (Parng et al., 1995, 1996).

The bovine IGL locus has previously been mapped to chromosome 17 and was estimated to contain more than 30 IGLV genes (Aitken et al., 1999; Tobin Janzen and Womack, 1996). The available bovine IGLV genes are classified into two gene families (Parng et al., 1996; Sinclair et al., 1995) or three gene families IGLV1, IGLV2 and IGLV3 (Saini et al., 2003), on the basis of sequence similarity. It has been suggested that the bovine heavy chains with exceptionally long CDR3 are preferentially paired with variable domains encoded by genes from the IGLV1 subgroup (Saini et al., 2003).

The recent generation of somatically cloned transchromosomic calves producing human polyclonal antibodies, provides a promising system for production of therapeutic human polyclonal antibodies (Kuroiwa et al., 2002; Robl et al., 2003). However, the presence of active endogenous immunoglobulin loci may interfere with the expression of the human antibodies and could give rise to human and bovine chimeric antibodies (Echelard and Meade, 2002; Kuroiwa et al., 2002). Hence, knocking out or inactivating the bovine immunoglobulin genes is a prerequisite for development of a system for commercial production of human antibodies in cattle for pharmaceutical use (Echelard and Meade, 2002; Kuroiwa et al., 2002). This, however, requires knowledge of the bovine immunoglobulin loci. In this paper, we have characterized the bovine IGLC genes and associated IGLJ genes, and it will provide some information for above mentioned aim. The understanding of the organization and expression of IGLC in cattle is directly relevant to the development of mammalian expressed light chain repertoire.

2. Materials and methods

2.1. Bovine BAC library and PCR screening

A bovine genomic bacterial artificial chromosome (BAC) library was constructed using high molecular weight DNA isolated from a genital ridge cell line derived from a male Holstein fetus (Eggen et al., 2001). The vector used was pBeloBAC11. The BAC library consisted of 105,984 clones with an average insert size of 120 kb. The BAC DNA was amplified in *Escherichia coli* DH10B and purified using the QIAEX II kit (Qiagen) following the manufacturer's instructions.

The bovine genomic BAC library was screened using a PCR based approach. The primers used for screening were IGL1F (5′-AGC TCA ACG GCA ACA AGG C-3′) and IGL1R (5′-GGT CTT ATT CAG GAG AAG CAG G-3′), both designed according to previously published bovine IGL mRNA sequences (GenBank accession numbers AF396698 and X62917). PCR reactions were performed under conditions of 95 °C 5 min, then 30 cycles of 94 °C 30 s, 58 °C 30 s, and 72 °C 40 s, finally holding at 72 °C for 10 min. Amplified products were cloned into a pGEM-T vector (Promega, Madison, USA) and verified by sequencing.

2.2. Pulsed field gel electrophoresis (PFGE)

Pulsed field gel electrophoresis (PFGE) was used to separate large DNA fragment (CHER-DR III system, Bio-Rad, Hercules, CA). The DNA was run on a 1% agarose gel in 0.5× TBE buffer (6 V/cm). The switch time was adjusted based on the size of DNA following

the manufacturer's instruction. The DNA size was estimated using the Midrange DNA Marker (New England Biolabs, Beverly, USA).

2.3. Shotgun sequencing strategy

A shotgun library of an isolated BAC clone was constructed in pUC18 to carry inserts with an average size of 1.5–3.0 kb (Genomics Institute of Chinese Academy of Sciences, Beijing). For quality clipping of sequences, PHRED (Washington University) and PHRAP package were coupled. The phred/phrap/consed package (Washington University) was used for sequence assembly and gap closure. Identities were calculated using the Identity Matrix of the BioEdit software, which showed the proportion of identical residues between all aligned sequences.

2.4. IGLC1 and IGLC4 specific PCR analysis

In order to confirm the mutations of the IGLC1 and IGLC4 observed in the BAC clone, primers RSSJ1F (5′-CAG GGC TTG GCA GAC GGG TGC TTG ATG G-3′) and RSSJ1R (5′-GAC CCA GGT CCC GCC GCC TAA GAC AAA A-3′) were designed to amplify the recombination signal sequences (RSS) of IGLJ1. Primers J1-1F (5′-GTC TTA GGC GGC GGG ACC TGG GTC ACC GTC-3′), and C1-1R (5′-TGC CTT TCG ATT TCC AGT CGC TGC CCG TCA-3′), J4-1F (5′-CTA TTT TCA TTG GCA GGA CCA GGC TGA CT-3′) and C4-1R (5′-TCC CAA AGG CTA AGA GGA TTT GTT TCC TT-3′) were designed to amplify the IGLC1 and IGLC4 genes. Genomic DNA isolated from two unrelated Holstein cattle were employed as templates. PCR products were separated on a 1% agarose gel and subsequently cloned into pGEM-T vector (Promega, Madison, CA) and sequenced.

2.5. Examination of the bovine IGLC genes expression by RT-PCR

Specific PCR reactions were conducted to examine whether or not the IGLC genes were functional. Four pairs of gene specific primers were used to examine the IGLJ–IGLC expression. J1F (5′-TCT TAG GCG GCG GGA CCT GG-3′) and C1R (5′-TTC GAT TTC CAG TCG CTG CC-3′) were IGLJ1–IGLC1 specific primers. Primers J2F (5′-TTT CGG CGG CGG GAC CAG AG-3′) and C2R (5′-TCG ATT TCC AGT CGC TGT CT-3′), J3F (5′-GGG ACC ACA CTG ACC GTC CT-3′) and C3R (5′-TTC GAT TTC CAG TCG CTG CT-3′), J4F (5′-TTT TCA TTG GCA GGA CCA GG-3′) and C4R (5′-CGA TTT CCA GTC GCT GCC CA-3′) were specific to IGLJ2–IGLC2, IGLJ3–IGLC3 and IGLJ4 – IGLC4, respectively. Spleen cDNA from two unrelated cattle were employed as templates for PCR under conditions of 94 °C 5 min, then 30 cycles of 94 °C 30 s, 52 °C 30 s, 72 °C 30 s, finally holding at 72 °C for 10 min. PCR products were separated on an agarose gel and subsequently cloned into pGEM-T vector (Promega, Madison, CA) and sequenced.

2.6. DNA sequence computations

DNA searches were carried out using the BLAST program at NCBI (http://www.ncbi.nlm.nih.gov). Sequence editing and alignment were performed using the MegAlign program (DNAStar, Madison, WI). Dot plot comparisons were performed using the same program with the following parameters: percentage, 80, window, 30. The exon/intron boundaries of the bovine IGLC genes were deduced by comparing the genomic DNA with the cDNA sequences.

2.7. Phylogenetic analysis

A phylogenetic tree was constructed using sequences either retrieved from the GenBank or obtained in the present study. The *ClustalX* program was used for sequence alignment (Thompson et al., 1997). The phylogenetic analysis was based on genetic distance matrices and neighbor-joining methods (Saitou and Nei, 1987). Phylogenetic trees were deduced using the DNADIST and NEIGHBOR programs from the PHYLIP package (version 3.65) and based on a bootstrapping of 1000 separate genetic distance matrices.

3. Results

3.1. Screening of a genomic BAC library and sequencing of the bovine IGLJ and IGLC genes from a shotgun library

Using a PCR based screening approach, we obtained one bovine IGL positive BAC clone from a bovine BAC genomic library (Eggen et al., 2001). The length of the insert in the isolated BAC clone was estimated to be approximately 120–140 kb as visualized by PFGE (data not shown). For analysis of the full sequence of the BAC clone, a shotgun library was constructed using pUC18, with an average insert size of 1.5–3.0 kb. In total, 2640 clones derived from the shotgun library were sequenced and most of the sequences were determined on both

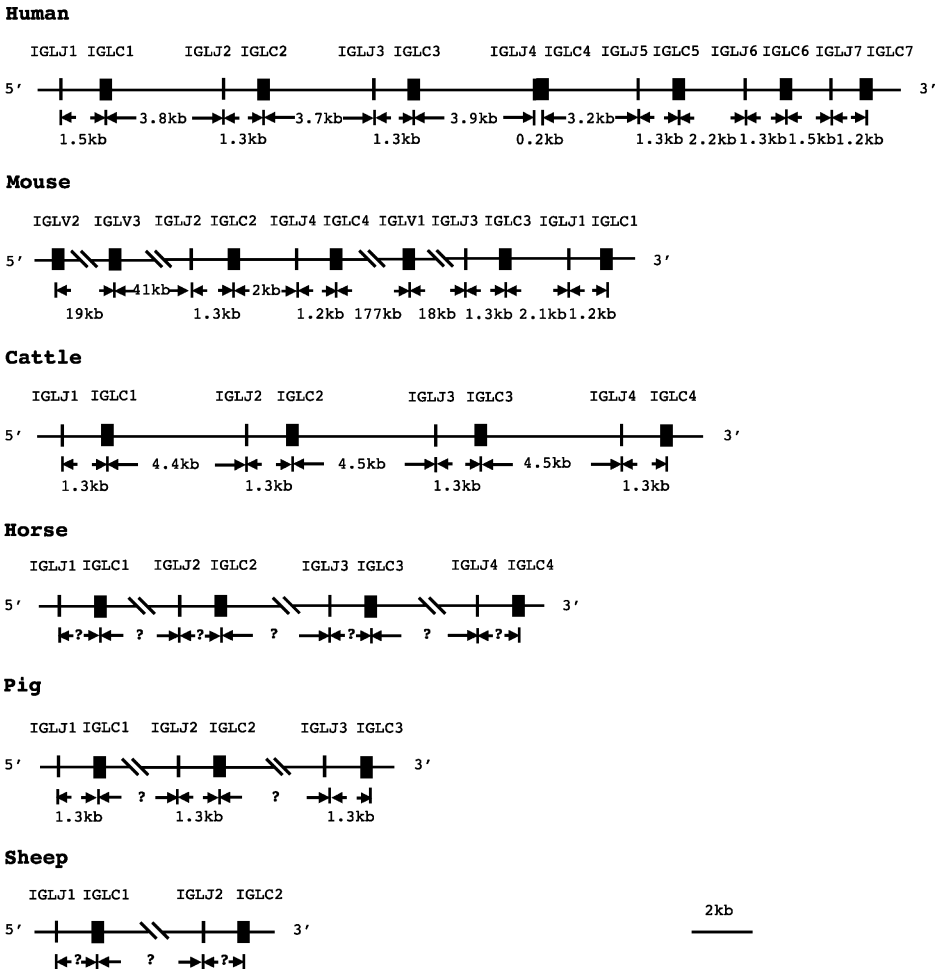

Fig. 1. Comparison of the genomic organization of IGLC regions of cattle with human (Lefranc, 2003; Vasicek and Leder, 1990), mouse (Lefranc, 2003), horse (Home et al., 1992), sheep (Jenne et al., 2006) and pig (Butler et al., 2006). The coding regions are indicated by black boxes. Schematics are drawn to scale.

strands (the read coverage was 7.59×). After editing, a complete insert of 138,080 bp was assembled. This sequence has been submitted to GenBank under the accession number DQ537487.

3.2. Genomic organization of the bovine IGLC gene locus

To analyze the bovine IGLC gene locus, we aligned a previously described bovine IGL sequence (B4, GenBank accession number AF396698) with the assembled 138 kb genomic sequence using the MegAlign program. Within the 138 kb contig, four distinct IGLC genes were identified. By the same strategy four IGLJ genes were also identified, each located upstream of one of the four distinct IGLC genes respectively. Thus, as found in humans and mice, the bovine IGLJ and IGLC genes are organized in pairs and the bovine IGL gene locus consists of four IGLJ–IGLC gene recombination units in a tandem array. Following the nomenclature of IGLC genes in the human and the mouse (Lefranc and Lefranc, 2001, 2004), the four IGLJ–IGLC gene recombination units were accordingly termed lambda1–lambda4, based on their sequential order in the contig (Fig. 1). Within the gene cluster, the average distance from the polyadenylation signal site of the IGLC gene to the downstream IGLJ was about 4450 bp, varying from 4437 bp to 4489 bp. The length of the intron between IGLJ and the adjacent IGLC ranges from 1275 bp to 1304 bp. These features appear similar to those of the human IGL gene locus (Schroeder, 2006).

There was only about 5.7 kb of DNA sequence upstream of the IGLJ1 in our BAC clone. Using this as a

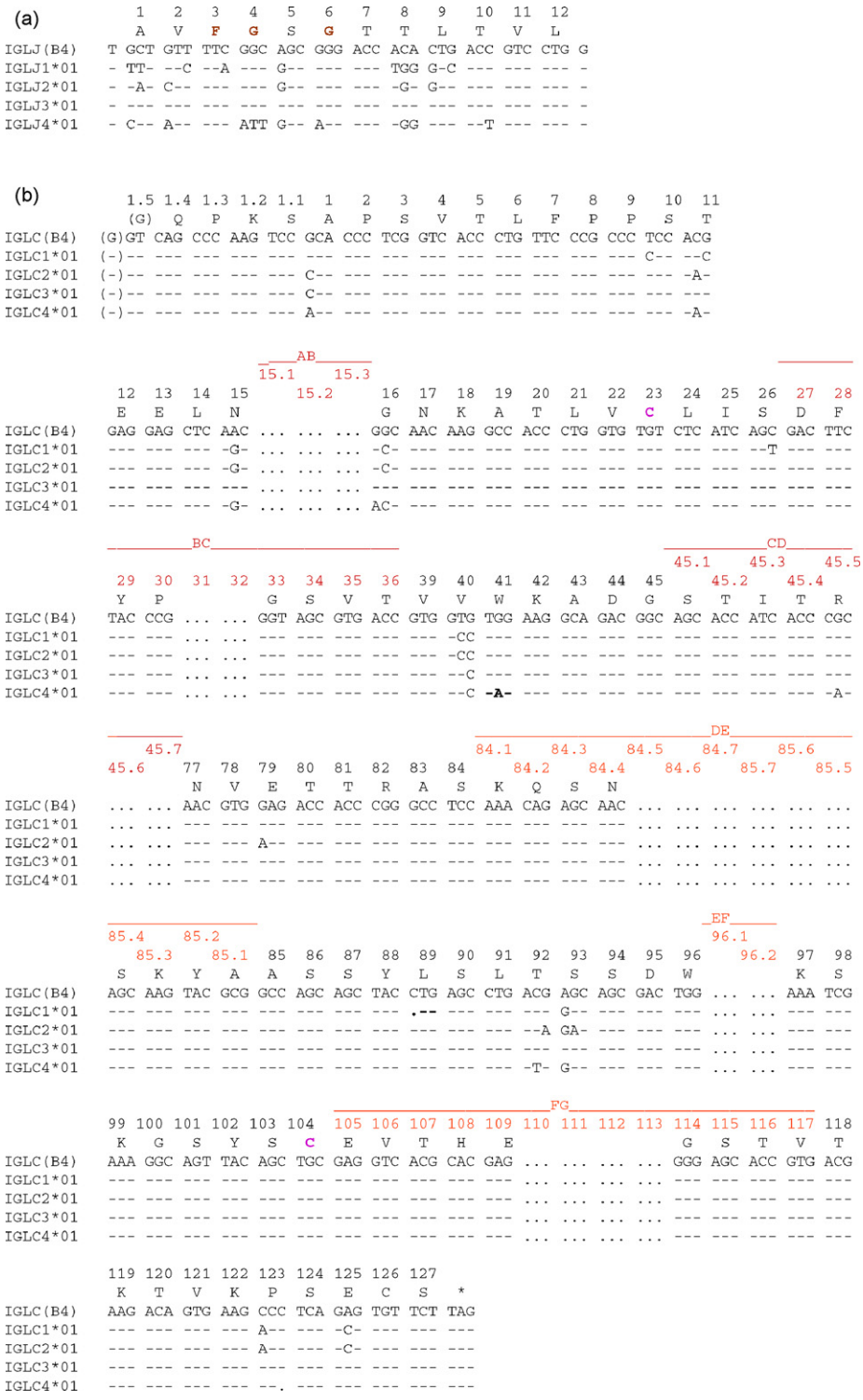

Fig. 2. Comparison of the bovine IGLJ and IGLC sequences. (a) An alignment of the bovine IGLJ1, IGLJ2, IGLJ3 and IGLJ4 nucleotide sequences with that in B4. Dashes indicate identical nucleotides and dots indicate gaps according to the IMGT numbering system. The F, G and G of the FGXG motif are shown in brown in the B4 amino acid sequence. (b) An alignment of the bovine IGLC1, IGLC2, IGLC3 and IGLC4 nucleotide sequences with that in B4. Numbering of the sequence is according to the IMGT numbering system for the constant domain. AB, BC, CD, DE, EF and FG correspond to the turns and loops between the sandwich fold beta strands. Dashes indicate identical

query sequence, we performed a BLAST search against the bovine genome database at NCBI. One contig (GenBank accession number NW_941002) was found to share approximately 10.5 kb of sequence with the 5′ end of our BAC clone and most likely overlapped with our clone on bovine chromosome 17. The sequence upstream of the IGLJ1 was thus extended to 13 kb. We were unable to identify potential IGLJ or IGLC genes in the region upstream of the IGLJ1 or in the 110 kb DNA sequence downstream of the IGLC4.

We further aligned the four bovine IGLJ and IGLC genes with the previously published B4 sequence and the result shows that IGLJ1, IGLJ2, IGLJ3 and IGLJ4 share 73.7%, 86.8%, 100% and 73.7% identify with the IGLJ of the B4 sequence (Fig. 2a). The four bovine IGLC genes in our clone are very similar in sequence with the IGLC of B4, sharing 96.9%, 96.3%, 99.4% and 96.0% identity respectively (Fig. 2b).

3.3. Mutations and validation of IGLC1 and IGLC4

From the alignment of the bovine IGLC gene sequences, a single nucleotide deletion in IGLC1 was identified at codon 89 (nucleotide 264) according to the IMGT numbering system for the constant domain (Lefranc et al., 2005), leading to a frame-shift in the coding sequence. It is predicted that this would cause premature translational termination and produce a truncated peptide (Fig. 2b). In the IGLC4 sequence, a G → A transition at codon 41 (nucleotide 122) generated a nonsense mutation (TGG to TAG) that would also result in translational termination (Lefranc et al., 2005) (Fig. 2b). As a result, IGLC4 would produce a much shorter polypeptide of only 40 amino acids. These observations suggested that IGLC1 and IGLC4 might be pseudogenes.

It has been shown that the V, D and J genes are flanked by conserved heptamer (CACTGTG) and nonamer (GGTTTTTGT) sequences, separated by either 12 bp or 23 bp non-conserved base pairs. These flanking sequences with the intervening sequences are together known as recombination signal sequences. Mutated RSS may sometimes account for non-functionality of an immunoglobulin gene segment (Akira et al., 1987). Fig. 3 shows that each bovine IGLJ has an intact copy of the consensus nonamer and heptamer separated by 12 nucleotides except for IGLJ1. The RSS signals of IGLJ2, IGLJ3 and IGLJ4 match the consensus sequence exactly, while three substitutions were found in the IGLJ1 RSS. As mutations in the heptamer sequence reduced recombination drastically (Hesse et al., 1989), this may serve as additional evidence supporting the notion that the IGLC1 is not used.

The splice sites are essential sequence signals for precise RNA splicing. Nearly all introns of genes contain a pair of highly conserved dinucleotides, GT/AG, as RNA splicing donor and receptor (Shapiro and Senapathy, 1987). We found that IGLJ2–IGLC2, IGLJ3–IGLC3 and IGLJ4–IGLC4 genes all contained these conserved splice sites but the 3′ splice acceptor site of the IGLC1 was GG instead of AG (Fig. 3). Although several non-canonical splice sites seem to be functional in RNA splicing (Burset et al., 2000; Hodge and Cumsky, 1989; Jackson, 1991), GG has not been observed as an acceptor sequence. It is thus unlikely that the primary IGLJ1–IGLC1 transcript (if it can be transcribed) could be appropriately spliced.

The consensus polyadenylation site (AATAAA) is also a pivotal signal for mRNA processing (Fitzgerald and Shenk, 1981). Point mutations in this sequence generally reduce the abundance of mRNA (Lucier et al., 1998; Wickens and Stephenson, 1984). Our sequence data show that each of the four IGLC genes had a single copy of the hexanucleotide in their putative 3′ untranslated regions (data not shown). However, the polyadenylation signal site of the IGLC4 was different from the others. In IGLC4, only 82 bp separated the TAG stop codon corresponding to the normal stop codon in IGLC2 and IGLC3 and the hexanucleotide. This was shorter than the distance (133 bp) found for the other IGLC genes.

To confirm RSS mutations and the aberrant splice acceptor of the IGLC1 sequence, we amplified two genomic fragments of the IGLC1 gene from two unrelated cattle using two pairs of specific primers respectively. The resulting 771 bp and 1556 bp PCR products were cloned and sequenced (10 clones of 771 bp PCR product and eight clones of 1556 bp PCR product). The data were consistent with the sequence derived from the BAC as described above (data not shown). These findings suggest that the aberrant RSS and splice acceptor found in IGLC1 are common features in cattle.

To verify the mutations in the IGLC4 gene and its distinct polyA site, we amplified a 1.8 kb DNA fragment covering the region from IGLJ4 to the polyA

nucleotides and dots indicate gaps. Conserved cysteines involved in the disulfide bridge are shown in purple. Stop codon is indicated by an asterisk. B4 is a previously published full-length bovine IGL cDNA (GenBank accession no. AF396698).

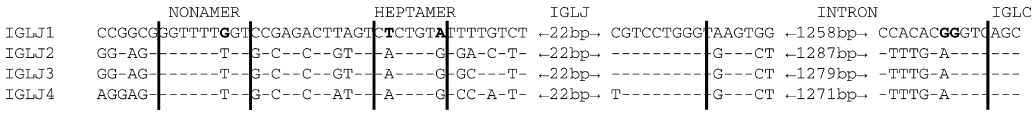

Fig. 3. The nonamer–heptamer recombination signals found 5′ to the bovine IGLJ regions and splice donor and acceptor sequences in the IGLJ–IGLC introns.

site from the unrelated cattle mentioned above using primers J4-1F and C4-1R. Ten recombinant clones derived from each individual animal were sequenced and compared. The data showed that all were identical to the IGLC4 sequence from the BAC clone (data not shown).

3.4. Expression of the bovine IGLC genes

To check for expression of the four bovine IGLC genes *in vivo*, four pairs of IGLJ–IGLC primers, specific to IGLJ1–IGLC1, IGLJ2–IGLC2, IGLJ3–IGLC3 and IGLJ4–IGLC4, were employed in RT-PCR using cDNA from two different spleens. The IGLJ2–IGLC2 and IGLJ3–IGLC3 specific primers amplified a fragment of approximately 300 bp, but the density of the amplified IGLJ3–IGLC3 band was substantially stronger than that of the IGLJ2–IGLC2 band (Fig. 4a). Cloning and sequencing of the amplified products clearly showed that they were IGLC2 and IGLC3 derived (data not shown). At the same time, the other two primer pairs did not give any PCR product. However, with genomic DNA as templates, these two primer pairs could both amplify a DNA fragment of about 1.6 kb as predicted from our analysis of the locus (Fig. 4b). This supports our hypothesis that both IGLC1 and IGLC4 are pseudogenes. The gene expression data also suggest that, although IGLC3 and IGLC2 are used in formation of the lambda light chain repertoire, IGLC3 is used preferentially.

A BLAST search against the bovine expressed sequence tag (EST) database (1,315,093 clones; May, 21, 2007) at NCBI found 99 IGLC3 and only five IGLC2 EST clones, but no IGLC1 or IGLC4 matches were obtained. A similar search using IGLJ revealed 99 IGLJ3, two IGLJ2 EST clones and again no IGLJ1 or IGLJ4 hits. Together with our RT-PCR data, these facts strongly support the notion that, in the bovine, IGLC3 is preferentially expressed and IGLC1 and IGLC4 are pseudogenes.

3.5. Phylogenetic analysis of the IGLC genes in vertebrates

A dot plot analysis showed that large areas of substantial similarity exist within the bovine IGLC locus (Fig. 5), indicating that the bovine IGLC genes could have been generated by duplication. In order to obtain more information with regard to the phylogeny of IGLC genes, a phylogenetic tree was thus constructed using the IGLC genes derived from different species (Fig. 6).

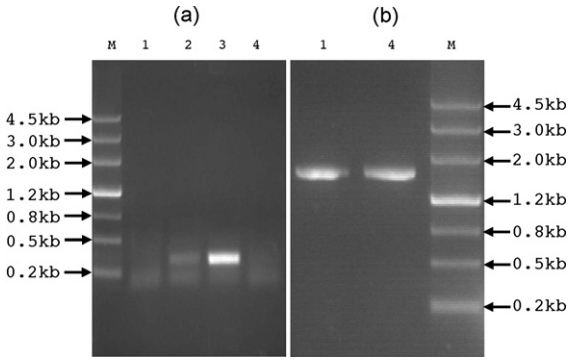

Fig. 4. PCR analysis of expression of the bovine IGLC genes. (a) RT-PCR amplifications of the bovine IGLC1, IGLC2, IGLC3 and IGLC4 (specific RT-PCR reactions in lanes 1–4, respectively). M, DNA marker. (b) PCR amplification of IGLC1 (labeled 1) and IGLC4 (labeled 4) from genomic DNA. Primers were those used for amplifications loaded to lanes 1 and 4 in panel a. M, DNA marker.

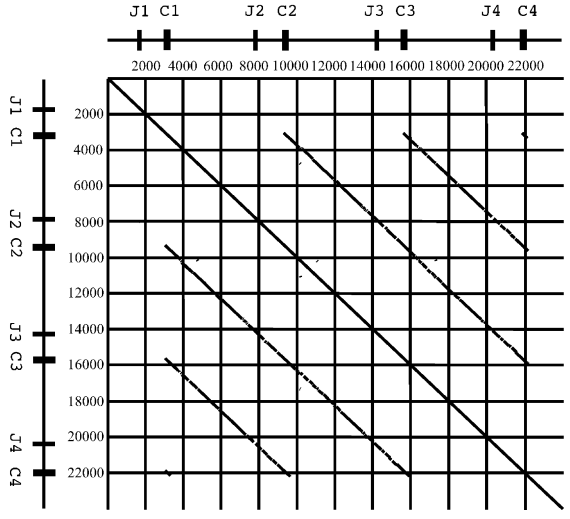

Fig. 5. A dot plot comparison of the bovine IGLJ–IGLC gene locus with its self. Dot plot parameters: window, 30; percentage, 80.

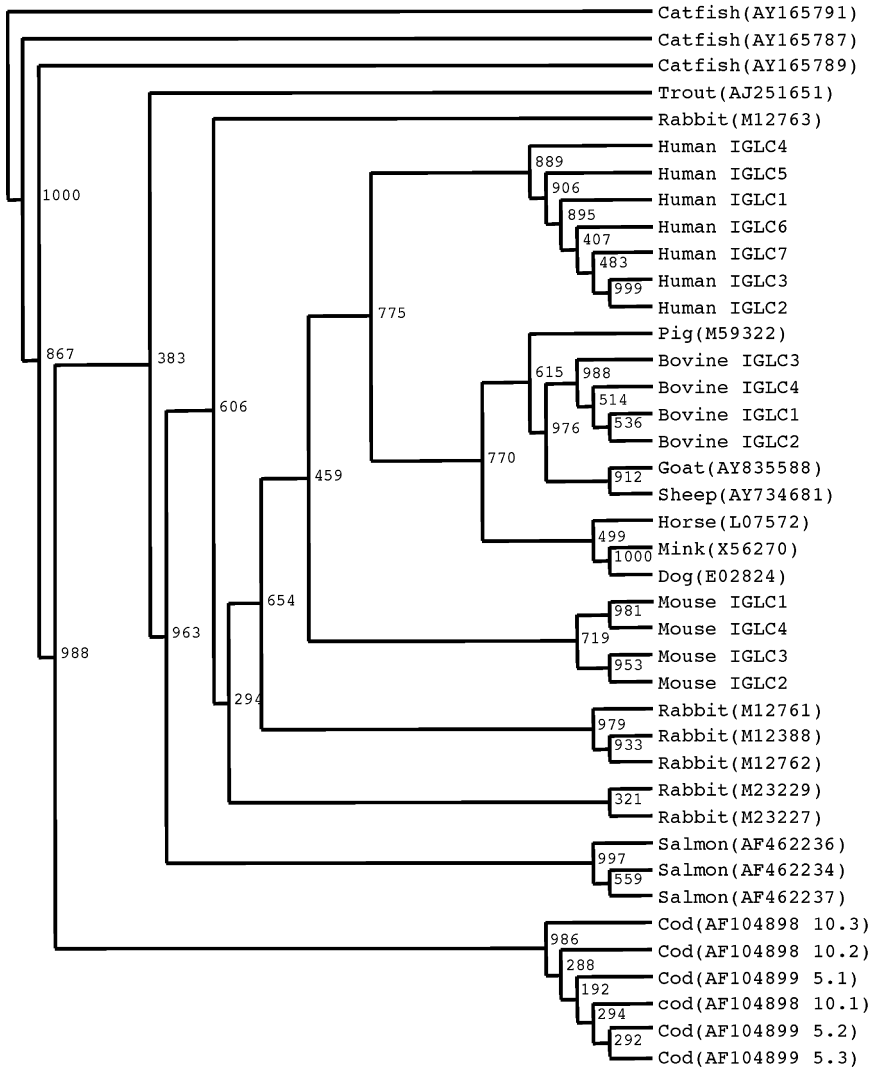

Fig. 6. Phylogenetic relationships of the IGLC genes in vertebrates. The tree was constructed using nucleic acid sequences aligned by CLUSTRAL_X. Accession numbers of the sequences used are shown within the parentheses. Human IGLC genes were derived from NG_000002, and mouse IGLC genes were derived from NG_004051.

As shown in Fig. 6, the bovine IGLC genes are phylogenetically closer to the IGLC genes in goats, sheep, pigs, dogs, minks and horses than that in salmons, cods and catfishes, just as the molecular timescale for vertebrate evolution would predict (Kumar and Hedges, 1998). The bovine IGLC genes were clearly clustered together with the IGLC genes from goats (*Capra hircus*) and sheep (*Ovis aries*), and this is consistent with the previous observations that the cattle and sheep immunoglobulins are very similar at the DNA or protein level (Zhao et al., 2006). A comparison of the most highly expressed bovine IGLC gene (IGLC3) with the sheep IGLC gene (GenBank accession no. AY734681) revealed 92.5% identity. However, this value is still lower than the degrees of similarity between the bovine IGLC genes themselves (Fig. 2b). This may imply that the duplications generating the clustered bovine IGLC genes might have occurred after the divergence of cattle and sheep about 20 million years ago (Kumar and Hedges, 1998). The similarities of the IGLC coding regions deduced peptide sequences between the cattle (IGLC3) and goat, sheep, pig, dog, human, mouse and trout were 88.8%, 86.0%, 81.3%, 74.7%, 75.7%, 65.4% and 37.0%, respectively.

4. Discussion

In the present study, we have completely analyzed the bovine IGLC region. It contains four IGLC genes,

each of which is preceded by a unique IGLJ gene. The number of the IGLC genes varies in different mammals with two in sheep (Jenne et al., 2006), three in pigs (Butler et al., 2006), four in both mice and horses (Home et al., 1992; Schroeder, 2006; Wagner, 2006), 7–11 in humans (Dariavach et al., 1987; Ghanem et al., 1988; Lefranc and Lefranc, 2001, 2004; Vasicek and Leder, 1990) and four in cattle as identified in the present study (Fig. 1). This may suggest that duplication of IGLC genes has occurred in these animals during the evolution of the IGLC region like duplication of the gamma genes in the IGHC locus (Zhao et al., 2003). The dot plot analysis of the bovine IGLC gene locus showed a high degree of similarity existed across the IGLC region beyond the protein coding sequences (Fig. 5).

The similarity of the immunoglobulin of cattle and sheep has led to speculation that the processes of generating repertoires in these two animals may share common features (Zhao et al., 2006). As far as the IGLC gene locus is concerned, cattle, sheep and horse are, in one way or another, all similar, when compared with humans and mice. Sheep have two IGLJ–IGLC recombination units, of which IGLJ1 always associates with IGLC1 and IGLJ2 always associates with IGLC2, a situation that is again analogous to that of humans and mice. IGLJ1–IGLC1 produces normal immunoglobulin and IGLJ2–IGLC2 produces a severely truncated protein (Jenne et al., 2006). In horses, four very similar IGLC genes were identified and only a single IGLJ gene was found to be associated with three functional IGLC genes and the fourth IGLC gene associates with a pseudo-IGLJ gene (Wagner, 2006). Our data show that cattle contain four IGLJ and four IGLC genes and among them IGLJ1, IGLC1 and IGLC4 appear to be non-functional. Immunoglobulin light chains are predominantly formed from rearrangement of IGLJ3, transcription and splicing to IGLC3. It seems that unlike the situation in humans and mice, the lambda light chain germline repertoires in these animals are all restricted.

To confirm the data obtained from the BAC clone, genomic DNA from two additional cattle was isolated and characterized, and results of these two samples have confirmed findings from analysis of the BAC clone. In addition, RT-PCR from splenic cDNA of two cattle showed that IGLC2 and IGLC3 were functional and that IGLC2 was expressed at a much lower level than IGLC3, a finding corroborated by BLAST searches against the bovine EST database and consistent with previous work indicating that only one IGLC gene appears to be expressed in the bovine lambda chain repertoire (Lucier et al., 1998; Parng et al., 1996). The mechanism underlying the preferential expression of IGLC3 is not clear. A sequence comparison showed that the coding regions of the IGLC2 and IGLC3 differed in 10 nucleotides (Fig. 2b), leading to an alternation of eight amino acids. These sequence variations provide no obvious explanation for the preferential expression of any one gene over the other. Equally, the RSS and spacer sequences flanking the IGLJ2 and IGLJ3 are identical, suggesting that the recombination efficiency of IGLV to IGLJ2 or IGLJ3 should not differ.

The expressed IGK: IGL ratio differs between species with a value of 60%: 40% in humans and 95%: 5% in mice (Alt et al., 1987; Hood et al., 1967). Although the exact ratio is not yet known in cattle, it is known that the expressed light chain repertoire is dominated by IGL (Butler, 1997; Max, 1993). As we have analyzed the bovine IGLC genes in this study, a future study of the bovine IGKC genes may explain why the bovine IGL genes are expressed at a higher level than the IGK genes in cattle.

Acknowledgements

We are grateful to Dr. Yaofeng Zhao (China Agricultural University, China) and Dr. Rob Aitken (University of Glasgow, UK) for critical reading of the manuscript. We thank Dr. Francois Piumi and his colleagues for their kind assistance in screening of the BAC library (Institut National de la Recherche Agronomique, France). This work was supported by the Chinese National High technology Research and Development Program (2002AA206311).

References

Aitken, R., Hosseini, A., MacDuff, R., 1999. Structure and diversification of the bovine immunoglobulin repertoire. Vet. Immunol. Immunopathol. 72, 21–29.

Akira, S., Okazaki, K., Sakano, H., 1987. Two pairs of recombination signals are sufficient to cause immunoglobulin V-(D)-J joining. Science 238, 1134–1138.

Alt, F.W., Blackwell, T.K., Yancopoulos, G.D., 1987. Development of the primary antibody repertoire. Science 238, 1079–1087.

Amrani, Y.M., Voegtle, D., Montagutelli, X., Cazenave, P.A., Six, A., 2002. The Ig light chain restricted B6.kappa(-)lambda(SEG) mouse strain suggests that the IGL locus genomic organization is subject to constant evolution. Immunogenetics 54, 106–119.

Arun, S.S., Breuer, W., Hermanns, W., 1996. Immunohistochemical examination of light-chain expression (lambda/kappa ratio) in canine, feline, equine, bovine and porcine plasma cells. Zentralbl Veterinarmed A 43, 573–576.

Burset, M., Seledtsov, I.A., Solovyev, V.V., 2000. Analysis of canonical and non-canonical splice sites in mammalian genomes. Nucl. Acids Res. 28, 4364–4375.

Butler, J.E., 1997. Immunoglobulin gene organization and the mechanism of repertoire development. Scand. J. Immunol. 45, 455–462.

Butler, J.E., Sun, J., Wertz, N., Sinkora, M., 2006. Antibody repertoire development in swine. Dev. Comp. Immunol. 30, 199–221.

Dariavach, P., Lefranc, G., Lefranc, M.P., 1987. Human immunoglobulin C lambda 6 gene encodes the Kern+Oz-lambda chain and C lambda 4 and C lambda 5 are pseudogenes. Proc. Natl. Acad. Sci. U. S. A. 84, 9074–9078.

Echelard, Y., Meade, H., 2002. Toward a new cash cow. Nat. Biotechnol. 20, 881–882.

Eggen, A., Gautier, M., Billaut, A., Petit, E., Hayes, H., Laurent, P., Urban, C., Pfister-Genskow, M., Eilertsen, K., Bishop, M.D., 2001. Construction and characterization of a bovine BAC library with four genome-equivalent coverage. Genet. Se. l Evol. 33, 543–548.

Fitzgerald, M., Shenk, T., 1981. The sequence 5′-AAUAAA-3′forms parts of the recognition site for polyadenylation of late SV40 mRNAs. Cell 24, 251–260.

Ford, J.E., Home, W.A., Gibson, D.M., 1994. Light chain isotype regulation in the horse. Characterization of Ig kappa genes. J. Immunol. 153, 1099–1111.

Ghanem, N., Dariavach, P., Bensmana, M., Chibani, J., Lefranc, G., Lefranc, M.P., 1988. Polymorphism of immunoglobulin lambda constant region genes in populations from France. Lebanon and Tunisia. Exp. Clin. Immunogenet. 5, 186–195.

Gibson, D., 1974. Structural studies on normal horse immunoglobulin light chains. Detection of k-type N-terminal sequences. Biochemistry 13, 2776–2785.

Griebel, P.J., Ferrari, G., 1994. Evidence for a stromal cell-dependent, self-renewing B cell population in lymphoid follicles of the ileal Peyer's patch of sheep. Eur. J. Immunol. 24, 401–409.

Hesse, J.E., Lieber, M.R., Mizuuchi, K., Gellert, M., 1989. V(D)J recombination: a functional definition of the joining signals. Genes Dev. 3, 1053–1061.

Hodge, M.R., Cumsky, M.G., 1989. Splicing of a yeast intron containing an unusual 5′ junction sequence. Mol. Cell Biol. 9, 2765–2770.

Home, W.A., Ford, J.E., Gibson, D.M., 1992. L chain isotype regulation in horse. I. Characterization of Ig lambda genes. J. Immunol. 149, 3927–3936.

Hood, L., Gray, W.R., Sanders, B.G., Dreyer, W.J., 1967, Light chain evolution, Vol. 32. Cold Spring Harbor Symposia on Quantitative Biology, 133–146 pp.

Ivanov, V.N., Karginov, V.A., Morozov, I.V., Gorodetsky, S.I., 1988. Molecular cloning of a bovine immunoglobulin lambda chain cDNA. Gene 67, 41–48.

Jackson, I.J., 1991. A reappraisal of non-consensus mRNA splice sites. Nucl. Acids Res. 19, 3795–3798.

Jackson, T., Morris, B.A., Sanders, P.G., 1992. Nucleotide sequences and expression of cDNAs for a bovine anti-testosterone monoclonal IgG1 antibody. Mol. Immunol. 29, 667–676.

Jenne, C.N., Kennedy, L.J., Reynolds, J.D., 2006. Antibody repertoire development in the sheep. Dev. Comp. Immunol. 30, 165–174.

Kumar, S., Hedges, S.B., 1998. A molecular timescale for vertebrate evolution. Nature 392, 917–920.

Kuroiwa, Y., Kasinathan, P., Choi, Y.J., Naeem, R., Tomizuka, K., Sullivan, E.J., Knott, J.G., Duteau, A., Goldsby, R.A., Osborne, B.A., Ishida, I., Robl, J.M., 2002. Cloned transchromosomic calves producing human immunoglobulin. Nat. Biotechnol. 20, 889–894.

Lefranc, M.P., 2003. IMGT, the international ImMunoGeneTics database. Nucl. Acids Res. 31, 307–310.

Lefranc, M.P., Lefranc, G., 2001. The Immunoglobulin Factsbook. Academic Press, London, UK, 458 p.

Lefranc, M.P., Lefranc, G., 2004. Immunoglobulin lambda (IGL) genes of human and mouse. In: Molecular Biology of B cells, Academic Press, Elsevier Science, 37–59 pp.

Lefranc, M.P., Pommie, C., Kaas, Q., Duprat, E., Bosc, N., Guiraudou, D., Jean, C., Ruiz, M., Da Piedade, I., Rouard, M., Foulquier, E., Thouvenin, V., Lefranc, G., 2005. IMGT unique numbering for immunoglobulin and T cell receptor constant domains and Ig superfamily C-like domains. Dev. Comp. Immunol. 29, 185–203.

Lucier, M.R., Thompson, R.E., Waire, J., Lin, A.W., Osborne, B.A., Goldsby, R.A., 1998. Multiple sites of V lambda diversification in cattle. J. Immunol. 161, 5438–5444.

Malcolm, S., Barton, P., Murphy, C., Ferguson-Smith, M.A., Bentley, D.L., Rabbitts, T.H., 1982. Localization of human immunoglobulin kappa light chain variable region genes to the short arm of chromosome 2 by in situ hybridisation. Proc. Natl. Acad. Sci. U. S. A. 79, 4957–4961.

Max, E.E., 1993. Immunoglobulins: molecular genetics. In: Fundamental Immunology, third ed. Raven Press, New York, 315–382 pp.

Parng, C.L., Hansal, S., Goldsby, R.A., Osborne, B.A., 1995. Diversification of bovine lambda-light chain genes. Ann. N Y. Acad. Sci. 764, 155–157.

Parng, C.L., Hansal, S., Goldsby, R.A., Osborne, B.A., 1996. Gene conversion contributes to Ig light chain diversity in cattle. J. Immunol. 157, 5478–5486.

Robl, J.M., Kasinathan, P., Sullivan, E., Kuroiwa, Y., Tomizuka, K., Ishida, I., 2003. Artificial chromosome vectors and expression of complex proteins in transgenic animals. Theriogenology 59, 107–113.

Saini, S.S., Farrugia, W., Ramsland, P.A., Kaushik, A.K., 2003. Bovine IgM antibodies with exceptionally long complementarity-determining region 3 of the heavy chain share unique structural properties conferring restricted VH + Vlambda pairings. Int. Immunol. 15, 845–853.

Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.

Schroeder Jr., H.W., 2006. Similarity and divergence in the development and expression of the mouse and human antibody repertoires. Dev. Comp. Immunol. 30, 119–135.

Shapiro, M.B., Senapathy, P., 1987. RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucl. Acids Res. 15, 7155–7174.

Sinclair, M.C., Gilchrist, J., Aitken, R., 1995. Molecular characterization of bovine V lambda regions. J. Immunol. 155, 3068–3078.

Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res. 25, 4876–4882.

Tobin Janzen, T.C., Womack, J.E., 1996. The immunoglobulin lambda light chain constant region maps to Bos taurus chromosome 17. Anim. Biotechnol. 7, 163–172.

Vasicek, T.J., Leder, P., 1990. Structure and expression of the human immunoglobulin lambda genes. J. Exp. Med. 172, 609–620.

Wagner, B., 2006. Immunoglobulins and immunoglobulin genes of the horse. Dev. Comp. Immunol. 30, 155–164.

Wickens, M., Stephenson, P., 1984. Role of the conserved AAUAAA sequence: four AAUAAA point mutants prevent messenger RNA 3′ end formation. Science 226, 1045–1051.

Zhao, Y., Kacskovics, I., Rabbani, H., Hammarstrom, L., 2003. Physical mapping of the bovine immunoglobulin heavy chain constant region gene locus. J. Biol. Chem. 278, 35024–35032.

Zhao, Y., Jackson, S.M., Aitken, R., 2006. The bovine antibody repertoire. Dev. Comp. Immunol. 30, 175–186.