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MOLECULAR IMMUNOGENETICS OF BOVINE ANTIBODY

A Thesis

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of

The University of Guelph

by

SURINDER SINGH SAINI

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for the degree of

Doctor of Philosophy

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ABSTRACT

MOLECULAR IMMUNOGENETICS OF BOVINE ANTIBODY

Surinder Singh Saini
University of Guelph, 1999

Advisor:
Dr. Azad Kaushik

A single predominantly expressed polymorphic bovine V_H gene family (Bov V_H1), comprising 13 to 15 genes, is identified in cattle. The Bov V_H1 gene family is closest to the murine V_H Q-52 and human V_H4 gene families and related to evolutionarily less conserved mammalian group I V_H genes. Approximately 9% of the VDJ rearrangements in B lymphocytes of adult cattle show exceptionally long CDR3H (56 to 61 codons) with multiple cysteine residues. Such an exceptionally long CDR3H in an antibody is the first ever to be documented in a species. The even numbered cysteine residues in the CDR3H appear to provide hitherto unknown configurational ability to the antigen combining site via probable intra-CDR3H disulfide bridging. The VDJ rearrangements with exceptionally long CDR3H are also noted in the splenic B cells of a 125-day-old fetus. Somatic hypermutations contribute significantly to the diversification of the IgM antibody repertoire in cattle. The bovine D_H genes are preferentially used in the hydrophilic reading frame. The CDR3H region of fetal VDJ rearrangements shows repetitive GGT (glycine) and TAT (tyrosine) codons that seem to favour the recruitment of somatic hypermutations. The expressed bovine J_H genes are most homologous to human J_H4 and J_H5 genes, suggesting the existence of, at least, two J_H genes in cattle.

The VDJ rearrangements resulting in exceptionally long CDR3H preferentially pair

with either the $V_\lambda 1x$ gene or novel $V_\lambda 1$ genes, designated as $V_\lambda 1d$ and $V_\lambda 1e$ subfamilies. Such preferential pairing may be related to unique conservation of alanine-serine-alanine residues in the $V_\lambda 1x$, $V_\lambda 1d$ and $V_\lambda 1e$ gene encoded 3' region of the CDR3.

The cDNA sequence of bovine Cμ gene showed that sporadic nucleotide substitutions resulting in amino acid replacements provide a structural basis for bovine IgM allotypes. The retention of nucleotides at the CH1-CH2 junction from the intervening intron sequence, as a result of selection of an alternate splice donor site, provides an additional source of IgM variants. Bovine IgM has fewer proline residues at the CH1-CH2 junction which may result in low flexibility in this region as compared to other species. Southern blot analysis using a bovine Cμ specific DNA probe revealed four allelic forms of the Cμ gene in the bovine genome.

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To my wife Navjot, daughter Ravneet and son Sukhmandeep

whose love has made it possible to complete this thesis

and

To the eternal memory of my grand parents

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LIST OF FREQUENTLY USED ABBREVIATIONS

Antibody (Ab)

Antigen (Ag)

Bovine leukemia virus (BLV)

Base pairs (bp)

Bovine serum albumin (BSA)

Complementarity determining region (CDR)

Third CDR of the heavy chain (CDR3H)

Heavy chain constant region gene (C_H)

Light chain constant region gene (C_L)

Heavy chain diversity gene (D_H)

Catalytic subunit of DNA-dependent protein kinase (DNA-PK_{CS})

Receptor for Fc region of antibody (FcR)

Framework region (FR)

Gut-associated lymphoid tissue (GALT)

Heavy chain (H)

Hour (h)

Heat shock protein (Hsp)

Immunoglobulin (Ig)

Ileal Peyer's patch (IPP)

Heavy chain joining gene (J_H)

Kilobase (kb)

Kilo dalton (kDa)

Light chain (L)

Major histocompatibility complex (MHC)

Microgram (μg)

Microlitre (μl)

Peripheral blood lymphocytes (PBL)

Phosphate buffered saline (PBS)

Polymerase chain reaction (PCR)

Recombination activating gene (RAG)

Restriction fragment length polymorphism (RFLP)

Recombination signal sequence (RSS)

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

T cell receptor (TCR)

Terminal deoxynucleotidyl transferase (TdT)

Trimethyl (p-aminophenyl)-ammonium chloride (TMA)

2,4,6-trinitrobenzenesulfonic acid (TNP)

Variable region heavy chain gene (V_H)

Variable region light chain gene (V_L)

Variable region lambda light chain gene (V_λ)

Variable region kappa light chain gene (V_κ)

Workshop cluster 1 (WC1)

PREFACE

This thesis is organized into seven chapters. The research work pertaining to this thesis was performed by the author unless otherwise stated.

Chapter one provides an introduction to the research topic and highlights the objectives of the study. Since the studies outlined in this thesis focused on the primary antibody repertoire that involved IgM-secreting polyclonally activated B-cell population, the term antibody and immunoglobulin are used interchangeably throughout the text.

Chapter two reviews current information on immunoglobulin genes across species and relevant information on the bovine immune system.

Chapter three describes the identification, classification and genomic complexity of bovine V_H genes. Discussions with Dr W. R. Hein, Basel Institute of Immunology, Basel, Switzerland, and a sheep V_H gene-specific DNA probe provided by him were helpful in the successful conduct of these experiments.

Chapter four outlines experiments that demonstrate contribution of somatic hypermutations and exceptionally long CDR3Hs to antibody diversification in cattle. Dr. Robert M. Jacobs provided blood samples from healthy and BLV-infected cows for making hetero-hybridomas. In addition, he helped in screening the hybridoma supernates for BLV antigens and also helped in setting up a PCR assay for detection of BLV *env* gene in hetero-hybridomas. Dr. Brian Allore, Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario, Canada, helped with DNA sequencing that included modified sequencing techniques to determine DNA sequence of VDJ rearrangements expressing exceptionally long CDR3H.

Chapter five describes unique structural features of VDJ rearrangements during B

lymphocyte ontogeny, especially the repetitive codons GGT and TAT in the D_H gene-encoded CDR3H region.

Chapter six describes the identification of two novel bovine $V_\lambda 1$ subfamilies, $V_\lambda 1d$ and $V_\lambda 1e$. In addition, it provides evidence for restricted V_H and V_λ gene pairings in polyclonally activated B lymphocytes. The available bovine V_λ genes are classified into three V_λ gene families.

Chapter seven provides structural basis of bovine IgM allotypes and, also, discusses differential splicing events that may also lead to generation of bovine IgM variants.

Chapter eight summarises the conclusions drawn from the research and provides perspectives on future research direction.

Chapters four to seven are written as individual manuscripts for publication and this is likely to result in some redundancy especially in Materials and methods sections. Chapter three is already published in Molecular Immunology **34**:641-651, 1997.

Surinder Singh Saini

Chapter 1

INTRODUCTION

The life cycle of a species imposes unique host defense requirements that essentially involve molecules whose specificity is encoded in the germline. Despite the fact that both vertebrate and invertebrate species use diverse strategies for host defense, some immune recognition characteristics are remarkably similar. The invertebrates diversify their immune recognition units, such as lectins, via combinatorial processes (e.g. pentraxins) but not via gene rearrangement mechanisms. The 'big bang' of gene duplication probably occurred at the time of separation of the chordates from the echinoderms and tunicates coincident with the appearance of the effector molecules of the immune system. The primordial immunoglobulin gene appears to have evolved earlier than the 'big bang' of gene duplication as some immune recognition molecules of invertebrates, such as neural cell adhesion molecules (N-CAMs) of arthropods, share remarkable homology with the immunoglobulin domain of higher vertebrates (Marchalonis and Schluter, 1994). Similarly, the recombination activating genes (RAGs), which play a pivotal role in the rearrangement of immunoglobulin (Ig) and T-cell receptor (TcR) genes in all vertebrates, share several structural and functional features with procaryotic transposons and suggests that the RAG-transposons might have been incorporated in the genome of a vertebrate ancestor even before the evolution of antigen-specific immune response (Agrawal *et al.*, 1998)

Advances in recombinant DNA technology, immunochemistry and cell biology have

contributed significantly to contemporary understanding of the genetic mechanisms that contribute to the ability of vertebrates to respond to an enormous array (10^8) of environmental antigens. Interestingly, antibodies of species as phylogenetically distant as shark and man are constructed similarly, i.e., as heavy and light chain polypeptides that show significant diversity in the variable-region that imparts specificity for a particular antigen. The antibody diversity in vertebrate immune system is generated via juxtaposition of separated germline gene segments, both for heavy (V_H, D_H and J_H) and light (V_L and J_L) chains, followed by the association of newly generated two identical heavy and two light chains (Tonegawa, 1983). The V(D)J rearrangement processes are common to all vertebrates but germline sequence diversity varies considerably across species. In mouse and human, several divergent germline V_H and V_κ or V_λ gene families generate remarkably diverse V(D)J rearrangements resulting in significant germline encoded combinatorial diversity (Kabat *et al.*, 1991). By contrast, a small number of functional germline V-genes contribute to possible V(D)J rearrangements leading to limited germline encoded combinatorial diversity in species like chicken (Reynaud *et al.*, 1989), rabbit (Becker and Knight, 1990; Weinstein *et al.*, 1994), pig (Sun *et al.*, 1994) and sheep (Dufour *et al.*, 1996). These species, however, employ other strategies to generate antibody diversity essential to normal immune function. For example, the process of gene conversion is involved in the diversification of the pre-immune antibody repertoire in the bursa of Fabricius of chicken (Reynaud *et al.*, 1989) and appendix of the rabbit (Weinstein *et al.*, 1994); non-antigen-dependent somatic hypermutations, occurring in ileal Peyer's patches, generate antibody diversity in sheep (Reynaud *et al.*, 1991, 1995). Several species-related differences exist with regard to the use of κ- and λ-light chains of the antibody

molecule. For example, λ-light chains in cattle, sheep and horse and κ-light chains in mice are expressed at >90% frequency. In humans, the κ- and λ-light chains are expressed at 60% and 40% frequencies, respectively (reviewed by Butler, 1998). By contrast, some camel antibodies exist as homodimers of V_H chains, without light chains (Hamers-Casterman *et al.*, 1993).

The bovine immune system develops in the absence of possible influences from maternal antibodies (Kearney and Vakil, 1986) since a syndesmochorial type of placenta prevents transplacental passage of immunoglobulins to the fetus (Tizard, 1996). In contrast to mice and humans, bovine B-cells do not express surface IgD and probably belong to a unique B-cell lineage with characteristics common to both B-1 and B-2 cells (Naessens and Williams, 1992; Naessens, 1997). Given such unique characteristics, studies on immunogenetic aspects of bovine antibody are essential to advance understanding of the genetic elements and mechanisms involved in the construction of the humoral immune system in cattle, an economically important species. From a practical perspective, such studies may contribute towards developing immunotherapeutic agents via genetically engineered antibodies or development of transgenic cattle for use in the diagnosis and treatment of infections and autoimmune or immunodeficiency diseases of humans and other species.

The focus of this thesis is the identification and characterization of genetic elements that encode heavy and light chains of antibodies that constitute the bovine humoral immune system and also to understand the processes involved in antibody diversification. The various hypotheses tested to achieve these objectives include- (i) restricted genetic elements encode bovine antibodies; (ii) novel antibody diversification strategies contribute to the generation of functional bovine IgM antibody repertoire; and (iii) bovine IgM allotypes originate from

sporadic nucleotide substitutions and/or novel genetic mechanisms. The experiments outlined here have led to the following conclusions:

1. A single polymorphic immunoglobulin V_H gene family (Bov V_H1), related to mammalian group I, clan II, is predominantly expressed in cattle. The genomic complexity of Bov V_H1 gene family comprises 13 to 15 genes.

2. In addition to single point mutations resulting in amino acid substitutions, generation of an exceptionally long CDR3H (55 to 61 amino acids), with multiple cysteine residues, provides another mechanism of antibody diversity in cattle.

3. The repititive codons GGT (glycine) and TAT (tyrosine) occur in D_H gene-encoded CDR3H regions of bovine fetal VDJ rearrangements and seem to favour recruitment of somatic hypermutations. The bovine D_H genes are preferentially used in the hydrophilic reading frame.

4. The VDJ rearrangements resulting in exceptionally long CDR3H, preferentially pair with the V_λ1x gene, hitherto reported to be not expressed, or novel V_λ1d and V_λ1e genes. Such preferential $V_H + V_\lambda$ pairings in polyclonally activated B cells are unique to cattle.

5. Sporadic nucleotide substitutions and use of an alternate splice donor site at the CH1 and CH2 junction resulting in retention of additional nucleotides from the intervening intron, provide a structural basis for bovine IgM allotypic variants.

Overall, these studies suggest that restricted germline sequence diversity, both at V_H and V_λ loci, contribute to the development of primary antibody repertoire in cattle and generation of an exceptionally long CDR3H (55 to 61 amino acids) provides a novel mechanism for antibody diversification in this species.

Chapter 2

LITERATURE REVIEW

2.1 The vertebrate immune system

The immune system of all organisms has the ability to mount a defensive response against invading pathogens or harmful antigens commonly referred to as immune response. The immune response falls into two categories: the innate or non-specific response and the adaptive or specific response.

The innate response is characterized by those defense mechanisms that are intrinsic to the host *per se* and do not require prior exposure to the pathogen or antigen. These responses potentially act upon all foreign substances without altered specificity or efficacy upon repeated exposure to the same pathogen. For example, the phagocytic cells, macrophages and neutrophils, can bind and internalize a wide variety of foreign substances and, therefore, constitute an innate immune defence. Accumulating evidence suggests that the innate response is essential for the activation of the acquired or specific immune response and its direction towards a particular effector type (Medzhitov and Janeway, 1997).

The adaptive or specific immune response is mediated by specific cellular interactions triggered by the entry of the pathogen or its components into the body of the host. The specificity and efficacy of the adaptive immune response is enhanced upon repeated exposures to the same antigen. Unlike the innate response, the adaptive immune response is characterized by its specificity, diversity and memory. The adaptive immune response is of

two types: cell-mediated immune response and humoral immune response. The former is mediated by lymphocytes that undergo maturation in the thymus (T), for example cytotoxic T cells (T_C) and helper T cells (T_H), whereas the the latter is mainly effected by antibodies secreted by bone marrow-derived B lymphocytes (Medzhitov and Janeway, 1997).

2.2 Immunoglobulin structure and function

Immunoglobulins (Ig) are antigen-binding glycoproteins and are, therefore, called antibodies (Abs). Abs are either expressed as membrane-bound receptors on the surface of B lymphocytes or are present in secreted form in various body fluids such as blood, lymph, mucous etc. The membrane-bound Ab confers antigen specificity to a B cell leading to its proliferation upon interaction with an antigen (Ag). Abs are produced by terminally differentiated B lymphocytes called plasma cells and act by specifically recognizing and eliminating the foreign or harmful Ag. All the Abs produced by a single cell have an identical Ag binding specificity. The total B cell population is capable of producing Abs of approximately 10^8 different Ag specificities, sufficient to recognize any antigen present in the universe (Alt *et al.*, 1987). Such an enormous Ab diversity is generated through random shuffling of separated germline heavy (H) and light (L) chain gene segments and/or various antibody diversification processes during B cell development as well as during the generation of an immune response (Tonegawa, 1983, Reynaud *et al.*, 1989; Weinstein *et al.*, 1994; Reynaud *et al.*, 1995).

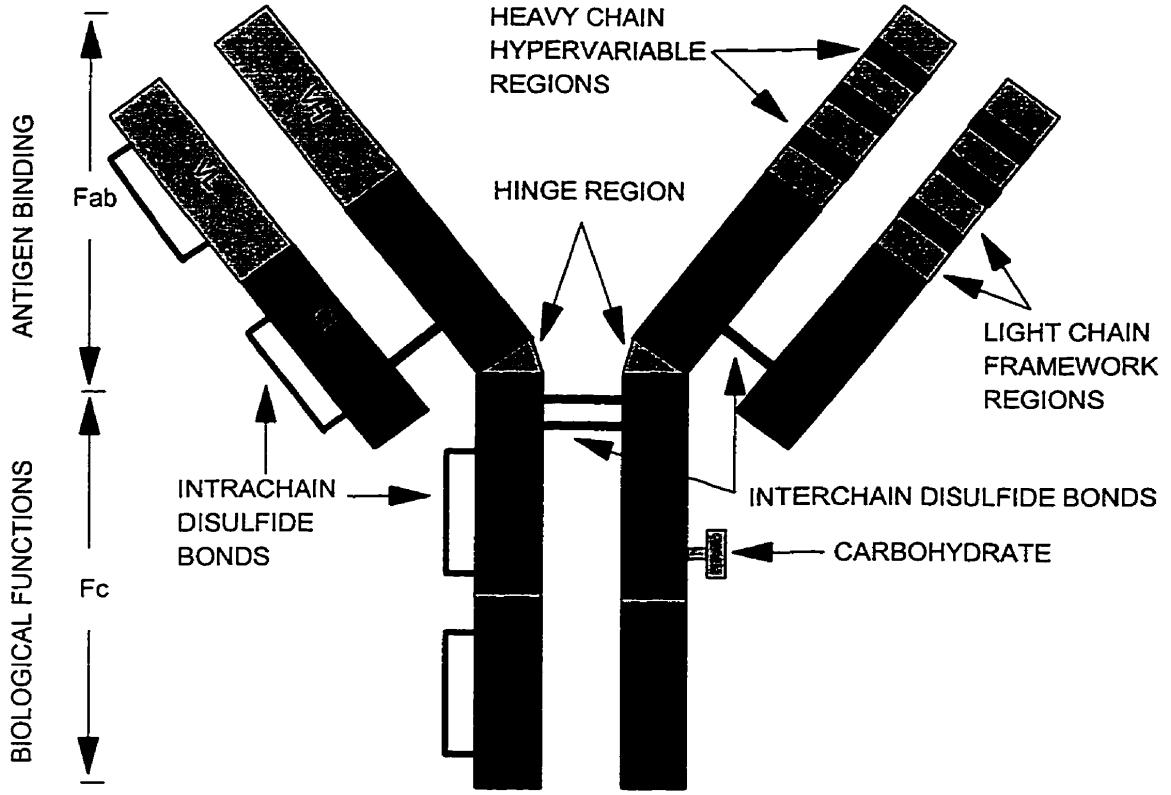
2.2.1 Immunoglobulin structure

The first experimental evidence of antibody mediated immunity was provided by Emil

von Behring and Shibasabura Kitasato in 1890 who successfully transferred immunity against diptheria to naive animals by infusing them with hyperimmune serum. They were not able to identify the active components of the immune serum and, therefore, named them 'serum antitoxins'. In 1939, A. Tiselius and E. A. Kabat discovered Ag binding proteins in the γ-globulin fraction of serum obtained from an immunized rabbit and named them immunoglobulins (cited by Porter, 1967; Edelman, 1973). The polypeptide structure of the Ab molecule, comprising two identical H and two identical light L chains held together by interchain disulfide bonds (Fig. 2.1), was established in the early 1960s by Porter (1967) and Edelman (1970). Later on, Wu and Kabat (1971) showed that some segments of the amino-terminal region of different Abs were relatively conserved while others exhibited great variation. They termed the latter hypervariable regions and proposed that these formed a contiguous structure at one end of the Ab and are involved in Ag recognition. Poljak *et al.* (1973) confirmed the proposed structure of the Ag binding site by X-ray crystallographic analysis of an Fab fragment of the antibody.

An Ig molecule consists of several interacting domains, each spanning approximately 100-110 amino acids. The L chain comprises two domains (V_L and C_L) whereas the H chain have either four (IgG, IgA and IgD) or five (IgM and IgE) domains. An intrachain disulfide bond between cysteine residues forms a loop of 60 amino acids within each domain. The amino-terminal domain of H and L chains of different Abs has significant variation in the amino acid composition and is, therefore, named the variable (V)-region (Dryer and Bennett, 1965). The V-region has three hypervariable regions which provide complementarity to the molecular shape of antigenic determinants or epitopes and, therefore, these are called

Fig. 2.1 Diagrammatic representation of an immunoglobulin molecule. Variable and constant-regions occur in heavy and light chain. Each variable-region is divided into three hypervariable regions known as complementarity determining regions (CDRs) and four relatively conserved regions known as framework regions (FRs); (Modified from Alt *et al.*, 1987).


ANTIGEN BINDING
BIOLOGICAL FUNCTIONS
Fab
Fc
VH
VL
HEAVY CHAIN
HYPERVARIABLE
REGIONS
HINGE REGION
LIGHT CHAIN
FRAMEWORK
REGIONS
INTRACHAIN
DISULFIDE
BONDS
INTERCHAIN DISULFIDE BONDS
CARBOHYDRATE

complementarity determining regions (CDRs). The relatively less variable V-region sequences, designated framework regions (FRs), provide the required configurational support (Wu and Kabat, 1971). The V-region is encoded by the juxtaposition of variable (V_H), diversity (D_H) and joining (J_H) gene segments for the H chain, and variable (V_L) and joining (J_L) gene segments for the L chain (Tonegawa, 1983). The carboxyl-terminal domains of both H and L chains represent the constant (C) region which determines the isotype or class of an Ig molecule. Five H chain isotypes, IgM, IgG, IgA, IgD and IgE exist among higher vertebrates which are encoded by Cμ, Cγ, Cα, Cδ and Cϵ germline genes, respectively. Minor differences in the amino acid sequences of the C_H region determine the subisotype or subclass of an Ig. The L chains are of two isotypes: kappa (κ) and lambda (λ). A single Ig molecule expresses only one isotype of L and H chain each. The H chain of IgG, IgA and IgD isotypes may contain an additional proline rich hinge region between the C_H1 and C_H2 domains. The hinge region adds flexibility to the Ag binding arms of the Ab molecule, enabling it to better engage an Ag (Davies and Metzger, 1983). The rearranged V_H-D_H-J_H-C_H1 and V_L-J_L-C_L units held together by interchain disulfide bond represent the Ag binding Fab fragment (Fig. 2.1). Two Fab fragments held together by an interchain disulfide bond constitute the $F(ab')_2$ fragment while the remaining part of the C-region is the Fc portion which mediates various biological functions such as complement activation and binding to cell surface Fc receptors (FcR) (Edelman, 1970; Davies and Metzger, 1983). The V-regions of the H- (V_H-D_H-J_H) and the L- (V_L-J_L) chains, held together by noncovalent interaction, constitute the Fv fragment. The V-region of the H chain alone is termed "dAb" (Winter and Milstein, 1991).

The amino acid sequence determines the primary structure of an Ig molecule. Two overlapping antiparallel β-pleated sheets, in which the hydrophobic amino acid residues are located in the interior and the hydrophilic amino acids are oriented outward, represent the secondary structure of Ig, called the immunoglobulin fold. The β-pleated sheet is stabilized by intrachain disulfide bonds and interchain hydrogen bonds, and is folded into a globular domain creating the tertiary structure of the Ig molecule. The globular domains of adjacent H and L chains interact to form the quaternary structure of the Ig (Amzel and Poljak, 1979).

2.2.2 Immunoglobulin function

The CDRs of the V-region participate in the formation of an Ag binding loop whereas the FRs constitute the β-pleated sheet structure of the Ab molecule. The CDRs are located on the loops connecting the β strands and, therefore, make contact with the Ag. The FRs provide support to the three dimensional structure of the Ag binding site and also contribute by making contact with the epitope in some instances (Wu and Kabat, 1971; Amzel and Poljak, 1979). Recent evidence suggest that conserved sequences in FR1 and FR2 interact with some B-cell superantigens (Silverman, 1997). The V-region of different Abs interact through an idiotypic network in which the V-region of one unique Ab will recognize and bind the V-region of a different Ab via complementarity molecular shape. Such idiotypic and anti-idiotypic interactions among Abs can regulate the immune response (Jerne, 1974).

The Fc portion of the C-region determines the biological effector functions mediated by the Ab molecule. The C_H2 domain of IgG and the C_H3 domain of the IgM isotype bear carbohydrate residues which interact with the C1q component of complement and initiate an Ab-mediated cascade of complement activation which recruits and activates phagocytic and

accessory cells, activates B-cells and mediates lytic pore formation resulting in complement-mediated lysis. The Ab acts as an opsonin by linking Ag to the phagocytic cells via FcR, thereby enhancing phagocytosis. The Ab is also involved in antibody-dependent cell-mediated cytotoxicity (ADCC) in which Ab-coated target cells are recognized by NK and other cells via FcRs resulting in cytotoxicity. The Abs also play a role in neutralization of toxins and viruses and also inhibit colonization and entry of potential pathogens into host cells. The crosslinking of IgE bound to the FcRs on the surface of basophils and mast cells mediates degranulation, an essential event in immune-mediated host defence against parasitic infections and allergic reactions. The FcRs specific for some Ig isotypes are present on the placental and mammary gland tissues that mediate transfer of maternal Abs to the fetus and the newborn (Davies and Metzger, 1983).

2.3 Phylogeny of immunoglobulins

Several immune recognition mechanisms have been identified in the invertebrate species. For example, the primitive marine sponge (*Microciona prolifera*) has the ability for allorecognition and, therefore, may reject tissues from other sponge species (Humphreys and Reinherz, 1994). The phagocytes present in the coelomic fluid of sea urchins exhibit cytotoxic killing (Bertheussen, 1979). Various species of sponges, nematodes, arthropodes, echinoderms and tunicates possess lectins which share sequence homologies with vertebrate C-type lectins or S-type lectins or pentraxins (C-reactive proteins and serum amyloid P) and interact with the carbohydrate ligands to act as agglutinins or opsonins (Vasta *et al.*, 1994). Most of the invertebrates have white blood cells in their fluid-filled body cavities which

probably represent the first blood cells that evolved from free living protozoan species (Horton and Ratcliffe, 1996). While lymphocyte-like cells exist in the jawless vertebrates, Igs and T cells occur in the jawed vertebrates (Du Pasquier, 1993). It has been proposed that adaptive immunity evolved in the jawed vertebrates to provide protection against increased frequency of physical injuries in the digestive tract (Matsunaga *et al.*, 1994). It is known that the major histocompatibility complex (MHC) molecules evolved earlier than the T cell antigen receptor (TCR; Marchalonis and Schluter, 1994) and the TCRs emerged earlier than the Ig (Matsunaga and Tormanen, 1990).

2.3.1 Immunoglobulin heavy chain genes

The available information on the organization and expression of Ig H chain genes in various classes of vertebrate species is as follows:

2.3.1.1 Agnatha (jawless fish)

Order: Myxiniformes (hagfish) and Petromyzontiformes (lamprey):

The hagfish and lamprey, the most ancient form of jawless vertebrates, do not have a thymus or spleen but possess lymphocyte-like cells which are produced in the gut-associated lymphoid tissues (GALT) (Du Pasquier, 1993). Earlier, an Ig-like heterodimer molecule was discovered in the hagfish (Kobayashi *et al.*, 1985; Varner *et al.*, 1991) which was later classified as a complement protein homologous to the C4 complement component of the higher vertebrates (Hanley *et al.*, 1992; Ishiguro *et al.*, 1992). Similarly, lamprey also has inducible humoral recognition molecules which do not share structural features with Ig (Litman *et al.*, 1993). It appears that these extant jawless vertebrates lack Ig homolog of

higher vertebrates and depend upon a complement-related humoral defence system.

2.3.1.2 Chondrichthyes (cartilaginous fish)

Subclass: Elasmobranchii (sharks, skates and rays) and Holocephali (ratfish):

The cartilaginous fish have thymus, spleen and B lymphocytes (Du Pasquier, 1993). The germline Ig genes of the horned shark (*Heterodontus francisci*) exist as closely linked clusters on the same or different chromosomes (Amemiya and Litman, 1991). Within these clusters, the Ig germline genes may be organized either as separated V-D1-D2-J gene segments or as VD-J and VDJ germline-joined forms. Each cluster of germline Ig genes has its own Cμ gene. (Hinds-Frey and Litman, 1986; Kokubu *et al.*, 1988b; Litman *et al.*, 1993). The horned shark V_H genes have been classified into two V_H gene families; V_H I and V_H II (Rast *et al.*, 1995). The H chain Ig genes of skate (*Raja erinacea*) and ratfish (*Hydrolagus colliei*) are organized either as unjoined germline clusters (V-D1-D2-J) or as germline joined VD1-D2J units in the skate and VDJ units in the ratfish (Hardings *et al.*, 1990b; Litman *et al.*, 1993; Rast *et al.*, 1995). The V_H genes of sandbar shark (*Carcharhinus plumbeus*) have been grouped into six V_H gene families (Shen *et al.*, 1996). The occurrence of joined germline gene clusters and the rearrangement of V, D and J genes within the clusters limit the combinatorial diversity in these species. The expressed VDJ genes of these species do not exhibit somatic hypermutations, probably due to the absence of germinal centres which are the sites for selection of high affinity Abs (Hinds-Frey *et al.*, 1993). The nurse shark (*Ginglymostoma cirratum*) V_H genes, associated with nurse shark antigen receptor (NAR), constitute a single V_H gene family. The nurse shark V, D and J genes undergo rearrangement as well as extensive somatic hypermutations (Greenberg *et al.*, 1995).

The IgM isotype in elasmobranchs is encoded by multiple germline Cμ genes, each associated with a different gene cluster (Rast *et al.*, 1995). Other H chain isotypes found among elasmobranchs include IgX (skate), NAR (nurse shark), IgNARC (new antigen receptor in cartilaginous fish) and IgW (sandbar shark). These isotypes differ from IgM in the number of C region domains. The IgX isotype is unique to skate and has only two C-region domains which share 60% nucleotide homology with the IgM isotype (Harding *et al.*, 1990b). The NAR isotype of the nurse shark has five C-region domains and is secreted as a dimer without light chains (Greenberg *et al.*, 1995). The IgNARC and IgW molecules have six C-region domains (Bernstein *et al.*, 1996; Greenberg *et al.*, 1996). Two amino terminal domains of IgNARC are homologous to skate IgX while the remaining four domains are homologous to the four carboxyl-terminal domains of NAR (Greenberg *et al.*, 1996). The homology between the V_H and C-regions of Cω is greater than that found between V_H and Cμ or V_L and $C_L$suggesting that Cω may be the primordial Ig gene during evolution whose V- and C-regions might have diverged from a common ancestral gene through gene duplication (Bernstein *et al.*, 1996).

2.3.1.3 Osteichthyes (Bony fish)

Subclass: Actinopterygii:

Teleost fishes: The organizational pattern of Ig genes of bony fish is similar to that found in mammals. The closely linked V_H genes of ladyfish (*Elops saurus*), a primitive teleost, are classified into two V_H gene families (Amemiya and Litman, 1990). The sequence analysis of channel catfish (*Ictalurus punctatus*) V_H genes has established the existence of, at least, seven different V_H gene families in this species (Ghaffari and Lobb, 1991; Warr *et al.*, 1991;

Ventura-Holman *et al.*, 1996). Nine germline J_H gene segments have been identified in channel catfish and the J_H locus is located only 1.8 kb upstream of the first C-region exon (Hayman *et al.*, 1993). The rainbow trout (*Oncorhynchus mykiss*), another teleost species, has eleven V_H gene families and six J_H gene segments (Roman and Charlemagne, 1994; Roman *et al.*, 1996). The expressed V_H genes of Atlantic cod (*Gadus morhua*) have been grouped into three V_H gene families (Bengten *et al.*, 1994). The functional V-region immunoglobulin genes as well as pseudogenes of goldfish (*Carassius auratus*) fall into three distinct V_H gene families (Wilson *et al.*, 1988; 1991).

The IgM H chain isotype is the only one detected, so far, among teleosts. The splicing pattern of the Cμ gene of teleosts is unique in that the transmembrane exon splices into the Cμ3 exon instead of the Cμ4 exon, thereby deleting the whole Cμ4 exon from the membrane-bound IgM (Kokubu *et al.*, 1988a; Wilson *et al.*, 1990).

Sublcass: Sarcopterygii:

Latimeria: The V_H genes of coelacanth (*Latimeria chalumnae*) belong to a single V_H gene family. The D_H genes are located only 190 nucleotides downstream of the most 3' end V_H gene, but are far apart from the J_H genes. This partial clustering of V_H and D_H genes is unique to Latimeria (Amemiya *et al.*, 1993).

2.3.1.4 Amphibia

Order: Anura:

Xenopus: The amphibians have better developed lymphoid organs. A primitive form of germinal centres, characterized by B-cell rich white pulp nodular areas surrounded by T-cell zones, is observed in the lymphoid organs of *Xenopus laevis* (Manning and Horton, 1982).

Amphibians have multiple V_H gene families which are organized at a single extended locus in the chromosomal DNA. As many as 11 V_H gene families, 17 D_H segments and 10 J_H segments, capable of generating significant combinatorial Ab diversity, have been identified in *Xenopus* (Haire *et al.*, 1990; Wilson *et al.*, 1992). The Ig H chain gene rearrangement in *Xenopus* occurs in two waves. First restricted Ab repertoire appears during larval life which exhibits short and less diverse CDR3H regions, without N-region insertions owing to the lack of the terminal deoxynucleotidyl transferase (TdT) enzyme expression. This is followed by the appearance of a diverse Ab repertoire during adulthood (Hadji-Azimi *et al.*, 1982, 1990; Du Pasquier and Schwager, 1991; Schwager *et al.*, 1991). *Xenopus* has all the elements needed for affinity maturation, but the absence of fully functional germinal centres results in an inefficient selection of the somatic mutants (Wilson *et al.*, 1992).

The Ig H chain isotypes identified in *Xenopus* include: IgM (Schwager *et al.*, 1988), IgX (Haire *et al.*, 1989) and IgY (Amemiya *et al.*, 1989). IgM and IgY isotypes predominate in the primary and secondary immune response, respectively (Du Pasquier, 1993).

Order: Caudata:

Axolotl: The V_H genes of the Mexican axolotl (*Ambystoma mexicanum*) have been classified into 11 gene families, each comprising 1 to 19 genes. Three V_H gene families, V_H7, V_H8 and V_H9, are expressed predominantly. The CDR1 contains several hot-spots that are potential sites for somatic hypermutations. Nine J_H gene segments have been identified, and of these two J_H genes, J_H6 and J_H9, are rarely expressed (Golub and Charlemagne, 1998). The germline J_H segments contribute significantly to the CDR3H region (Patel and Hsu, 1997).

The axolotl Cμ and Cυ genes, encoding IgM and IgY isotypes, share high nucleotide

homology with the corresponding genes of *Xenopus* (Fellah *et al.*, 1992, 1993).

2.3.1.5 Reptilia

Order: Crocodylia:

Caiman: Homolog of murine V_H S107 genes are identified in Caiman (*Caiman crocodylus*) which share 65-70% nucleotide homology with the corresponding mammalian V_H genes. The recombination signal sequences (RSS) of some germline V_H genes are noted to be embedded within the leader intron (Litman *et al.*, 1983, 1985).

Order: Testudines:

Turtle: The turtle (*Pseudodemys scripta*) has a single Ig H chain locus that comprises multiple V_H genes classified into 4 V_H gene families. The CDR3H length varies between 5 to 14 amino acids. Extensive variations observed in the FR4 of the rearranged VDJ genes suggest that large number of J_H segments exist in the genome of this species (Turchin and Hsu, 1996).

2.3.1.6 Aves

Chicken: Chicken H chain locus has only one functional V_H1 gene. Approximately 80-100 pseudogenes, some of which lack RSS and express fused D_H elements, are located towards the 5' end of the V_H1 gene. The chicken has 16 highly homologous D_H genes and only one J_H gene (Reynaud *et al.*, 1989, 1991; Masteller *et al.*, 1997). The D-D fusions contribute significantly to the CDR3H region diversity (Reynaud *et al.*, 1991; Masteller *et al.*, 1997). The progenitor B lymphocytes first appear in the yolk sac of the 5-6 day old embryo and undergo D_H-J_H rearrangement. Developing B lymphocytes migrate to the spleen and other lymphoid organs and undergo complete V(D)J rearrangement (Reynaud *et al.*, 1994). B-cells localize into the bursa of Fabricius around 10-15 days of embryonic life and diversify the

antibody repertoire through gene conversion (Reynaud *et al.*, 1987; Masteller *et al.*, 1997).

Chicken C-region genes have been cloned and sequenced for Cμ (Dahan *et al.*, 1983), Cγ (Parvari *et al.*, 1988) and Cα (Mansikk, 1992) H chains. A possible homolog of mammalian IgD has also been identified (Chen *et al.*, 1982).

2.3.1.7 Mammalia

Order: Marsupiala:

Opossum: The V_H genes of short-tailed opossum (*Monodelphis domestica*), related to mammalian group III V_H genes, have been classified into two V_H gene families. One V_H gene family, with a genomic complexity of 10 to 12 genes, is expressed predominantly (Miller *et al.*, 1998).

Order: Perissodactyla:

Horse: The expressed horse V_H genes are most homologous to sheep and cattle V_H genes that belong to the evolutionarily less conserved mammalian group I V_H genes. Five J_H gene segments are identified (Schrenzel *et al.*, 1997). The horse genome contains one copy each of Cμ (Schrenzel *et al.*, 1997), Cϵ and Cα genes (Butler, 1998). Four IgG subclasses, IgGa, IgGb, IgGc and IgG(T), have been identified in horse (Lunn *et al.*, 1995).

Order: Artiodactyla:

Swine: The expressed V_H genes of swine constitute a single V_H gene family which, similar to chicken and rabbit, belongs to mammalian group III V_H genes. The swine genome bears less than 20 germline V_H genes, and a single J_H gene segment (Sun *et al.*, 1994; Butler *et al.*, 1996). Two germline D_H genes (D_HA and D_HB) are preferentially expressed in 95% of VDJ rearrangements in fetal and neonatal piglets (Sun and Butler, 1996).

The structure of the swine Cμ is highly homologous to the human Cμ gene (Sun and Butler, 1997) and the Cδ gene does not exist in swine (Butler *et al.*, 1996). Eight subclasses of IgG are identified in swine, five of which have been characterized (Kackovics *et al.*, 1994).

Camel: Three IgG subclasses are known to exist in camel (*Camelus dromedarius*). The IgG1 subclass expresses a typical four domain structure and comprises two light and two heavy chains. However, the IgG2 and IgG3 subclasses lack the C_H1 domain and exist as homodimers of two heavy chains only (Hamers-Casterman *et al.*, 1993). Analysis of camel V_H genes from antibodies lacking the light chain, revealed remarkable structural differences as compared to other species. For example, the amino acids Leu45 and Leu11, which interact with the C_H1 domain, were substituted by Arg or Cys and Ser, respectively. These substitutions increase the solubility of isolated camel V_H domains. The CDR3H region of camel antibodies varies between 10 and 24 amino acids (Muyldermans *et al.*, 1994). The camel V_H genes belong to the mammalian group III V_H genes (Nguyen *et al.*, 1998).

Sheep: The sheep ileal Peyer's patch (IPP) is considered to be the primary B-cell lymphoid organ. The progenitor B-cells colonize IPPs around 110 days of fetal life, divide extensively and produce a large population of surface IgM^+ B lymphocytes. The lymphopoiesis in IPPs continues until 3 months after birth (Reynolds, 1987). A limited number of sheep germline and expressed V_H genes analysed so far belong to a single V_H gene family related to the murine Q-52 and human V_H4 gene families of the mammalian group I V_H genes. Based on Southern blot analysis, the genomic complexity of sheep V_H genes seems to be restricted to approximately 10 members (Dufour *et al.*, 1996). The sheep genome contains six germline J_H genes of which two (J_H1 and J_H2) are functional and the remaining four are pseudogenes.

The J_H1 gene is expressed in >90% VDJ rearrangements (Dufour and Nau, 1997).

The C_H-region genes characterized in sheep include: Cμ (Patri and Nau, 1992), Cγ1 (Foley and Beh, 1989), Cγ2 (Clarkson *et al.*, 1993) and Cε (Engwerda *et al.*, 1992).

Order: Rodentia:

Mouse: The murine H chain genes have been mapped to chromosome 12 and approximately 100-500 germline V_H genes are classified into 15 V_H gene families (Brodeur and Riblet, 1984; Kofler *et al.*, 1992; Mannville *et al.*, 1996). The V_H gene families range in genomic complexity from one member in V_H12 to an estimated 50-500 members in the largest V_HJ558 gene family (Kofler *et al.*, 1992). According to Meek *et al.* (1990) and Tutter *et al.* (1991), the relative position of V_H gene families on chromosome 12 is: 5'-(J558-3609)-V_H10-J606-V_H12-3609N-36-60-VGAM-3660-S107-VGAM-V_H11-3660-V_H11-X24-SM7-S107-(Q52-7183)-3'. The murine V_H gene families are classified into three major protein groups (Tutter and Riblet, 1989; Kofler *et al.*, 1992; Honjo and Matsuda, 1995): group I (V_H3609, V_H3660, V_HQ-52 and V_H12), group II (V_HJ558, V_HVGAM3-8, V_H14) and group III (V_H7183, V_HS107, V_HJ606, V_HX24, V_H10, $V_H11$and V_H13). The 3' V_H families, V_H7183 and V_HQ52, are expressed preferentially in the antibody repertoire of fetal and neonatal mice, while stoichastic V_H gene usage is noted in the adult antibody repertoire (Schulze and Kelsoe, 1987; Jeong and Teale, 1988; Yancopoulos *et al.*, 1988; Malynn *et al.*, 1990; Kaushik and Lim, 1996).

All the murine C_H genes have been characterized which are organized in the genome as: 5'-Cμ-Cδ-Cγ3-Cγ1-Cγ2b-Cγ2a-Cε-Cα-3' (Shimizu *et al.*, 1982).

Order: Lagomorpha:

Rabbit: The rabbit H chain locus has one Cμ, one Cγ, one Cε and 13 Cα functional genes,

but no Cδ gene (Burnett *et al.*, 1989; Knight and Winstead, 1997). The Cα4 gene occupies the most 5' position in the genome and is preferentially expressed in tonsils, lungs and Peyer's patches, whereas 11 of the remaining 12 Cα genes predominate in the gut, appendix, mesenteric lymph nodes and mammary tissues (Knight and Winstead, 1997). Approximately 100 germline V_H genes exist in the rabbit genome, of which 50% are pseudogenes. All V_H genes belong to a single gene family related to the mammalian group III V_H genes (Knight and Becker, 1990; Tutter and Riblet, 1989). Five germline J_H and 11 D_H gene segments have been identified (Becker *et al.*, 1989). Four V_H genes are expressed in the antibody repertoire with V_H1 being the most utilized (80%; Knight and Becker, 1990). Similarly, D_H2a, D_H2b, D_H3, D_H5 and J_H4 gene segments are expressed preferentially (Friedman *et al.*, 1994; Knight and Winstead, 1997). B-cell repertoire in rabbit is generated via somatic hypermutations and gene conversions occurring in the germinal centres of the appendix of neonatal animals and is maintained in adult animals by self-renewing B lymphocytes (Becker and Knight, 1990; Weinstein *et al.*, 1994). Recent evidence, however, suggests that some B lymphocytes in adult animal diversify their antibody repertoire in the splenic germinal centres (Sehgal *et al.*, 1998).

Order: Primates:

Human: The human H chain locus is located on chromosome 14. Based upon the criteria of ≥80% nucleotide homology, about 100 human germline V_H genes have been grouped into seven distinct V_H gene families (Berman *et al.*, 1988; Van Dijk *et al.*, 1993). The human V_H genes are further classified into three protein subgroups: group I (V_H2, V_H4 and V_H6), group II (V_H1 and V_H5) and group III (V_H3). The germline V_H genes are followed by 30 D_H gene segments and further downstream 9 J_H gene segments exist of which only six are functional

(Kabat *et al.*, 1991).

The human germline C_H genes are organized in the genome as: 5'-Cμ-Cδ-Cγ3-Cγ1-Cϵ2-Cα1-ψCγ-Cγ2-Cγ4-Cϵ1-Cα2-3' (Honjo and Matsuda, 1995).

2.3.2 Immunoglobulin light chain genes

The diversity and organization of L chain genes is more complex than the H chain genes. Two L chain isotypes, κ and λ, exist in vertebrates. The genes encoding κ- and λ-L chains are located on different chromosomes. In a species, both κ- and λ-L chains may contribute significantly to the expressed antibody repertoire or one or the other isotype may predominate. The organization and expression of L chain genes across vertebrate classes is discussed below.

2.3.2.1 Chondrichthyes (cartilaginous fish)

Subclass: Elasmobranchii (sharks, skates and rays) and Holocephali (ratfish):

Similar to the H chain locus, the V_L, J_L and C_L genes are arranged in clusters (Rast *et al.*, 1994). Based on nucleotide homology and clustering patterns, three distinct types of L chain genes are noted in the cartilaginous fish. The type I L chain genes are organized as unjoined V_L, J_L and C_L clusters in horned shark, *Heterodontus francisci* (Rast *et al.*, 1994), and as germline joined V_L-J_L units in little skate, *Raja erinacea* (Anderson *et al.*, 1995). The type II L chain genes exist as V_L-J_L germline-joined genes and are predominantly expressed in the cartilaginous fish, such as sandbar shark (*Carcharhinus plumbeus*), horned shark, little skate and spotted ratfish, *Hydrolagus colliei* (Hohman *et al.*, 1992, Rast *et al.*, 1994). Two distinct families of V_L genes, that share 61% nucleotide homology, are identified in the spotted ratfish (Rast *et al.*, 1994). The type III L chain genes, first reported in nurse shark

(*Ginglymostoma cirratum*), share 60% amino acid homology with the mammalian V_κ genes (Greenberg *et al.*, 1993). The germline unjoined type III genes are also noted in the horned shark (Rast *et al.*, 1994). Phylogenetic analysis has revealed that type I and II genes do not cluster with κ- and λ-L chain genes of other vertebrates. But, type III V_L genes cluster with the mammalian κ-L chain genes suggesting that chondrichthyan type III V_L genes are members of the κ-L chain family and that the κ-L chains probably diverged within or prior to the evolution of elasmobranchs (Rast *et al.*, 1994).

2.3.2.2 Osteichthyes (Bony fish)

Subclass: Actinopterygii:

Teleost fishes: The Ig L chain genes isolated from Atlantic cod (*Gadus morhua*) and rainbow trout (*Oncorhynchus mykiss*) share 55% amino acid homology in the C_L region but cannot be classified either as κ- or λ-L chains (Daggfeldt *et al.*, 1993). Two L chain isotypes, IL1 and IL2, have been described in the rainbow trout. The C_L regions of these isotypes share 29% amino acid homology and associate with different V_L and J_L gene families (Partula *et al.*, 1996). Two L chain isotypes have also been identified in channel catfish (*Ictalurus punctatus*). The L chain gene segments are arranged in clusters. Within each cluster, the V_L segments are located in opposite transcriptional polarity upstream of a single J_L and C_L element and, thus, undergo rearrangement by inversion. The second type of L chain gene(s) shows high homology with mammalian κ-L chain genes (Ghaffari and Lobb, 1993; 1997).

2.3.2.3 Amphibia

Order: Anura:

Xenopus: Three L chain isotypes, ρ (L I), σ (L II) and type III, are identified in *Xenopus*

laevis. Interestingly, the ρ-L chains associate preferentially with the IgY H chains and σ-L chains associate with the IgM H chains (Rast *et al.*, 1995). The ρ locus comprises a single family of several Vρ genes (Stewart *et al.*, 1993). A single Cρ and five Jρ segments share high nucleotide homology with the corresponding mammalian κ-L chain genes (Zezza *et al.*, 1992). The σ-L chain gene locus has two sets of genetic element: Vσ1, Jσ1 and Cσ1; and Vσ2, Jσ2 and Cσ2. These genes undergo rearrangement within the same set. The *Xenopus* genome has multiple copies of Vσ1 genes but only a few Vσ2 genes (Schwager *et al.*, 1991). The *Xenopus* type III genes are the most diversified and include six families of V_L genes, two J_L and two C_L elements. Interestingly, the type III genes are closely related to the λ-L chain genes of higher vertebrates (Haire *et al.*, 1996).

2.3.2.4 Aves

Chicken: The chicken exclusively uses λ-L chains in the antibody repertoire. Similar to the H chain locus, the chicken λ-L chain locus has only one functional $V_\lambda 1$ gene. Approximately 25 V_λ pseudogenes are located in alternate transcriptional polarities upstream of the $V_\lambda 1$ gene. Further, a single J_λ and C_λ gene, each, exists in the chicken genome (Reynaud *et al.*, 1985; 1987).

2.3.2.5 Mammalia

Order: Perissodactyla:

Horse: Approximately 93% of horse antibodies express λ-L chain. The expressed V_λ genes share >88% nucleotide homology amongst themselves. Four C_λ genes exist in the horse genome, of which three are expressed in the antibody repertoire. Southern blot analysis has revealed a limited number (20-30) of V_λ germline genes in the horse genome (Home *et al.*,

1992).

The organization of the horse κ-L chain locus is similar to that in mice and humans. Both germline and expressed V_κ sequences have been characterized. The horse genome contains as many as twentyfive V_κ, five J_κ and one C_κ genes (Ford *et al.*, 1994).

Order: Artiodactyla:

Swine: Swine, similar to humans, express κ- and λ-L chains at 60% and 40% frequencies, respectively (Butler, 1998). The cDNAs encoding swine κ- and λ-L chains have been cloned. Swine C_κ and C_λ genes share 64% and 74% amino acid homology with the corresponding genes of human, whereas swine J_κ and J_λ gene segments show 80% and 89% homology, respectively, with the consensus human J_κ and J_λ genes (Lammers *et al.*, 1991).

Sheep: Sheep predominantly expresses λ-L chain (>95%) in the expressed antibody repertoire. Approximately, 60 to 90 V_λ genes, one-fifth of which are pseudogenes, are classified into six distinct V_λ gene families. The V_λI gene family is further sub-divided into four sub-families. Two V_λI genes, V_λ5.1 and V_λ16.1, predominate in the expressed antibody repertoire (Reynaud *et al.*, 1997). The λ-L chain repertoire in sheep is diversified via non-antigen-dependent somatic hypermutations in the IPPs during fetal and neonatal life. A single J-C unit is expressed predominantly, though multiple C_λ genes exist in the sheep genome (Reynaud *et al.*,1991; Reynaud *et al.*,1995).

The sheep V_κ and C_κ regions share 76% and 58% amino acid homology with the corresponding human genes, respectively. Southern hybridization has revealed a single C_κ gene and three to five V_κ genes at the sheep κ-L chain locus (Foley and Beh, 1992).

Order: Rodentia:

Mouse: Murine antibodies predominantly express κ-L chain (95%). The κ-L chain locus, located on chromosome 6, comprises 200-300 V_κ genes followed by five J_κ genes and a single C_κ gene. The murine V_κ gene segments have been classified into 19 V_κ gene families. One of the J_κ genes is a pseudogene (Kofler *et al.*, 1992).

The murine λ-L chain locus is located on chromosome 16 and comprises four recombination units: ($V_\lambda 1$, $J_\lambda 1$, $C_\lambda 1$), ($V_\lambda 2$, $J_\lambda 2$, $C_\lambda 2$), ($V_\lambda 1$, $J_\lambda 3$, $C_\lambda 3$) and ($V_\lambda x$, $J_\lambda 2$, $C_\lambda 2$). The V_λ genes differ by only a few amino acids. The VJC rearrangements are usually restricted within the cluster (Sanchez *et al.*, 1991).

Order: Lagomorpha:

Rabbit: The κ-L chains predominate (80-90%) in the expressed antibody repertoire of the rabbit (Dray *et al.*, 1963). Two C_κ genes, $C_\kappa 1$ and $C_\kappa 2$, exist in the rabbit genome which undergo rearrangement with separate J_κ genes (Hole *et al.*, 1991).

The rabbit λ-L chain locus has as many as eight C_λ genes, of which six have been cloned. The $C_\lambda 1$, $C_\lambda 2$, $C_\lambda 3$ and $C_\lambda 4$ genes are pseudogenes (Duvoisin *et al.*, 1986), whereas $C_\lambda 5$ and $C_\lambda 6$ genes are functional (Hayzer *et al.*, 1987). At least five V_λ genes exist in the rabbit genome, of which two are pseudogenes (Hayzer and Jaton, 1989).

Order: Primates:

Human: The κ-L chains are found in 60% of human antibodies. The κ-L chain locus on chromosome 2 comprises 76 V_κ genes followed by 5 J_κ genes and one C_κ gene. However, only 32 V_κ genes are known to be functional (Klein and Zachau, 1995). Based upon protein sequence, human V_κ genes are classified into four subgroups (V_κI, II, III and IV) (Kabat *et al.*, 1991).

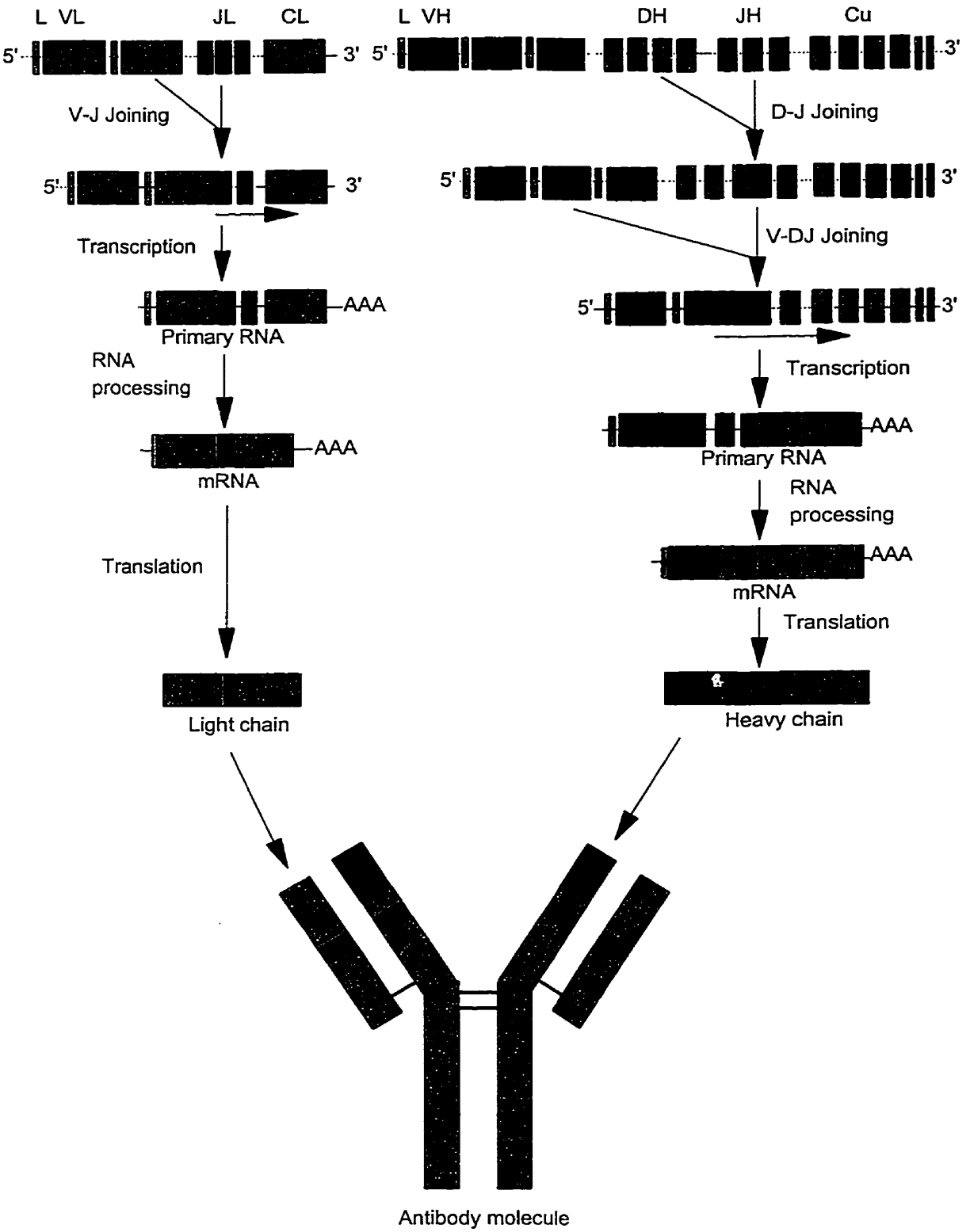
The human λ-L chain locus, located on chromosome 22, comprises approximately 70 V_λ genes. Based upon the criterion of >80% amino acid homology within the FR1 region, the human V_λ genes are divided into six (V_λ I-VI) subgroups (Kabat *et al.*, 1991), but additional V_λ subgroups (V_λVII-X and V_λT1) have now been identified (Blomberg *et al.*, 1995). The J_λ and C_λ genes are organized into seven J_λ-C_λ clusters. The $JC_\lambda4$, $JC_\lambda5$ and $JC_\lambda6$ genes are identified as pseudogenes (Blomberg *et al.*, 1995).

Overall, studies in phylogenetically distant species clearly demonstrate differences in the structure and organization of V_H and V_L genes. Further, some species, like nurse shark and camel can produce Abs as homodimers of H chains only. Obviously, these observations provide clues to different strategies that may be used to generate Ab diversity across species.

2.4 Immunoglobulin variable-region gene rearrangement

The process of V(D)J joining has been studied extensively in mice and humans. Both H and L chain V-region germline genes undergo rearrangement using similar site-specific recombination mechanisms. During H chain gene rearrangement, one of the available D_H genes joins with one of the downstream J_H genes, followed by the joining of one of the upstream V_H genes to the pre-assembled D_H-J_H segment (Fig. 2.2). However, some exceptions to this general rule are known to exist. As for L chain, the V_L genes directly recombine with downstream J_L genes. These somatic recombination events occur during the early stages of B-cell differentiation in the fetal liver and adult bone marrow (Tonegawa, 1983). The variable-region gene segments are flanked by recombination signal sequences (RSS) that are composed of conserved palindromic heptamer and A-T rich nonamer

Fig. 2.2 Immunoglobulin H and L chain gene rearrangement and expression. The H chain gene rearrangement begins with the joining of one of the available D_H genes with one of the downstream J_H genes, followed by the joining of one of the upstream V_H genes with the pre-assembled D_H-J_H segment. The L chain$_L$V gene directly recombine with one of the downstream J_L genes. The rearranged H (V_H-D_H-J_H-C_H) and L (V_L-J_L-C_L) chain genes are transcribed and processed to yield functional mRNAs which are translated independently into H and L chain polypeptides. H and L chains join together to form a monomeric subunit of the Ig molecule (Modified from Okada and Alt, 1995).


L VL
JL
CL
5'
3'
V-J Joining
Transcription
AAA
Primary RNA
RNA
processing
mRNA
Translation
Light chain
L VH
DH
JH
Cu
D-J Joining
V-DJ Joining
Heavy chain
Antibody molecule

sequences separated by a less conserved spacer sequence of 12 or 23 bp (Early *et al.*, 1980; Ramsden *et al.*, 1994). The V_H and J_H gene segments have 23 bp spacers, whereas $_HD$ segments are flanked by 12 bp spacers. The V_κ and J_κ genes are flanked by RSS separated by 12 and 23 bp spacers, respectively, while the V_λ genes have a 23 bp spacer and the J_λ genes have 12 bp spacers. During the process of V(D)J joining, the segment with a 12 bp (one turn) spacer can only recombine with those gene segments that have a 23 bp (two turn) spacer (Sakano *et al.*, 1980; Alt *et al.*, 1984). The V(D)J recombination process is initiated by the recognition of RSS by the lymphocyte-specific recombination-activation genes, RAG1 and RAG2. The recombination activity requires the expression of both RAG1 and RAG2 genes (Oettinger *et al.*, 1990; Shinkai *et al.*, 1992). The RAG1 and RAG2 proteins bind two RSS and bring them into close juxtaposition. Cleavage occurs in two steps, with a nick first introduced adjacent to the heptamer to expose a 3'-hydroxyl group on the coding side, followed by a direct nucleophilic attack of the 3'-hydroxyl group on the opposite DNA strand, thereby, generating 5'-phosphorylated signal ends and covalently sealed hairpin coding ends. In the second phase, two signal ends are joined to form a signal joint whereas the coding ends are processed to form an imprecise coding joint (McBlane *et al.*, 1995). Several components of the DNA strand break repair mechanisms, such as a double strand DNA-end binding Ku (Ku86/Ku70) autoantigen (Taccioli *et al.*, 1994) and a 450 kDa catalytic subunit of DNA-dependent protein kinase (DNA-PK_{CS}) (Gottlieb and Jackson, 1993) are implicated in the resolution of the coding ends. The mutations resulting in truncation of DNA-PK_{CS} do not permit V(D)J rearrangements and block B and T cell lymphopoiesis leading to severe combined immune deficiency (SCID) in mice (Blunt *et al.*, 1995) and Arabian horses (Shin

et al., 1997). When two recombining RSS are in the opposite transcriptional orientation, the V(D)J recombination occurs by inversion and the intervening signal joint sequences are retained in the chromosome. However, when two genes are in the same transcriptional orientation, joining occurs by a deletional method as the intervening sequences are deleted (Gauss and Lieber, 1992).

The process of V(D)J recombination generates antibody diversity via juxtaposition of separated germline genes; however, other factors also contribute to diversity in the Ag-combining CDR3 region of antibodies. Imprecise joining of the gene segments during recombination, due to flexibility at the joining site, may result in either addition or deletion of nucleotides at the joining regions (Max *et al.*, 1979). The template-independent G and C rich N-region nucleotides are added randomly during the VDJ rearrangement process by terminal deoxynucleotidyl transferase (TdT) enzyme (Alt and Baltimore, 1982; Landau *et al.*, 1987). The N-region additions are not detected in the VDJ rearrangements of fetus and neonatal animals owing to the lack of TdT expression in the lymphoid organs at early stages of B cell development (Lafaille *et al.*, 1989; Feeney, 1990). The P-nucleotides, palindromic to the terminal nucleotides of the coding segments, may be included if the hairpin structure formed by the complimentary RSS base pairing is nicked near the bottom, leaving a short single strand sequence (Lafaille *et al.*, 1989; Lieber, 1992). Thus, significant diversity is generated as a result of imprecise joining as well as addition of N or P nucleotides.

During B-cell development, the process of VDJ rearrangement is followed by the expression of a functional H chain protein that prevents H chain rearrangement at the second allele (allelic exclusion) and serves as a signal for κ-L chain rearrangement to begin. If

rearrangements at both the κ alleles are non-productive, the λ-L chain genes undergo rearrangement, though, this is not a requirement for rearrangement at the λ-L chain locus (Berg *et al.*, 1990; Chen *et al.*, 1993). The phenomenon of allelic exclusion prevents the expression of both alleles in a single B-cell (Yancopoulos and Alt, 1986; Alt *et al.*, 1987). The *cis*-acting enhancer elements activate the transcription of V-D-J-C and V-J-C rearrangements from the promoter sequences located upstream of the V_H and V_L leader sequences. The RNA-processing enzymes remove the introns from the primary RNA transcripts and the mRNA is transported to the cytoplasm. The leader sequence guides the mRNA into the rough endoplasmic reticulum for translation into a polypeptide chain. Both H and L chain polypeptides are transported to the cell surface, via the Golgi apparatus, and form a functional antibody molecule. The differential splicing of H chain precursor RNA generates mRNA which is translated into either secreted or membrane-bound antibodies. The IgM isotype (in some species IgD also) is expressed on the surface of mature B-cells. However, upon antigenic stimulation the rearranged VDJ genes recombine with a different downstream C_H gene (antibody class-switching) resulting in the production of other antibody isotypes. In addition, the affinity of an antibody for a particular antigen is selectively increased through somatic hypermutations (affinity maturation) during the course of class-switching in the germinal centres of the secondary lymphoid organs (Alt *et al.*, 1987; Kuby, 1994).

2.5 Antibody diversification across species

The V(D)J rearrangement processes are common to all vertebrates but the germline sequence diversity varies considerably across species. In mouse and human, a large pool of

divergent germline V-genes (Kabat *et al.*, 1991), grouped into several V_H and V_κ or V_λ gene families, generate significant combinatorial diversity via V(D)J rearrangements. In contrast, limited combinatorial diversity is generated in species like chicken (Reynaud *et al.*, 1989), rabbit (Becker and Knight, 1990), pig (Sun *et al.*, 1994) and sheep (Dufour *et al.*, 1996) because of a small number of functional V-genes. These species employ other strategies to generate antibody diversity essential to normal immune function and host defense. For example, gene conversion mechanisms are involved in the diversification of pre-immune antibody repertoire in the bursa of Fabricius of chickens (Reynaud *et al.*, 1989) and in the appendix of the rabbit (Weinstein *et al.*, 1994). Somatic hypermutations, occurring in ileal Peyer's patches without prior exposure to exogenous antigens, generate diversity in the primary antibody repertoire of sheep (Reynaud *et al.*, 1991; 1995). Nevertheless, single point somatic hypermutations occurring upon exposure to antigen are common to these species and selection of clones secreting high affinity antibody occurs in the germinal centre.

2.6 The bovine immune system

In this section, the information relevant to the development of antibody-based immunity is reviewed.

2.6.1 Ontogeny

The thymus is the first lymphoid organ to develop in the bovine fetus followed by progressive development of other lymphoid organs and cells of the immune system (Table 2.1). The bovine fetal blood lymphocytes can respond to pokeweed mitogen after 90 days of gestation and to concanvalin A and phytohemagglutinins at 121 days of gestation. The

Table 2.1. Progressive development of the organs and cells of the immune system in the bovine fetus.

Organs/cells of the immune system	Age of the fetus (days)
Thymus	42[a]
Blood lymphocytes	45[b]
Bone marrow/spleen	55[a]
IgM^+ B lymphocytes	59[b]
Peripheral lymph nodes	60[a]
Serum complement component C3	90[c]
Mesenteric lymph nodes	100[a]
Serum IgM	130[d]
Blood granulocytes	130[c]
IgG^+ B lymphocytes	145[b]
Serum IgG	145[a]
IgA^+ B lymphocytes	180[e]
Ig^+ B lymphocytes in Peyer's patches	180[e]
IgM^+ B lymphocytes in tonsils	240[e]

[a]Schultz *et al.* (1974), [b]Schultz *et al.* (1973), [c]Osburn *et al.* (1982), [d]Schultz *et al.* (1971), [e]Ishino *et al.* (1991).

antibodies against parainfluenza-3 virus, parvovirus and blue tongue virus have been detected in the bovine fetus at 120, 140 and 145 days of gestation, respectively (Osburn *et al.*, 1982). The IgM^+ B-cells are not detected in bone marrow of the fetus and neonatal calf suggesting that the bone marrow may not be the site of B-cell lymphopoiesis. The liver and spleen of the fetus and the IPPs of neonatal animals are likely to act as the primary lymphoid organ in cattle (Meyer *et al.*, 1997). The newborn calf is hypogammaglobulinemic because the syndesmochorial type of placenta prevents the transfer of maternal antibodies to the fetus. The immune system of the newborn calf is capable of responding to antigens encountered in the environment, but the immune response has a prolonged lag period and produces small amounts of antibodies. Therefore, passive transfer of maternal antibodies, via colostrum, provides immunological resistance during early neonatal life (Tizard, 1996).

2.6.2 Lymphocyte subpopulations

The development of monoclonal antibodies that recognize cattle leukocyte antigens has allowed characterization of bovine lymphocyte subpopulations. B lymphocytes constitute a significantly low proportion of blood lymphocytes in the fetus (1%) and newborn calves (6%) as compared to adult cattle (21.7-34%); (Senogles *et al.*, 1979; Duhamel *et al.*, 1987; Wyatt *et al.*, 1994). In healthy cattle, 5-35% of circulating B lymphocytes and 3-9% of splenic B lymphocytes express CD5 antigen. However, $CD5^+$ B lymphocytes may constitute up to 80% of blood B-cells in *Trypanosoma congolense* infected cattle and secrete non-parasite-specific IgM antibodies (Naessens and Williams, 1992). Whether CD5 antigen simply reflects a differentiation marker in cattle, similar to humans, or provides a marker for a unique lineage of B cells remains an open question.

In adult cattle, the frequency of circulating T lymphocytes expressing various cell surface markers are as follows: $CD2^+$-52-66%, $CD4^+$-30-32%, $CD8^+$-15-29% and γδ T cell receptor-3-6% (Wilson *et al.*, 1996). The γδ T lymphocytes comprise 26-40% of the circulating T cells in 1-4 week old calves (Clevers *et al.*, 1990; Wilson *et al.*, 1996). It has been proposed that the γδ T lymphocytes in neonatal ruminants provide non-specific cellular immunity until the maturation of αβ T cell system (Hein and Mackay, 1991). The γδ T lymphocytes are also prominent in the skin and the gastrointestinal tract of adult cattle but are rare in the thymus, spleen and lymph nodes. Approximately 82% of skin T cells are localized in the dermis, of which 44% express γδ TCR (Davis *et al.*, 1993; Wilson *et al.*, 1996; Hein and Dudler, 1997). The circulating bovine γδ T lymphocytes predominantly express cell surface WC1 antigen (Workshop cluster 1), a member of the scavenger receptor cysteine-rich domain family, not yet identified in humans and mice (Morrison and Davis, 1991; Wijngaard *et al.*, 1992). A majority of the γδ T lymphocytes express a $CD2^-CD3^+CD5^+CD4^-CD8^-$ phenotype (Davis *et al.*, 1993; Hein and Dudler, 1997). A small population of circulating γδ T lymphocytes (2-9%) and a substantial proportion of splenic γδ T lymphocytes (10-38%) do not express WC1 but express bovine CD2 (Wyatt *et al.*, 1994). Approximately 9.4% of blood lymphocytes in adult cattle do not express either B or T cell antigen receptors and represent the "null cell" population (Duhamel *et al.*, 1987). The frequency of blood monocytes in the bovine fetus varies between 1 to 5% (Senogles *et al.*, 1979) but increases to 15-31% in adult cattle (Park *et al.*, 1991).

2.6.3 Immunoglobulins

Cattle have IgG, IgM, IgA and IgE immunoglobulin isotypes (Butler, 1998), but

similar to sheep and swine, cattle B-cells do not express surface-IgD (Butler *et al.*, 1996; Naessens, 1997). The bovine IgG isotype is further subdivided into three subclasses, IgG1, IgG2 and IgG3 (Butler, 1995, 1998). IgG1 is the predominant serum Ig (11.2 mg/ml) followed by IgG2 (9.2 mg/ml), IgM (3.05 mg/ml) and IgA (0.37 mg/ml) (Butler, 1995, 1998). IgG1 also constitutes a major proportion of colostrum Ig (46.9 mg/ml) as a consequence of selective transport of the serum IgG1 to mammary gland via IgG1-specific transport receptors (Brandon *et al.*, 1971). Serum IgA predominantly exists in dimeric form in contrast to monomeric IgA in humans (Duncan *et al.*, 1972). Unlike other species, the transport of serum IgA into the mammary gland of cows is not efficient (Butler *et al.*, 1986). Serum IgM is also poorly transported into the mammary glands of cows (Frenyo *et al.*, 1987). It has been suggested that colostral and milk IgA and IgM are synthesized locally in the mammary glands of cows (Butler, 1998).

2.6.4 Immunoglobulin genes

2.6.4.1 Heavy chain constant-region genes

The bovine heavy chain locus spans approximately 400 kb from Cμ to Cα (Rabbani *et al.*, 1997). The Cμ and Cγ regions are separated by 6 kb while the Cα and Cϵ genes are 15 kb apart in the germline DNA (Knight and Becker, 1987). Bovine Cγ, Cϵ and Cα genes have been expressed as transfectomas to produce bovine-murine chimeric antibodies (Knight *et al.*, 1988). The bovine Cγ genes have been mapped to chromosome 21q24 (Gu *et al.*, 1992). The nucleotide sequence of bovine Cγ1 and Cγ2 has been described by Symons *et al.* (1989). Each of these genes is encoded by four exons corresponding to three C_H domains and a hinge region. The hinge region of IgG2 is shorter than that of IgG1. Two allotypic variants

of IgG2, IgG2a and IgG2b, are identified which differ significantly in the hinge region and C_H3 domain (Butler and Heyermann, 1986; Heyermann and Butler, 1987). The most N-terminal disulfide bridge is absent in IgG2a and its C_H3 domain has an immunodominant epitope which results in biased detection of IgG2a by various polyclonal and monoclonal antibodies (Butler *et al.*, 1994). The hinge region of IgG3 is suggested to be encoded by two exons, a remarkable difference from IgG1 and IgG2. Two alleles of IgG3, IgG3a and IgG3b, are reported (Rabbani *et al.*, 1997). The IgG3b differs from IgG3a by 6 amino acid substitutions in the coding region and a 84 bp insertion in the intron located between the C_H2 and C_H3 exons (Rabbani *et al.*, 1997). The IgG3b shares high nucleotide homology with a previousely described Cγ pseudogene (Symons *et al.*, 1989). Allotypic variants with minor structural differences have also been reported for IgG1 (Rapacz and Hasler-Rapacz, 1972; Wegrezyn and Wegrezyn, 1978; Jackson *et al.*, 1992; Kacskovics and Butler, 1996).

The existence of bovine IgM allotypes has been reported with isotype-specific monoclonal anti-IgM antibodies that recognize different epitopes on bovine IgM antibodies (Naessens *et al.*, 1988; Williams *et al.*, 1990). Recently, germline Cμ exons have been characterized from the Brown Swiss breed of cattle and it has been shown that, similar to other mammalian species, the TM1 exon is spliced into the Cμ4 exon in the membrane form of bovine IgM antibody (Mousavi *et al.*, 1998).

The bovine Cα gene exists in, at least, two allelic forms and bovine IgA shares high amino acid homology with swine IgA (Brown *et al.*, 1997).

The bovine Cϵ, similar to other species, has four exons and shares high nucleotide homology (87%) with the sheep Cϵ gene. Allelic polymorphism has also been demonstrated

at the bovine Cϵ-locus (Mousavi *et al.*, 1997).

2.6.4.2 Heavy chain variable-region genes

Based on Southern blot analysis of bovine genomic DNA, the homologs of the murine V_H11 (Saini *et al.*, 1996), V_H7183, V_H S107 and V_H 36-60 genes are detected in the bovine genome (Tutter and Riblet,1989). However, a single predominantly expressed V_H gene family, designated as bovine V_H1 (Bov V_H1), has been identified in cattle (Jackson *et al.*,1992; Armour *et al.*,1994, Berens *et al.*, 1997; Saini *et al.*, 1997, see Chapter 3; Sinclair *et al.*, 1997).

2.6.4.3 Light chain variable-region genes

The λ-light chain predominates (90-95%) in the expressed antibody repertoire of cattle (Meyer *et al.*, 1997). Two bovine λ-light chain gene families, $V_\lambda1$ and $V_\lambda2$, have been identified (Sinclair *et al.*, 1995). The $V_\lambda1$ gene family is further grouped into four subfamilies, $V_\lambda1a$, $V_\lambda1b$, $V_\lambda1c$ and $V_\lambda1x$. The $V_\lambda1a$ and $V_\lambda1b$ genes are expressed predominantly in the antibody repertoire of adult cattle. The $V_\lambda1x$ genes constitute 50% of the bovine germline V_λ genes, but have been noted to be not expressed (Sinclair *et al.*, 1995). Four germline C_λ and two J_λ gene segments are identified, but a single J-C unit is expressed preferentially. Many germline V_λ genes are pseudogenes and exist as fused VJ gene segments. Limited evidence suggests that the process of gene conversion may contribute to bovine λ-light chain diversity (Parng *et al.*, 1996).

Chapter 3

A SINGLE PREDOMINANTLY EXPRESSED POLYMORPHIC IMMUNOGLOBULIN V_H GENE FAMILY, RELATED TO MAMMALIAN GROUP I, CLAN II, IS IDENTIFIED IN CATTLE*

3.1 Abstract

In order to understand the generation of antibody diversity in cattle, seven cDNAs, from hetero-hybridomas secreting bovine IgM and IgG1 antibodies, were cloned and structurally analyzed for rearranged bovine VDJ genes. All of the seven bovine V_H genes, together with four available bovine V_H gene sequences, shared a high nucleotide sequence homology (84.2-93.5%). Based upon the criteria of nucleic acid homology ≥80%, all of the bovine V_H gene sequences isolated from the expressed antibody repertoire constitute a single V_H gene family, designated as bovine V_H1 (Bov V_H1). Analysis of 44 bovine IgM-secreting mouse x cattle hetero-hybridomas, originating from polyclonally-activated PBLs from bovine leukemia virus-infected cow, revealed that all of these expressed Bov V_H1 (100%) based upon DNA sequencing and Northern dot blot. The bovine V_H genes showed highest DNA sequence similarity, ranging between 81.5 and 87.6%, with a single sheep V_H gene family (related to human V_H4 gene family) and are, thus, closest to the V_H genes from another ruminant species. The Bov V_H1 gene family is most homologous to the murine V_H Q-52 (71.8-78%).

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and human V_H4 (67.4-69.8%) gene families, which belong to mammalian group I, clan II, V_H genes. The CDR3H of rearranged bovine VDJ genes is characteristically long (15 to 23 amino acids). The expressed bovine J_H gene segments were most homologous to human J_H4 (82.1-87.2%) and J_H5 (84.6-89.7%) genes, suggesting the existence of at least two J_H genes. An analysis of CDRs provides evidence that somatic hypermutations contribute significantly to the generation of antibody diversity in cattle. Southern blot analysis of *Bam*H I, *Eco*R I and *Hin*d III digested genomic DNA from four cattle breeds (Holstein, Jersey, Hereford and Charolais) revealed three RFLP patterns; the genomic complexity of Bov V_H1 ranged between 13 and 15 genes. These observations provide evidence for polymorphism at the bovine Ig-V_H locus, similar to that seen in mice and humans.

3.2 Introduction

Antibody diversity in the vertebrate immune system is generated through rearrangement of separated germline gene segments (i.e., variable (V_H), diversity (D) and joining (J_H) for the heavy and $V_{\kappa/\lambda}$ and $J_{\kappa/\lambda}$ for the light chain) and through subsequent heavy and light chain pairings. The variable region antibody diversity is primarily determined by two factors : (a) the number and sequence diversity of germline genes, and (b) somatic diversity, resulting from rearrangements, junctional deletions and additions (N or P nucleotides) and somatic mutations (Tonegawa,1983).

The process of gene rearrangement is common to all vertebrates, but the number and sequence diversity of V_H gene segments varies across the species. Based on nucleotide homology of ≥80%, about 100 human V_H genes are grouped into seven V_H gene families

(Berman *et al.*,1988; Van-Dijk *et al.*,1993) while 100 to 500 murine V_H genes are classified into 15 V_H gene families (Brodeur and Riblet,1984; Kofler *et al.*,1992; Mainville *et al.*,1996). The human and murine V_H genes are further classified into three major groups, group I (humanV_H2, V_H4, V_H6, murine V_H 3609, V_H 3660 and V_HQ-52), group II (Human V_H1, V_H5, murine V_H J558 and V_HVGam 3-8) and group III (humanV_H3, murine V_H7183, V_HS107, V_H J606 and V_HX24), according to evolutionary divergence across species (Tutter and Riblet,1988a; Tutter and Riblet,1989; Kofler *et al.*,1992). In general, group III V_H genes are conserved during evolution and have been suggested to constitute an obligatory component of heavy chain loci in widely divergent mammalian and non-mammalian lineages (Tutter and Riblet,1988a; Tutter and Riblet,1989).

In contrast to the mouse and to humans, chickens have approximately 100 V_H genes, but only one V_H gene, proximal to the D genes, is functional; it contributes to antibody diversity via gene conversion in the bursa of Fabricius (Reynaud *et al.*,1989; Reynaud *et al.*,1994). Similarly, the rabbit has a germline pool of at least 100 V_H genes, corresponding to the human V_H3 gene family, but approximately half of these are functional (Becker and Knight,1990; Knight,1992). However, a single 3' V_H gene is preferentially rearranged and undergoes gene conversion and somatic hypermutations in the appendix leading to the generation of antibody diversity (Weinstein *et al.*,1994). The swine V_H genes expressed in the peripheral antibody repertoire also belong to one V_H gene family and, like the rabbit, are closest to human V_H3 genes (Sun *et al.*,1994). In sheep, a single V_H gene family, a homologue of the human V_H4 gene family, is consistently isolated from the immunoglobulin variable region heavy chain repertoire (Dufour *et al.*,1996). The λ-light chain repertoire in sheep is

diversified via somatic hypermutations in the ileal Peyer's patches during fetal and neonatal life (Reynaud *et al.*,1991; Reynaud *et al.*,1995). Thus, multiple divergent V_H gene families contribute to the generation of antibody diversity in the primary antibody repertoire of mice and humans but few V_H genes appear to generate antibody diversity in other species such as the chicken, rabbit, sheep and pig.

In order to understand the development of antibody repertoire during evolution, studies of V_H genes of other species are necessary. The bovine immune system develops in the absence of any influence from maternal antibodies (Kearney and Vakil, 1986) due to the presence of a syndesmochorial type of placentation (Tizard,1996). Approximately 5 to 35% of bovine peripheral B lymphocytes are $CD5^+$(Naessens and Williams,1992), similar to murine B-1 cells, which are suggested to constitute a separate B cell lineage (Herzenberg *et al.*,1986). Homologs of the murine V_H J558 gene family, which constitute up to 50% of mouse V_H genes, do not exist in the bovine genome (Tutter and Riblet,1989). The bovine $C\gamma1$ and $C\gamma2$ genes have been cloned and sequenced (Symons *et al.*,1989; Jackson *et al.*,1992). The bovine V_λ light chain genes have been characterized and grouped into two families, $V_\lambda1$ and $V_\lambda2$, with $V_\lambda1$ predominating in the expressed antibody repertoire (Sinclair *et al.*,1995). The primary λ-light chain repertoire in cattle has been suggested to be diversified by gene conversion (Parng *et al.*, 1996). Few DNA sequences of bovine V_H genes are available (Jackson *et al.*,1992; Armour *et al.*,1994), but further studies are essential to characterize the VDJ gene rearrangements in the bovine peripheral antibody repertoire. This will help to elucidate the mechanisms involved in the generation of antibody diversity in the bovine immune system.

Seven cDNA sequences were analysed from mouse x cattle hetero-hybridomas secreting bovine IgM and IgG1 antibody. All the bovine V_H genes isolated so far from different sources constitute a single V_H gene family, designated as bovine V_H1 (Bov V_H1), which is most homologous to human V_H4 and murine Q-52 gene families. The expressed bovine J_H genes are most homologous to human J_H4 and J_H5 gene segments. The CDR3H length of rearranged bovine V_H genes is unusually long (15-23 amino acids) and somatic hypermutations appear to contribute significantly to the generation of antibody diversity in cattle. Finally, the Bov V_H1 gene family is polymorphic and its genomic complexity varies between 13 and 15 genes.

3.3 Materials and Methods

3.3.1 B-cell hetero-hybridomas

Two mouse x cattle hetero-hybridomas, alpha-BL5C2.870005 (HB-9907) and alpha-BL5C2.870009 (HB-9908), originating from lymph node B-cells of a Holstein cow and secreting anti-bovine herpesvirus 1 IgG1 antibody, were obtained from ATCC (Rockville, Maryland, USA). The hetero-hybridomas were grown in RPMI 1640 medium supplemented with 20% heat inactivated horse serum, 5 mM sodium pyruvate, 1 mM glutamine, 0.5 mM MEM non-essential amino acids, 1% 100x antibiotic-antimycotic solution and $5x10^{-5}$ M 2-mercaptoethanol (all from GIBCO, BRL, Gaithersburg, MD, USA).

Additional mouse x cattle hetero-hybridomas were developed by somatically fusing bovine PBLs, stimulated *in-vitro* for 48 h with mitogenic concentrations of *Escherichia coli* serotype 0127:B8 lipopolysaccharide (25 µg/ml) and phorbol 12-myristate 13-acetate (300

ng/ml) (Sigma Chemical Co., St. Louis, MO, USA), with non-immunoglobulin secreting X63.Ag8.653 mouse myeloma cells under limiting dilution conditions (Kaushik *et al.*,1988). The PBLs were obtained from a two-year old Holstein heifer suffering from BLV infection which causes generalized transformation and expansion of $CD5^+$, IgM^+ B lymphocytes (Meirom *et al.*, 1993). The supernates from actively growing hetero-hybridomas (400) were tested for bovine IgM secretion in a sandwich ELISA. The ELISA plates (Dynatech, Alexandria, VA, USA) were coated overnight (4°C) using 1:1000 dilution of unlabeled murine monoclonal anti-bovine IgM (Sigma Chemical Co.) in 0.05 M carbonate buffer (pH 9.6, 0.05% sodium azide). Between each step, plates were washed 5x with washing buffer (PBS, 0.05% Tween 20, 0.05% sodium azide). Wells were blocked with 1% BSA solution for 1 h at room temperature before adding, in triplicate, 50 µl of hybridoma supernates, along with the positive (1:500 bovine serum) and negative (PBS and RPMI) controls. Plates were incubated for 2 h at room temperature and after washing, biotin-conjugated murine monoclonal anti-bovine IgM (Sigma Chemical Co.) was added in 1:25000 dilution. Plates were washed after 1 h and avidin-conjugated alkaline phosphatase (Pierce, Rockford, IL, USA) was added in 1:150 dilution followed by incubation for 1 h at room temperature. The alkaline phosphatase substrate p-nitrophenyl phosphate (Sigma Chemical Co.), diluted to a concentration of 1 mg/ml in 10% diethanolamine buffer, was added. The resulting colour reaction was measured at 405 nm using an ELISA reader (Ceres UV 900 Hdi, Bio-Tek Instruments Inc., Winooski, VT). Forty four tested positive for bovine IgM secretion.

3.3.2 cDNA synthesis, PCR amplification and cloning of rearranged bovine VDJ genes

Total RNA was extracted from two bovine IgG1-secreting and seven IgM-secreting

hetero-hybridomas following the method of Chomczynski and Sacchi (1987). The cDNA was synthesized from 7 μg of total RNA using polydT primer (Pharmacia LKB Biotechnology, Uppsala, Sweden) and amplified with PCR (Perkin Elmer, Branchburg, NJ) using 5' primer (5'AGCTCGAGATGAACCCACTGTG 3') from the leader sequence and 3' primer from either the conserved 5' sequence of C_H1 region of bovine Cγ1(5' AGACTAGTGGCTGTGGTGGAGGC 3') or from the 3' sequences of bovine J_H gene segment (5' AGACTAGTGAGGAGACGGTGACC 3'); (Jackson *et al.*,1992). The 5' and 3' primers have built-in restriction sites for *Xho* I and *Spe* I endonucleases, respectively, and were synthesized at the Biotechnology Centre, University of Toronto, Toronto, Ontario, Canada. The PCR steps involved a hot start at 95°C for 2 min, denaturation at 95 °C for 1 min and combined annealing and extension steps at 72°C for 2.5 min up to a total of 30 cycles. The reaction conditions included 1.5 mM $MgCl_2$ concentration, 0.8 μM of each primer and 2.5 U of Taq polymerase (Perkin Elmer, Branchburg, NJ) in 100 μl volume.

The rearranged bovine VDJ gene amplification was confirmed in an agarose gel electrophoresis and Southern blot by hybridization with a [α-^{32}P]dCTP (Amersham Canada Ltd., Oakville, ON) radiolabeled DNA probe specific for sheep V_H gene. The sheep V_H gene specific DNA probe was a 255 bp *Pst* I and *Eco*52 I fragment from clone 4839 VH2 (Dr. W.R. Hein, Basel Institute of Immunology, Basel, Switzerland). The PCR product was digested with *Xho* I and *Spe* I, fractionated by gel electophoresis, purified with Gene Clean II (Bio 101, Vista, CA, USA) and ligated into the dephosphorylated (calf intestinal alkaline phosphatase from Boehringer Mannheim, Germany) multiple cloning site of Bluescript KS +/- vector (Stratagene, La Jolla, CA, USA) as described by Lipsanen *et al.* (1997). High

efficiency *E coli*, DH10B cells (GIBCO, BRL) were transformed with the ligated DNA by electroporation (GIBCO, BRL). The bacterial colonies were screened in a Southern blot of restriction enzyme-digested plasmid DNA (Sambrook *et al.*, 1989) by hybridizing with a sheep V_H gene-specific DNA probe radiolabeled with [α-^{32}P]dCTP by random priming (Boehringer Mannheim, Germany). Briefly, the restriction-digested plasmid DNA was transferred onto a nitrocellulose membrane (Schleicher and Schuell Inc., Keene, NH, USA) and UV cross-linked. The membranes were pre-hybridized for 6 hrs at 42^{0}C followed by overnight hybridization in 50% formamide, 5X Denhardt's solution, 5X SSPE, 0.1% sodium dodecyl sulfate (SDS), 100 µg/ml sonicated and denatured salmon sperm DNA and 10^6 dpm of radiolabeled DNA probe/ml. The membranes were washed three times in 2X SSC, 0.1% SDS at 20°C; once in 1X SSC, 0.1% SDS for 10-15 min at 65^0 C; and finally in 0.1X SSC, 0.1% SDS for 15-20 min at 65^{0}C. The autoradiography was performed using XAR-5 films (Eastman Kodak Company, Rochester, NY, USA).

3.3.3 DNA sequencing and structural analysis

The recombinant plasmids extracted from at least two clones originating from each hetero-hybridoma were purified on mini-affinity columns (Qiagen Inc., Stanford, CA, USA) and sequenced in both directions with T7 and T3 primers, using an automated DNA sequencing facility at MOBIX, McMaster University, Hamilton, Ontario, Canada. The clone p4H9.1 has a restriction site for *Spe*I enzyme at the 5' end of CDR3; its nucleotide sequence was thus determined by sequencing with T3 and T7 primers from nucleotide 1 to 291 from the recombinant plasmid, and the remaining sequence was obtained by direct sequencing of the PCR product. The nucleotide sequences of all the rearranged V_H genes were analysed for

the FWs and CDRs, and numbered according to Kabat *et al.* (1991). The DNA sequences were compared with nucleotide sequences from other species using the GenBank BLAST program (Altschul *et al.*,1990), and the amino acid analysis was performed using the GPMAW (version 2.1) program (Kyte and Doolittle,1982).

3.3.4 Southern blot hybridization

Genomic DNA was extracted from the blood cells of Holstein Friesian, Jersey, Hereford and Charolais cattle using a DNA extraction kit (Stratagene, La Jolla, USA). Additionally, sperm genomic DNA was extracted from fresh semen samples collected from seven Holstein bulls. About 50 µl of washed sperm were resuspended in 580 µl of 0.01 M Tris (pH 7.2) containing 50 mM EDTA and digested at 37°C for 24 h in 60 µl of proteinase K (20 mg/ml), 80 µl of SDS (20%) and 80 µl of dithiothreitol (0.39 M). The DNA was extracted twice with phenol:chloroform (1:1) and twice with chloroform:isoamylalcohol (24:1) and precipitated with 1/10th volume of 5 M NaCl and two volumes of chilled 100% ethanol. About 10-15 µg of DNA, digested to completion with *Bam*H I, *Eco*R I and *Hin*d III restriction enzymes, was electrophoresed at 18 V for 36 h in 0.8% agarose gel. The DNA was depurinated in 0.2 N HCl for 30 min and transferred onto maximum strength nytran membrane (Schleicher and Schuell Inc.) using an alkaline transfer method (1 M NaOH) by VacuGene (Pharmacia LKB Biotechnology, Uppsala, Sweden). The membranes were pre-hybridized overnight at 42°C followed by hybridization (50% formamide, 5X Denhardt's solution, 5X SSPE, 0.5% SDS, 100 µg/ml sonicated and denatured salmon sperm DNA and 2.5-3.0 x10^6 dpm of [α-^{32}P]dCTP radiolabeled Bov V_H1 gene family-specific DNA probe/ml.).

The Bov V_H1 gene family-specific DNA probe was prepared from recombinant

plasmid pB7S2 by double restriction digestion. First, a 463 bp fragment containing rearranged VDJ genes was isolated by restriction digestion with *Xho* I and *Xba* I enzymes; following purification, it was further digested with *Hae* III enzyme to obtain a 248 bp V_H specific DNA probe spanning nucleotides 23 to 270. The bovine V_H specific DNA probe was radiolabeled by random priming (Boehringer Mannheim, Germany). Following overnight hybridization, the membranes were washed three times in 2X SSC, 0.1% SDS solution at room temperature; once in 1X SSC, 0.1% SDS for 10-15 min at 65^{0}C; and finally in 0.1X SSC, 0.1% SDS at 65^{0}C for 10-15 min. The membranes were exposed to XAR-5 films (Eastman Kodak Company) for 2 to 5 days at -70^{0}C.

3.3.5 RNA Dot-blot

The total RNA was extracted from all hetero-hybridomas using Trizol reagent according to manufacturer's instructions (GIBCO, BRL). The RNA was blotted at the concentration of 1, 3 and 5 µg onto a nitrocellulose membrane and UV cross-linked. The RNAs from a bovine IgG1-secreting hetero-hybridoma (HB 9907) and non-secreting fusion partner (X63.Ag8.653) as well as from non-secreting hetero-hybridomas (BL2G11 and BL6A2) were included in the assay as positive and negative controls, respectively. The RNA blots were hybridized with a [α-^{32}P]dCTP radiolabeled Bov V_H1 gene-specific DNA probe, followed by stringent washing as described (Kaushik *et al.*,1990). The filters were exposed to XAR-5 films (Eastman Kodak Company) overnight at -70^{0}C. The specificity of RNA dot-blot assay was confirmed by cloning and sequencing cDNAs prepared from seven randomly selected RNAs which showed nucleotide sequence homology ≥80% with pB7S2 clone.

3.4 Results

3.4.1 Structural analysis of rearranged bovine Ig variable region heavy chain genes

The cDNAs prepared from seven mouse x cattle hetero-hybridomas, two secreting bovine IgG1 antibody against bovine herpesvirus 1 (pB7S2 and pB8S1) and five secreting bovine IgM antibody, derived from polyclonally-activated PBLs from BLV-infected cow (p2C7.5, p4H9.1, p5H4.1, p7B7.2 and p7G1.5), were cloned and structurally analysed for rearranged bovine VDJ genes (Fig. 3.1). A high nucleotide sequence homology (84.2-93.5%) was observed among all the cloned bovine V_H genes and four other available bovine V_H genes (Fig. 3.2). As the nucleotide sequences of all the bovine V_H genes from different sources analysed thus far shared a nucleotide homology of ≥80%, these correspond to a single bovine V_H gene family designated as Bov V_H1. In addition, an analysis of 44 bovine IgM-secreting mouse x cattle hetero-hybridomas prepared from polyclonally activated PBLs from a BLV-infected cow revealed that the Bov V_H1 gene family was expressed in 100% of IgM-secreting hetero-hybridomas (Fig. 3.3). Nucleotide sequences analysed from five cloned cDNAs from randomly selected Bov V_H1^+ RNAs (Fig. 3.3) confirmed their classification in the Bov V_H1 gene family (Fig. 3.1a). Other V_H genes may exist in the bovine genome. Some faintly hybridizing restriction fragments of genomic DNA were noted in Southern blots (Fig. 3.4), which may reflect divergent bovine V_H gene sequences.

The bovine V_H genes shared 81.8 to 86.6% nucleotide homology with available sheep V_H genes, 71.8 to 78% homology with the murine Q-52 gene family and 67.4 to 69.8% homology with the human V_H4 gene family (Table 3.1). The bovine V_H genes show a conserved CDR1 length of five amino acids, similar to murine V_H genes. However, the CDR2

Fig. 3.1 The nucleotide sequences of seven rearranged bovine VDJ genes cloned from *anti-bovine herpesvirus 1 IgG1-secreting hetero-hybridomas and **bovine IgM-secreting hetero-hybridomas from polyclonally-activated PBLs. The codons are numbered according to Kabat *et al.* (1991). The CDRs are shown in bold letters. The sequences presented here are available from GenBank under accession numbers: U36823 (pB7S2), U36824 (pB8S1), AF12 (p2C7.5), AF13 (p4H9.1), AF14 (p5H4.1), AF15 (p7B7.2) and AF16 (p7G1.5) **(a)**. The deduced amino acid sequences of rearranged bovine VDJ genes are presented in three-letter codes **(b)**. The dash (-) indicates identical nucleotide base or amino acid and dot (.) indicates lack of nucleotide base or amino acid at that particular position. The PI values were determined by GPMAW (version 2.1) programme (Kyte and Doolittle, 1982).

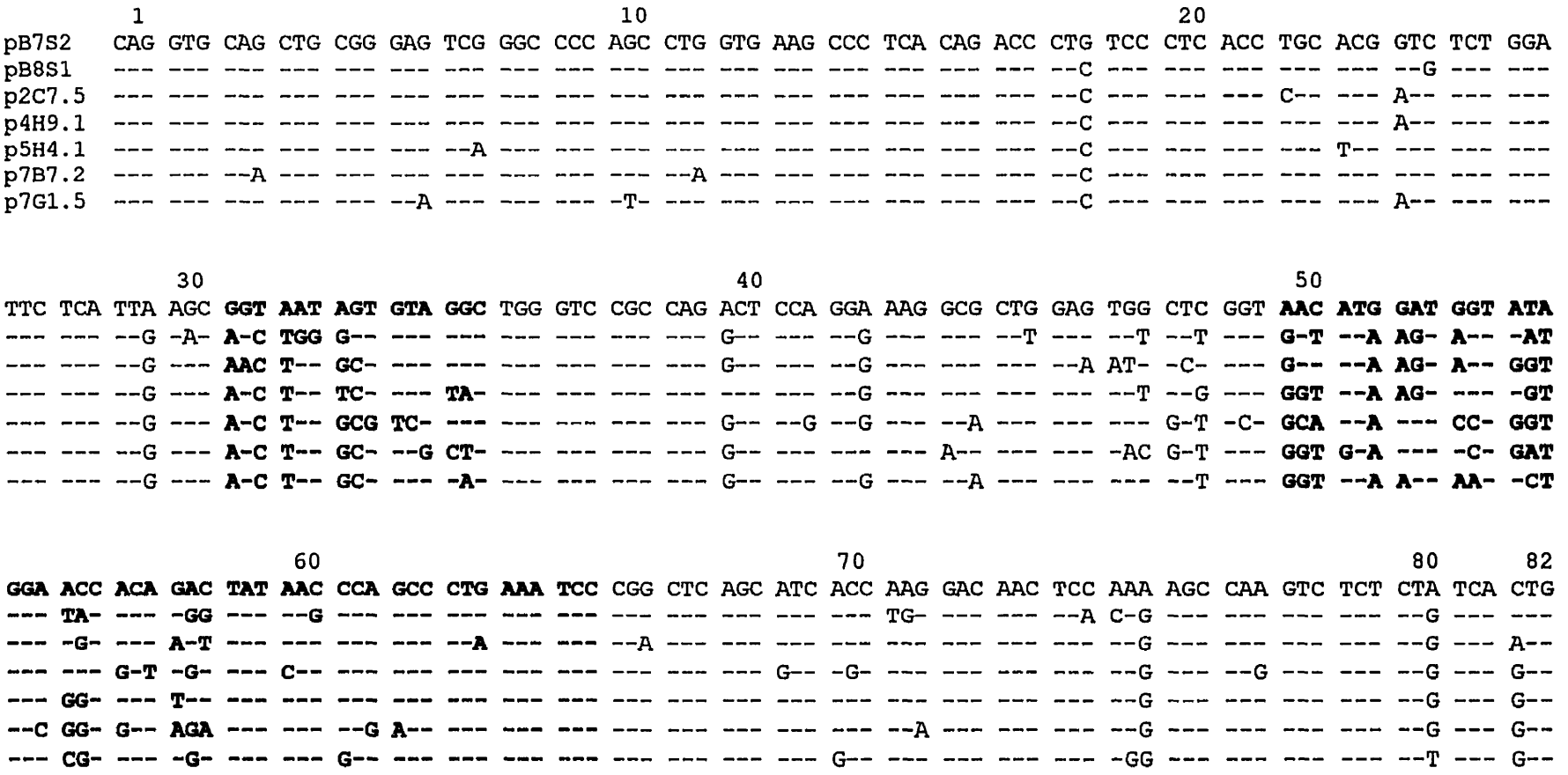


Fig. 3.1a (Continued)

```
 a   b   c                                  90                                                  100  a   b   c   d   e   f   g
AGC AGC GTA ACA ACT GAG GAC ACG GCC ACA TAC TAT TGT GCG AAG TGT ACT GGT GCT TAT TGC TGG AGG TTT GAT GAC GCT TAT
--- --- --G --- -G- --C --- --- --- --- --- --C --- -T- --A -C- GG- A-- AT- GGC -T- A-T G-C GG- -G- TGT -GA GTG
--- --- --G --- --- --- --- --- --- --- --- --C --- --- --- -A- --- TA- A-G -C- A-T G-T TTC C-- TG- --- -G- --C
--- --- --G --- --- --- --- --- --- --- --- --C --- --- --- -CA --- A-- -G- GC- GCT -AT --T AG- AG- TGT AG- GC-
--G --- --G --- C-- --- --- --- --- --- --- --C --- A-- -CA GAC TAC A-- -G- AG- G-T -AT -AA C-- AC- TGG AG- GTC
--- --- --G --- --G --A --- --- --- --- --- --C --- --A --A AA- T-G TTA TT- A-- G-- GAT G-T GAC -CC TGT -G- CGG
--- --- --G --- --- --- --- --- --- --- --- --C --- --- --- --- --- TT- -G- GG- GA- CGT GGT GG- TG- -GT TA- GCG

 h   i   j   k   l   m   n   o                                                  110
GGT TAT ... ... ... ... ... ... GAT GAC TGG GGC CAA GGA CTC CTG GTC ACC GTC TCC TCG  pB7S2  [Bovine IgG1]*
--- GG- GTT GCT TAT GCT TAC GTC --- -C- --- --- G-- --- --- -G- --- --- --- --- --A  pB8S1  [Bovine IgG1]*
AA- C-C TAT CGC ... ... ... ... --- -C- --- --- --- --- --- --- --- --- --- --- --A  p2C7.5 [Bovine IgM]**
--- A-- TTT GCT TCC TAC CTC ... --- -C- --- --- --- --- --- --- --- --- --- --- --A  P4H9.1 [Bovine IgM]**
... ... ... ... ... ... ... ... -G- -T- --- --- --- --- --- --- --- --- --- --- --A  p5H4.1 [Bovine IgM]**
CTC ... ... ... ... ... ... ... --A -T- --- --- --- --- --- --- --- --- --- --- --A  p7B7.2 [Bovine IgM]**
-A- GC- TAT ... ... ... ... ... --- -C- --- --- --- --- --- --- --- --- --- --- --A  p7G1.5 [Bovine IgM]**
```

Fig. 3.1a

	1									10										20						
pB7S2	Gln	Val	Gln	Leu	Arg	Glu	Ser	Gly	Pro	Ser	Leu	Val	Lys	Pro	Ser	Gln	Thr	Leu	Ser	Leu	Thr	Cys	Thr	Val	Ser	Gly
pB8S1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
p2C7.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Arg	-	Ile	-	--
p4H9.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Ile	-	--
p5H4.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Ser	-	-	--
p7B7.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	--
p7G1.5	-	-	-	-	-	-	-	-	-	Ile	-	-	-	-	-	-	-	-	-	-	-	-	-	Ile	-	--

			30									40									50				
Phe	Ser	Leu	Ser	**Gly**	**Asn**	**Ser**	**Val**	**Gly**	Trp	Val	Arg	Gln	Thr	Pro	Gly	Lys	Ala	Leu	Glu	Trp	Leu	Gly	**Asn**	**Met**	**Asp**	**Gly**	**Ile**
-	-	-	Asn	**Ser**	**Trp**	**Gly**	-	-	-	-	-	-	Ala	-	-	-	-	-	-	Cys	-	-	**Asp**	**Ile**	**Ser**	**Ser**	**Asn**
-	-	-	-	**Asn**	**Tyr**	**Ala**	-	-	-	-	-	-	Ala	-	-	-	-	-	-	Met	Pro	-	**Asp**	**Ile**	**Ser**	**Ser**	**Gly**
-	-	-	-	**Ser**	**Tyr**	**Ala**	-	**Tyr**	-	-	-	-	-	-	-	-	-	-	-	Cys	-	-	**Gly**	**Ile**	**Ser**	-	**Ser**
-	-	-	-	**Ser**	**Tyr**	**Ala**	**Ser**	-	-	-	-	-	Ala	-	-	-	-	-	-	-	Val	Ala	**Ala**	**Ile**	-	**Pro**	**Gly**
-	-	-	-	**Ser**	**Tyr**	**Ala**	-	**Leu**	-	-	-	-	Ala	-	-	-	Thr	-	-	Tyr	Val	-	**Gly**	**Val**	-	**Ala**	**Asp**
-	-	-	-	**Ser**	**Tyr**	**Ala**	-	**Asp**	-	-	-	-	Ala	-	-	-	-	-	-	-	-	-	**Gly**	**Ile**	**Asn**	**Asn**	**Thr**

				60										70									80		82
Gly	**Thr**	**Thr**	**Asp**	**Tyr**	**Asn**	**Pro**	**Ala**	**Leu**	**Lys**	**Ser**	Arg	Leu	Ser	Ile	Thr	Lys	Asp	Asn	Ser	Lys	Ser	Gln	Val	Ser	Leu	Ser	Leu
-	**Tyr**	-	**Gly**	-	**Lys**	-	-	-	-	-	-	-	-	-	-	Trp	-	-	-	Gln	-	-	-	-	-	-	--
-	**Ser**	-	**Asn**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Met
-	-	**Ala**	**Gly**	-	**His**	-	-	-	-	-	-	-	-	Val	Ser	-	-	-	-	-	-	-	-	-	-	-	Val
-	**Gly**	-	**Tyr**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Val
-	**Gly**	**Ala**	**Arg**	-	-	-	**Thr**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Val
-	**Arg**	-	**Gly**	-	-	**Ala**	-	-	-	-	-	-	-	-	Ala	-	-	-	-	Arg	-	-	-	-	-	-	Val

Fig. 3.1b (Continued)

a	b	c								90										100	a	b	c	d	e	f	g
Ser	Ser	Val	Thr	Thr	Glu	Asp	Thr	Ala	Thr	Tyr	Tyr	Cys	Ala	Lys	**Cys**	**Thr**	**Gly**	**Ala**	**Tyr**	**Cys**	**Trp**	**Arg**	**Phe**	**Asp**	**Asp**	**Ala**	**Tyr**
-	-	-	-	Ser	Asp	-	-	-	-	-	-	-	Val	-	**Ser**	**Gly**	**Ser**	**Ile**	**Gly**	**Phe**	**Ser**	**Gly**	**Gly**	**Gly**	**Cys**	**Gly**	**Val**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**Tyr**	**-**	**Tyr**	**Thr**	**Ser**	**Ser**	**Gly**	**Phe**	**Leu**	**Cys**	**-**	**Gly**	**--**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**Ser**	**-**	**Ser**	**Gly**	**Ala**	**Ala**	**Tyr**	**Ser**	**Ser**	**Ser**	**Cys**	**Ser**	**Ala**
Arg	-	-	-	Pro	-	-	-	-	-	-	-	-	Thr	Thr	**Asp**	**Tyr**	**Ser**	**Gly**	**Ser**	**Gly**	**Tyr**	**Lys**	**Leu**	**Thr**	**Trp**	**Ser**	**Val**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**Asn**	**Ser**	**Leu**	**Phe**	**Asn**	**Gly**	**Asp**	**Gly**	**Asp**	**Ala**	**Cys**	**Gly**	**Arg**
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**-**	**-**	**phe**	**Gly**	**Gly**	**Asp**	**Arg**	**Gly**	**Gly**	**Cys**	**Gly**	**Tyr**	**Arg**

h	i	j	k	l	m	n	o										100					
Gly	**Tyr**	.	.	.	.	.	.	**Asp**	**Asp**	Trp	Gly	Gln	Gly	Leu	Leu	Val	Thr	Val	Ser	Ser	pB7S2	(PI 5.04)
-	**Gly**	**Val**	**Ala**	**Tyr**	**Ala**	**Tyr**	**Val**	**-**	**Ala**	-	-	Glu	-	-	Arg	-	-	-	-	-	PB8S1	(PI 7.77)
Asn	**His**	**Tyr**	**Arg**	...	...	...	...	**-**	**Ala**	-	-	-	-	-	-	-	-	-	-	-	P2C7.5	(PI 9.04)
-	**Asn**	**Phe**	**Ala**	**Ser**	**Tyr**	**Leu**	...	**-**	**Ala**	-	-	-	-	-	-	-	-	-	-	-	p4H9.1	(PI 8.20)
...	...	...	...	...	...	...	...	**Glu**	**Val**	-	-	-	-	-	-	-	-	-	-	-	P5H4.1	(PI 8.66)
Leu	...	...	...	...	...	...	...	**Glu**	**Val**	-	-	-	-	-	-	-	-	-	-	-	P7B7.2	(PI 7.92)
Asp	**Ala**	**Tyr**	...	...	...	...	...	**-**	**Ala**	-	-	-	-	-	-	-	-	-	-	-	P7G1.5	(PI 8.55)

Fig. 3.1b

Fig. 3.2 Percent nucleotide homologies between rearranged bovine V_H gene sequences spanning the 5' end of FR1 to the 3' end of FR3. [a]GenBank U49772, [b]GenBank U49773, [c]GenBank U11629 and [d]Jackson *et al.*, (1992).

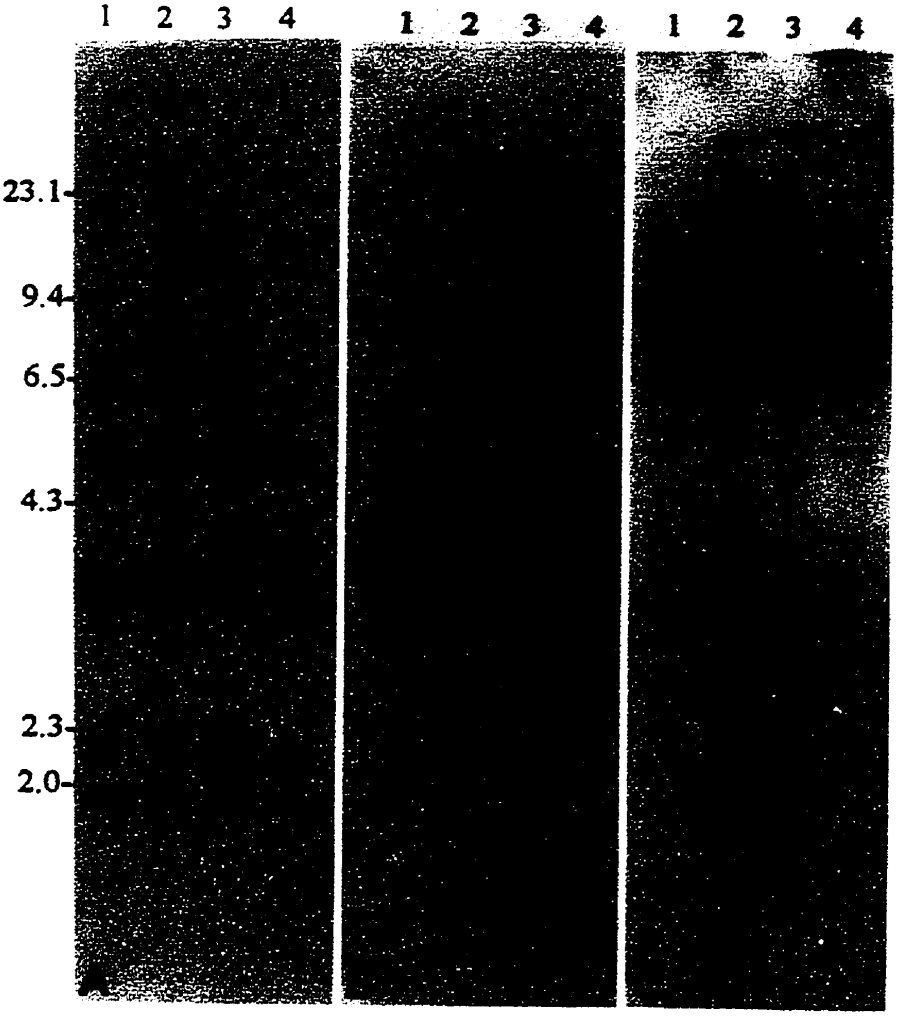
Clone	pB8S1	p2C7.5	p4H9.1	p5H4.1	p7B7.2	p7G1.5	U49772[a]	U49773[b]	B-11[c]	pUC13.8.75[d]
pB7S2	86.3	88.7	88.7	85.9	84.2	88.0	86.3	88.0	86.5	86.9
pB8S1		88.0	88.3	85.2	85.2	88.7	85.9	90.4	88.3	87.9
p2C7.5			89.7	88.3	86.3	90.4	89.3	91.8	90.0	89.7
p4H9.1				86.9	87.3	91.8	89.0	92.8	90.4	90.0
p5H4.1					87.3	89.3	87.6	89.0	92.4	90.4
p7B7.2						88.0	86.9	88.7	88.7	87.6
p7G1.5							89.7	92.1	91.1	89.7
U49772[a]								90.4	90.7	87.6
U49773[b]									92.4	93.5
B-11[c]										92.1

Fig. 3.2

Fig. 3.3 RNA dot blot analyses of RNA from bovine IgM-secreting mouse x cattle hetero-hybridomas probed with a [α-^{32}P]dCTP radiolabeled Bov V_H1 gene family-specific DNA probe (pB7S2). *RNA from bovine IgG1 secreting hetero-hybridoma HB-9907 (positive control), **RNA from X63.Ag8.653, mouse myeloma fusion partner (negative control) and ***RNA from non-Ig secreting hetero-hybridomas (negative controls). The nucleotide sequences for five clones (p2C7.5, p4H9.1, p5H4.1, p7B7.2 and p7G1.5) are presented in Fig. 3.1a.

Clone No.	RNA Conc.			Clone No	RNA Conc			Clone No.	RNA Conc.			Clone No.	RNA Conc.		
	1	3	5µg		1	3	5µg		1	3	5µg		1	3	5µg
HB-990**				BL3D11				BL6D10				BL8G4			
P3x63Ag 8.653**				BL3E5				BL7A2				BL8H1			
BL2G11***				BL3F12				BL7A8				BL9A7			
BL2A2***				BL4A5				BL7B*				BL9B8			
BL1F3				BL4B8				BL7D7				BL9C12			
BL1G9				BL4H9				BL7F8				BL9F2			
BL1H12				BL5B6				BL7G1				BL9H3			
BL2A9				BL5B8				BL8A5				BL10A11			
BL2C7				BL5B12				BL8B3				BL10E12			
BL2G4				BL5D3				BL8C11				BL10H2			
BL2H6				BL5H1				BL8E10				BL10H8			
BL3D7				BL5H4				BL8F3				BL11F3			

Fig. 3.4 Southern blot hybridization of bovine genomic DNA with [α-^{32}P]dCTP radiolabeled Bov V_H1 gene family-specific DNA probe (pB7S2). Genomic DNA extracted from blood of individual (1) Holstein Friesian, (2) Jersey, (3) Hereford and (4) Charolais cattle was digested with *Bam*H I (A), *Eco*R I (B) and *Hin*d III (C) restriction enzymes. The arrows indicate polymorphic bands. The size of molecular weight standards is shown in kilobase pairs.


1 2 3 4
1 2 3 4
1 2 3 4
23.1
9.4
6.5
4.3
2.3
2.0

length comprised 16 amino acid residues, longer than that of murine Q-52 genes, with a CDR2 length of 11 amino acids, but similar to the CDR2 length of the human V_H4 gene family (Kabat *et al.*,1991). In contrast to the murine CDR3 length distribution of 7 to 12 amino acid residues (Kabat *et al.*,1991), the bovine CDR3 length of rearranged VDJ genes ranged between 15 (p5H4.1) and 23 amino acids (pB8S1). The exceptionally long CDR3H length of rearranged bovine V_H genes reflects unique characteristics of rearranged bovine Ig variable region heavy chain genes; because of the limited germline diversity in cattle, these appear to be important in antigen recognition and in the development of antibody combining sites. The contribution of heavy chain D gene segments to the CDR3 length must await the identification of bovine D genes as both germline and rearranged nucleotide sequences become available.

The bovine J_H gene sequence show highest nucleotide similarity with the human J_H4 (82.1-87.8%), human J_H5 (84.6-89.7%), murine J_H1 (74.4-79.5%) and murine J_H4 (74.3-76.9%) genes. When compared with the sheep J_H gene sequence, the bovine J_H genes shared nucleotide homology ranging between 82.1 and 87.2%. All the bovine J_H gene segments shared a relatively low level of nucleotide homology with the single swine J_H gene segment (66.7-69.2%); (Table 3.2).

An analysis of deduced amino acid sequences revealed extensive amino acid substitutions in the CDR1 and CDR2 of both IgM and IgG1 antibodies (Fig. 3.1b), which is consistent with the somatic hypermutations which appear to contribute significantly to generation of antibody diversity in cattle. Amino acids serine, leucine, threonine and glycine contributed most to the Ig heavy chain variable regions of bovine antibodies. The amino acids

Table 3.1 Comparative analysis of nucleotide sequences of the cloned bovine V_H genes with other species.

Species	Clone Number	Per cent Homology							Reference
		pB7S2	pB8S1	p2C7.5	p4H9.1	p5H4.1	p7B7.2	p7G1.5	
Sheep	V5a *	86.2	87.3	86.2	85.6	86.6	86.6	87.6	Dufour *et al.*(1996)
Sheep	VR-22	83.2	84.9	84.5	83.5	83.2	83.4	85.6	Genbank Z49157
Sheep	SHμ7	82.8	81.5	82.5	81.8	81.8	83.5	83.2	Patri and Nau (1992)
Mouse	PCG1-1	78.0	73.2	75.3	74.9	72.9	71.8	74.2	Stenzel-Poore *et al.* (1987)
Mouse	N-92	75.6	74.2	76.6	76.6	74.9	73.5	76.3	Czerwinski *et al.* (1994)
Human	58 P2	69.4	67.4	68.4	68.7	69.8	67.6	69.4	Schroeder *et al.* (1987)

* Germline gene sequence.

Table 3.2 Comparative analysis of nucleotide sequences of bovine J_H gene segments with other species.

Species	Clone Number	Per cent Homology							Reference
		pB7S2	pB8S1	p2C7.5	p4H9.1	p5H4.1	p7B7.2	p7G1.5	
Sheep J_H	Vr 15	87.2	82.1	84.6	84.6	82.1	84.6	84.6	Dufour *et al.* (1996)
Pig J_H	Consensus	66.7	66.7	69.2	69.2	69.2	69.2	69.2	Sun *et al.* (1994)
Mouse J_H1	MEP 203	74.4	79.5	76.9	76.9	76.9	76.9	76.9	Czerwinski *et al.* (1994)
Mouse J_H4	MEP 203	76.9	74.4	76.9	76.9	74.3	76.9	76.9	Czerwinski *et al.* (1994)
Human J_H4	-	87.2	82.1	87.2	87.2	84.6	87.8	87.2	Schroeder *et al.* (1987)
Human J_H5	-	84.6	84.6	89.7	89.7	84.6	87.8	89.7	Schroeder *et al.* (1987)

methionine and histidine were rarely used. Four of the seven antibodies, both IgM and IgG1, had arginine (pB7S2, p2C7.5, p7B7.2 and p7G1.5), while six had cysteine (pB7S2, pB8S1,p2C7.5, p4H9.1, p7B7.2 and p7G1.5) in their CDRH3 (Fig. 3.1b). The clone pB7S2 had two cysteine residues at positions 95 and 100, separated by threonine, glycine, alanine and tyrosine. As compared to FR1 and FR2, the FR3 region of the protein sequences of all antibodies show a higher degree of hydrophilicity. The PI values of these antibodies ranged between 5.04 and 9.04 (Fig. 3.1b).

3.4.2 Genomic complexity and polymorphism in the Bov V_H1 gene family

The genomic complexity of cloned Bov V_H1 gene (pB7S2) was determined in a Southern blot using *Bam*H I, *Eco*R I and *Hin*d III digested bovine genomic DNA. The genomic DNA extracted from blood cells of four different breeds of cattle (Holstein, Jersey, Hereford and Charolais) and sperm DNA from seven Holstein bulls (Fig. not shown) was analysed. Maximum number of Bov V_H1-specific DNA hybridizing fragments, ranging from 13 to 15, were noted in *Hin*d III digested DNA (Fig. 3.4). Three polymorphic RFLP patterns were evident in all four breeds that involved either deletion or addition of V_H gene segments (Fig. 3.4). Such polymorphism may be associated with inter- or intra-breed recombination as a result of cattle breeding practices. These observations provide evidence that bovine V_H genes, similar to murine and human V_H genes, are polymorphic and the genomic complexity of the Bov V_H1 gene family varies between 13 and 15 genes.

3.5 Discussion

The human and murine variable region genes of both heavy and light chains are

grouped into a number of multi-gene families based on the criteria of ≥80% nucleotide homology among the members of a gene family (Brodeur and Riblet,1984; Berman *et al.*,1988; Kofler *et al.*,1992; Van-Dijk *et al.*,1993; Mainville *et al.*,1996). However, certain other vertebrates such as the chicken (Reynaud *et al.*,1989; Reynaud *et al.*,1994), rabbit (Becker and Knight, 1990; Knight,1992), sheep (Dufour *et al.*,1996) and pig (Sun *et al.*,1994) predominantly utilize a single V_H gene family in the expressed antibody repertoire. For these reasons, elucidation of the structure and organization of Ig loci from phylogenetically distant species is essential to understand the development of antibody diversity and the evolution of the complex multigene variable region gene families. In order to understand the structural features of bovine V_H genes, the VDJ rearrangements were analyzed from two anti-bovine herpesvirus1 IgG1-secreting hetero-hybridomas and five bovine IgM-secreting hetero-hybridomas. Because of nucleotide homology of ≥80%, all the cloned V_H genes, as well as four available cattle V_H genes isolated from different sources (Jackson *et al.*, 1992; Armour *et al.*,1994), were found to constitute a single V_H gene family designated as bovine V_H1 (Bov V_H1). The Bov V_H1gene family is a homolog of the human V_H4 and murine Q-52 gene families which belong to mammalian group I (Tutter and Riblet,1988a; Tutter and Riblet,1989; Kofler *et al.*,1992) and clan II (Kirkham *et al.*,1992) V_H genes. In addition, the Bov V_H 1 gene family was observed to be expressed at 100% frequency among 44 mouse x cattle hetero-hybridomas originating from polyclonally-activated PBLs. The nucleotide sequence of five cDNAs (Fig. 3.1) prepared from randomly selected RNAs which tested positive for Bov V_H1 gene in the dot-blot assay showed them to belong to Bov V_H1 gene family. These observations suggest that a single Bov V_H 1 gene

family is predominantly expressed in the antibody repertoire of cattle.

Unusually long CDR3 regions noted in the rearranged bovine VDJ gene sequences are consistent with previous reports (Jackson *et al.*,1992; Armour *et al.*,1994, Chapter 4). The identification of bovine D genes is required to ascertain their possible contribution to this unusually long CDR3, possibly via D-D fusions. This is especially important as the CDR3 is located in the middle of the antibody combining site and its length is important in fitting the contour of the antigen molecule.

The amino acid substitutions in the CDR1 and CDR2 of the cloned bovine V_H genes of both IgM and IgG1 antibodies suggest that somatic hypermutations contribute significantly to the generation of antibody diversity in cattle. However, comparison with germline V_H gene sequences is essential to confirm the contribution of somatic hypermutations, and to determine if other mechanisms such as gene conversion also contribute to antibody diversity in cattle. The nucleotide sequences encoding FR4 of seven rearranged VDJ genes are most homologous to human J_H4 and J_H5 gene segments suggesting the existence of at least two J_H gene segments in the bovine genome.The PI values of these antibodies ranged between 5.04 and 9.04. The differences in PI values noted in pB7S2 (PI 5.04) and pB8S1 (PI 8.2) are probably related to the heterogenous epitopes on bovine herpesvirus 1 recognized by these antibodies.

The genomic complexity of the designated Bov V_H1 gene family ranges from 13 to 15 members. Three polymorphic RFLP patterns were noted among the four cattle breeds tested. By RFLP analysis of sperm genomic DNA from seven Holstein bulls, polymorphism was detected at the bovine Ig-V_H locus consistent with the results from genomic DNA

prepared from blood cells. Overall, these results suggest that, similar to mouse (Tutter and Riblet,1988b) and human (Pincus,1987), polymorphism exists at bovine Ig-V_H locus.

In contrast to the previous observation that homologs of the murine V_H11 gene were detected in a Southern blot of bovine genomic DNA (Saini *et al.*,1996), and the observation that the homologs of group III V_H genes (murine V_H 7183, V_H S107, V_H J606 and V_H X24 and human V_H3 gene families) are conserved during evolution (Tutter and Riblet,1988a; Tutter and Riblet,1989), a single bovine V_H gene family has been consistently isolated from the expressed antibody repertoire from different sources (Jackson *et al.*,1992; Armour *et al.*,1994). It is unknown at present whether the homologues of V_H11 and other group III V_H genes, which appear by Southern blotting to be present in bovine genome, are actually expressed.

The single bovine V_H gene family (Bov V_H1) defined here is closest in sequence to the single sheep V_H gene family, a homolog of the human V_H4 gene family, which predominates in the Ig variable region heavy chain repertoire of sheep (Dufour *et al.*,1996). Swine also express a single V_H gene family, related to human V_H 3 gene family (Sun *et al.*,1994). Similarly, a 3' gene from a single V_H gene family related to human V_H3 family preferentially rearranges in the rabbit Ig repertoire (Becker and Knight,1990; Knight,1992; Weinstein *et al.*,1994). Chicken B cells utilize a single available functional V_H gene (and one functional V_λ gene) to achieve antibody diversity via gene conversion (Reynaud *et al.*,1989; Reynaud *et al.*,1994). As in horse (Home *et al.*,1992) and sheep (Reynaud *et al.*,1991; Reynaud *et al.*,1995), the λ-light chain predominates in the primary antibody repertoire of cattle (Butler,1983). Further, of the two V_λ light chain gene families, the V_λ 1 gene family is

predominantly expressed in the bovine light chain repertoire (Sinclair *et al.*, 1995). The diversity of the bovine λ-light chain repertoire has been suggested to be generated through gene conversion (Parng *et al.*,1996). It has also been suggested that the bovine V_λ light chain repertoire contributes relatively little to the recognition of antigens (Sinclair *et al.*,1995) and, therefore, bovine V_H genes are expected to contribute most to antigen recognition. It thus appears that the single V_H gene family (Bov V_H1) and the single V_λ1 gene family contribute most to the antibody repertoire in cattle. This is in contrast to both mice and humans in which multigene families, representing divergent germline sequences, contribute most to both heavy and light chain diversity. The ruminant immune system also differs from that of rabbits and swine, in which a single V_H gene family, related to the human V_H3 gene family (mammalian group III), is consistently expressed (Knight,1992; Sun *et al.*,1994). Both the bovine and ovine V_H genes are homologs of human V_H4 and murine Q-52 gene families, corresponding to mammalian group I V_H genes which, unlike group III genes, are not highly conserved during evolution (Tutter and Riblet,1988a; Tutter and Riblet,1989).

The conservation of mammalian group III V_H gene families throughout the vertebrate immune system has led to the suggestion that members of this group might be closest to the primordial Ig V_H genes. They may have an important non-coding function related to the nucleotide sequences in the FR1 and FR3 regions, which target a series of recombinogenic events (Tutter and Riblet,1989). The present observations do not support such a hypothesis, as the expressed bovine and ovine V_H genes were found to belong to relatively less conserved group I V_H genes. Further studies on other mammalian and non-mammalian Ig-V_H loci would be essential to better understand the origin of the V_H genes during phylogeny.

To conclude, the studies outlined here demonstrate that (i) a single bovine V_H gene family (Bov V_H1), a homolog of human V_H4 and murine V_H Q-52 gene families related to mammalian group I and clan II genes, is consistently isolated from cattle and is predominantly expressed among antigen-induced and polyclonally-activated PBLs from BLV-infected cattle; (ii) the CDR3H of the rearranged bovine VDJ genes is unusually long (15 to 23 amino acids); (iii) the FR4 of rearranged bovine VDJ sequences appears to be encoded by two J_H gene segments which are most homologous to human J_H4 and J_H 5 genes; (iv) the genomic complexity of the Bov V_H1 gene family ranges between 13 and 15 genes, and (v) polymorphism exists at the Ig-V_H locus of cattle.

Chapter 4

VDJ REARRANGEMENTS RESULTING IN EXCEPTIONALLY LONG CDR3H, WITH MULTIPLE CYSTEINE RESIDUES, ARE NOTED IN FUNCTIONAL BOVINE IgM ANTIBODIES.

4.1 Abstract

In order to understand antibody diversification in cattle, a species with restricted germline diversity both at V_H and V_λ loci, the VDJ and VJ rearrangements were analysed in IgM-secreting hybridomas originating from the peripheral blood lymphocytes of a cow infected with bovine leukemia virus (BLV). The BLV is known to cause generalized expansion of $CD5^+$ and IgM^+ B lymphocytes regardless of antigen specificity. A comparison of the V_H encoded region with the available bovine germline genes showed that single point mutations contributed to the diversification of IgM antibodies. The most striking observation is that approximately 9% of the VDJ rearrangements in IgM-secreting B cells encode exceptionally long CDR3H (56 to 61 amino acids) with multiple cysteine residues. Such an exceptionally long CDR3H in an antibody is first ever to be documented in a species. These VDJ rearrangements indeed encode functional IgM antibodies as some of these show polyreactive binding specificity. The presence of even numbered cysteine residues in the CDR3H may provide hitherto unknown configurational ability to the antigen combining site via intra-CDR3H disulfide bridging. In addition, the VDJ rearrangements resulting in long CDR3H paired with either novel $V_\lambda 1$ or $V_\lambda 1x$ genes, earlier noted to be not expressed.

Overall, these experiments provide evidence that somatic hypermutations and generation of an exceptionally long CDR3H contribute to the diversification of IgM antibodies in cattle.

4.2 Introduction

Antibody diversity in vertebrates is generated via juxtaposition of separated germline gene segments, both for heavy (V, D and J) and light (V and J) chains, followed by subsequent association of two identical heavy (VDJ) and light chain (VJ) rearrangements (Tonegawa, 1983). Further diversification results from junctional flexibility involving nucleotide deletions and/or additions (N or P nucleotides) intrinsic to the VDJ rearrangement processes. Somatic hypermutations, including point mutations (Wagner and Neuberger, 1996) as well as sporadic insertions or deletions (Wilson *et al.*, 1998), also contribute significantly to antibody diversification.

The V(D)J rearrangement processes are common to all vertebrates but the germline sequence diversity varies considerably across species. In mice and humans, a large pool of divergent germline V-genes (Kabat *et al.*, 1991), grouped into several V_H and V_κ or V_λ gene families based on ≥80% nucleotide homology, exist that generate significant combinatorial diversity via V(D)J rearrangements. By contrast, limited combinatorial diversity is generated in species like chicken (Reynaud *et al.*, 1989), rabbit (Becker and Knight, 1990), pig (Sun *et al.*, 1994), sheep (Dufour *et al.*, 1996) and cattle (Berens *et al.*, 1997; Saini *et al.*, 1997; Sinclair *et al.*, 1997) because of restricted functional germline sequence diversity. These species employ other strategies to generate antibody diversity essential to normal immune function and host defense. For example, gene conversion mechanisms are involved in the

diversification of pre-immune antibody repertoire in the bursa of Fabricius of chicken (Reynaud *et al.*, 1989) and in the appendix of rabbit (Weinstein *et al.*, 1994). In addition, somatic hypermutations, occurring in ileal Peyer's patches without prior exposure to exogenous antigens, are noted to generate diversity in the primary antibody repertoire of sheep (Reynaud *et al.*, 1991; 1995).

Species-related differences exist with regard to the use of κ- and λ-light chains of the antibody molecule. Cattle, sheep and horse express >90% λ-light chains while mice express 95% κ-light chains. In humans, the κ- and λ-light chains are expressed at 60% and 40% frequencies, respectively (Butler, 1998). Camel IgG2 and IgG3 subclasses exist as homodimers of V_H chains, devoid of light chains altogether (Hamers-Casterman *et al.*, 1993). In contrast to mice and humans, bovine B-cells do not express surface IgD and probably belong to a unique B-cell lineage with characteristics common to both B-1 and B-2 cells (Naessens and Williams, 1992; Naessens, 1997). Earlier, a single predominantly expressed polymorphic V_H gene family (Bov V_H1), related to mammalian group I (Tutter and Riblet, 1989) or clan II (Kirkham *et al.*, 1992) V_H genes, was identified in cattle (Berens *et al.*, 1997; Saini *et al.*, 1997; Sinclair *et al.*, 1997). Two bovine λ-light chain gene families, V_λ1 and V_λ2, are identified. The V_λ1 gene family is grouped into four subfamilies, V_λ1a, V_λ1b, V_λ1c and V_λ1x, of which V_λ1a and V_λ1b subfamilies are expressed predominantly (Sinclair *et al.*, 1995).

To understand the diversification of the primary antibody repertoire in cattle, the VDJ and VJ rearrangements were analysed among bovine IgM-secreting hetero-hybridomas originating from the PBLs of a BLV-infected cow. The BLV, an oncovirus, causes

generalized transformation and expansion of $CD5^+$, IgM^+ B lymphocytes (Meirom *et al.*, 1993) and thus provides an useful source of bovine B lymphocytes for developing hetero-hybridomas. The experiments outlined here provide evidence that generation of a remarkably long CDR3H (up to 61 amino acids), with multiple cysteine residues, provides an additional mechanism for antibody diversification. Further, a comparison of germline and expressed V_H gene sequences shows that the somatic hypermutations contribute to the diversification of the primary antibody repertoire in cattle.

4.3 Materials and Methods

4.3.1 Hybridomas

Mouse x bovine hetero-hybridomas were developed by somatic cell fusion of PBLs from a BLV-infected cow, stimulated *in vitro* with 25 µg/ml of lipopolysaccharide from *Escherichia coli* (serotype 0127:B8) and 300 ng/ml of phorbol 12-myristate 13-acetate (Sigma Chemical Co., St. Louis. MO. USA) for 48 hours, with X63.Ag8.653 mouse myeloma cells (Saini *et al.*, 1997). Forty-four clones tested positive for bovine IgM secretion in ELISA (Saini *et al.*, 1997; Chapter 3) using unlabeled murine monoclonal anti-bovine IgM (Sigma Chemical Co.), biotin-conjugated murine monoclonal anti-bovine IgM (Sigma Chemical Co.), and avidin-conjugated alkaline phosphatase (Pierce, Rockford, IL). None of the hetero-hybridomas expressed surface CD5 glycoprotein by flow cytometry (Becton Dickinson Canada Inc., Mississauga, ON, Canada) using murine anti-bovine CD5 (VMRD Inc., Pullman, WA, USA) and FITC-labeled goat anti-mouse IgG (Southern Biotechnology, Birmingham, AL, USA).

4.3.2 PCR amplification and cloning

Total RNA was extracted from eighteen hetero-hybridomas using Trizol reagent (GIBCO BRL Gaithersburg, MD, USA). The cDNA synthesized from 7 μg of total RNA using polydT primer (Pharmacia LKB Biotechnology, Uppsala, Sweden) was amplified by PCR (Saini *et al.*, 1997) using a 5' primer (5'AGCTCGAGATGAACCCACTGTG 3') from the bovine V_H leader sequence (Jackson *et al.*, 1992) and a 3' primer (5' AGACTAGTGAAGACTCTCGGGTGTG 3') from the C_H1 region of the bovine Cμ gene (Chapter 7) with built-in restriction sites for *Xho* I and *Spe* I (or *Xba* I) endonucleases, respectively. The PCR products from hetero-hybridomas, BLV1H12, BLV5B8, BLV5D3 and BLV8C11 were cloned in the Bluescript KS +/- vector (Stratagene, La Jolla, CA, USA) as described (Saini *et al.*, 1997). The rearranged bovine λ-light chain genes were amplified using a 5' primer (5' AGGTCGACTCTGCACAGGATCCTG 3') from the bovine V_λ leader sequence and a 3' primer (5' AGTCTAGAACAGGGTGACCGAG 3') from the bovine C_λ gene (Jackson *et al.*, 1992). The PCR conditions included a hot start, denaturation at 95 ^{0}C for 1 min, annealing at 68^{0}C for 1 min and extension at 72^0 C for 1 min up to a total of 30 cycles. All the PCR reactions were performed in 100 μl volume using 1.5 mM $MgCl_2$ concentration, 0.8 μM of each primer and 2.5 U of Taq polymerase (Perkin Elmer, Branchburg, NJ, USA).

4.3.3 DNA sequencing and structural analysis

The recombinant plasmids extracted from at least two colonies positive for the insert, were purified using mini-affinity column (Qiagen Inc., Stanford, CA, USA) and sequenced in both directions with T7 and T3 primers, by automated DNA sequencing (MOBIX,

McMaster University, Hamilton, Ontario, Canada). The nucleotide sequence of some hetero-hybridomas was determined by direct sequencing of the PCR product, from two or three individual PCR amplifications. The nucleotide sequence was numbered according to Kabat *et al.* (1991) and analysed using the GenBank BLAST program (Altschul *et al.*, 1990). The protein sequence analysis was performed using the GPMAW program (Kyte and Doolittle, 1982).

4.3.4 Detection of BLV *env* gene

A 434 bp fragment of the BLV *env* gene was PCR amplified from the genomic DNA and cDNA using 5' sense primer (5' GTCTCCCAGATACACCTTGG 3') and the 3' anti-sense primer (5' GAAGGTTCCCAACATATAGC 3') corresponding to the BLV genome sequence spanning 5042-5061 bp and 5457-5476 bp, respectively (Murtaugh *et al.*, 1991). The PCR reaction conditions involved denaturation at 94^{0}C for 1 min, annealing at 56^{0}C for 0.5 min and extension at 72^{0}C for 0.5 min for 30 cycles. The PCR products were analysed by agarose gel electrophoresis. The DNA samples from the PBLs of BLV-infected and normal cattle and X63.Ag8.653 mouse myeloma cells were included as positive and negative controls.

4.3.5 ^{35}S-cysteine internal radiolabeling of antibodies and SDS-PAGE

The IgM secreting hetero-hybridomas, BLV1H12 and BLV2C7 (Saini *et al.*, 1997), and X63.Ag8.653 mouse myeloma cells were grown overnight in cysteine-free medium (RPMI-1640 Select-Amine Kit; GIBCO, BRL) supplemented with 100 μCi of ^{35}S-labeled L-cysteine (Du Pont Canada Inc., Markham, ON, Canada).The supernates were dialysed extensively in PBS containing cold 10^{-2} M cysteine. Fifty microlitres of 2-mercaptoethanol-denatured supernates were run in 10% SDS-PAGE at 180 volts for 2 h. The gel was fixed

(40% methanol and 10% acetic acid) for 30 min, soaked in Amplify solution (Amersham Canada Ltd., Oakville, ON, Canada) for 30 min, dried in a gel dryer (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada) at 80°C for 2 h and exposed to XAR-5 film (Eastman Kodak Com., Rochester, NY, USA).

4.3.6 Antibody specificity

The specificity of IgM antibodies was determined against a panel of test antigens including bovine serum albumin (BSA) fraction V (Boehringer Mannheim, Germany), bovine thyroglobulin, ubiquitin, trimethyl (p-aminophenyl)-ammonium chloride (TMA) conjugated to BSA (substitution of 10 TMA molecules/molecule of BSA), TNP (2,4,6-trinitrobenzenesulfonic acid) conjugated to BSA (substitution of 26 TNP molecules/molecule of BSA), actin (all from Sigma Chemical Co.), mouse IgG1 (Chemicon International Inc., Temecula, CA, USA), recombinant mycobacterial heat shock protein hsp65 and hsp70 (courtesy of Dr. J.D.A. van Embden, UNDP/World Bank/ WHO program, Geneva, Switzerland) in an ELISA (Chapter 3). The ELISA plates were coated overnight (4°C) with test antigens at coating concentrations: 5μg/ml (BSA), 2.5μg/ml (actin, bovine thyroglobulin, hsp65, hsp70, mouse IgG1 and ubiquitin) and 10 μg/ml (TMA and TNP). The biotin-conjugated murine monoclonal anti-bovine IgM (Sigma Chemical Co.) was used in 1:25000 dilution as second antibody followed by addition of 1:150 dilution of avidin-conjugated alkaline phosphatase (Pierce, Rockford, IL, USA). The alkaline phosphatase substrate p-nitrophenyl phosphate (Sigma Chemical Co.), diluted to a concentration of 1 mg/ml in 10% diethanolamine buffer, was added. The colour reaction was measured at 405 nm using an ELISA reader (Ceres UV 900 Hdi, Bio-Tek Instruments Inc., Winooski, VT). Bovine serum

(1:500 dilution) and RPMI were included as positive and negative controls, respectively.

4.4 Results

4.4.1 VDJ rearrangements encoding exceptionally long CDR3H occur in cattle

Two types of VDJ rearrangements were noted in bovine IgM-secreting hybridomas, originating from the blood lymphocytes of a BLV-infected cow. The first type of rearrangements noted in four clones, BLV1H12, BLV5B8, BLV5D3 and BLV8C11 (approximately 9% frequency), show a characteristic CDR3H length ranging from 56 to 61 codons (Fig. 4.1; Table 4.1). The codon TGT, that encodes cysteine, was conserved in the middle of CDR3H (position 100j11; Fig. 4.1) of these VDJ rearrangements. All the four clones showed nucleotide homology ranging from 95.9 to 97.7% (Table 4.1) with a single germline bovine V_H gene, gl.110.20 (Dr. R. A. Goldsby, Amherst College, Amherst, MA, USA) and, thus seem to be encoded by a single or closely related germline V_H genes. Few single point mutations resulting in amino acid replacements were noted in the CDR2H but such nucleotide substitutions were not evident in the CDR1H (Fig. 4.2). The second group of VDJ rearrangements comprised a majority of clones with the CDR3H length ranging from 4 to 31 codons. None of these clones were encoded by the g1.110.20 germline gene, but shared highest nucleotide homology (85.6 to 97.2 %) with three available germline V_H gene sequences, clone 5 (GenBank BTU55168), clone 6 (GenBank BTU55172) and clone 10 (GenBank BTU55171); (Table 4.1). Single point mutations resulting in amino acid replacements were evident both in CDR1H and CDR2H of these IgM antibodies (Fig. 4.2). Two clones, BLV1F3 and BLV3D7, expressed identical VDJ rearrangements but these paired

Fig. 4.1 The nucleotide (A) and the deduced amino acid (B) sequences of bovine VDJ rearrangements from IgM-secreting mouse x cattle hetero-hybridomas. The codons are numbered according to Kabat *et al.* (1991) and the CDRs are shown in bold letters. The sequences are available in GenBank (Accession numbers: AF015490 to AF015506). The dash (-) indicates identical nucleotide base or amino acid and dot (.) indicates a lack of nucleotide base or amino acid at the particular position.

```
          1                                   10                                  20
BLV1H12 CAG GTG CAG CTG CGC GAG TCG GGC CCC AGC CTG GTG AAG CCG TCA CAG ACC CTC TCG CTC ACC TGC ACG GCC TCT GGA
BLV5B8  --- --- --- --- --G --- --- --- --- --- --- --- C-- --C --- --- --- --- --C --- --- --- --- --- --- ---
BLV5D3  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV8C11 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

BLV1F3  --- --- --- --- --G --- --A --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --- -T- --- ---
BLV1G9  --- --- --- --- --G --- --- --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --- AT- --- ---
BLV3D7  --- --- --- --- --G --- --A --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --- -T- --- ---
BLV3D11 --- --- --- --- --G --- --A --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --- -T- --- ---
BLV4B8  --- --- --- --- --G --- --A --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --- -T- --- ---
BLV6D10 --- --- --- --- --G --- --- --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --- -T- --- ---
BLV7A8  --- --- --- --- --G --- --- --- --- -A- --- --- --- --A --- -T- --- --- --C --- --- --- --- -T- --- ---
BLV7D7  --- --- --- --- --G --- --A --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --- -T- --- ---
BLV7F8  --- --- --- --- --G --- --- --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --- -T- --- ---
BLV9B8  --- --- --- --- --G --- --- --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --- AT- --- ---
BLV9C12 --- --- --- --- --G --- --- --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --- AT- --- ---
BLV9H3  --- --- --- --- --G --- --- --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --A -T- --- ---
BLV10H2 --- --- --- --- --G --- --- --- --- --- --- --- --- --- --C --- --- --- --- --C --- --- --- --- A-- --- ---
BLV11F3 --- --- --- --- --G --- --- --- --- G-- --- --- --- --C --- --- --- --- --C --- --- --- --- A-- --- ---


                    30                                  40                                  50
BLV1H12 TTC TCA TTG AGC GAC AAG GCT GTA GGC TGG GTC CGC CAG GCT CCA GGG AAG GCG CTG GAG TGG CTC GGT AGT ATA GAC
BLV5B8  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV5D3  --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -C- -C- ---
BLV8C11 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

BLV1F3  --- --- --- -A- --- T-T -G- --T AAA --- --- --- --- --- --G --- --- --A --- --- --- G-T --- GT- --G TGG
BLV1G9  --- --- --- --- AG- T-T T-- --- -T- --- --- --- --- --- --- --- --- --- --- --- --A --T --T -C- -C- --T -GT
BLV3D7  --- --- --- -A- --- T-T -G- --T AAA --- --- --- --- --- --G --- --- --A --- --- --- G-T --- GT- --G TGG
BLV3D11 --- --- --- --- AT- T-T A-- --- -C- --- --- --- --- --- --G --- --- --A --- --- --- G-T --- -CA --T CGT
BLV4B8  --- --- --- --- AG- T-T --- --- --- --- --- --- --- --G --- --- --A --- --- --- G-T --- G-- --- ACG
BLV6D10 --- --- C-- --- AGA T-T T-- --- -T- --- --- --- --- --- --- --- --- --- --- --- --- G-T --- G-- --G TTA
```

Fig. 4.1 A (Continued)

```
BLV7A8  --- --- --A --- -G- GCT A-- --- --- --- --- --- --- --- T-- --A --- --- --- --- --- --G A-- GTC TAC ACT
BLV7D7  --- --- --- --- AGT T-T --- --- --- --- --- --- --- --- --G --- --- --A --- --- --- G-T --- G-- --G AG-
BLV7F8  --- --- --- --- AG- T-T A-- --- AT- --- --- --- --- --- --- --A --- --- --- --- --- --T --- --- GG- AGG
BLV9B8  --- --- --- --- AG- T-T A-- --- -A- --- --- --- --- --- --G --- --- --- --- --- --- G-T --- CAG --- A-T
BLV9C12 --T --- A-- --- AG- T-T -G- --- -A- --- --- --- --- --- --G --- --- --- --- --- --- G-T --- G-A --- C-T
BLV9H3  --- --- --- --- A-- --T -G- --- TT- --- --- --- --- --- --- --- --- --- --- --- --- --- --- G-- --- TG-
BLV10H2 --- --- C-- --- AG- GCT A-- --- -A- --- --- --- --- --- --G --- --- --- --- --- --- G-T --- G-- --- --T
BLV11F3 --- --- --- --- -G- TG- -A- --- --- --- --- --- --- --- --G --- --- --- --- --- --- A-T --- G-- --T A-T
```

```
                                                  60                                                  70
BLV1H12 ACT GGT GGA AAC ACA GGC TAT AAC CCA GGC CTG AAA TCC CGG CTC AGC ATC ACC AAG GAC AAC TCC AAG AGT CAA GTC
BLV5B8  --- --- --- -G- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- ---
BLV5D3  --- --- --- -G- G-- -C- --- --- --- --- --- --- --- --A --- --- --- --- --- --- --- --- --C --- ---
BLV8C11 G-- -CA --- -G- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- ---

BLV1F3  -G- --A --G --- T-C -AT --- --- --- -C- --- --- --- --- --- --- --- --- --- --- --- --- --C --- ---
BLV1G9  -G- CTC --- GG- --- -A- --C --- --- -C- --- --- --- --- --- --- --- --- --- --- --- --- --C --- ---
BLV3D7  -G- --A --G --- T-C -AT --- --- --- -C- --- --- --- --- --- --- --- --- --- --- --- --- --C --- ---
BLV3D11 G-- --- --- --- -T- -A- CG- --- --- -C- --A G-- --- --T G-- --- --- -G- --- --- --- --- -G- --C --- ---
BLV4B8  -G- --- --- -GG --- -T- --- --T --- -C- --- --- --- --- --- -C- --- --- --- --- --- --- --- --C --- ---
BLV6D10 --- -A- --- CT- --- TA- --- --T --- -C- --- --- --- --- --- G-- --- --- -G- --- -C- --- --- --C -CC ---
BLV7A8  TA- AAC --- -TA G-- -A- --- --- --- -C- --- --- --- --- --- -C- --- --- --- --- -GT --- --- --C --- T--
BLV7D7  -G- --- --- -G- --- AA- --- --- --- -C- --- --- --- --- --- --G --G --- --- --- --G --- --- --C --- ---
BLV7F8  TA- -A- --- T-- --- TCG --- --T T-- -C- --- --- --- --- --- --- --- --G --- --- --- --- C-- --C --- ---
BLV9B8  -G- --- --- -G- --- T-- CT- --- --- -C- --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- ---
BLV9C12 G-- A-- --- -GT --- TA- CG- --- -T- -C- --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- ---
BLV9H3  GG- --- --C GG- --- ACA -T- --- --- -C- --- --- --- --- --- --- --- --- --- --- -C- --- --- -AC --G ---
BLV10H2 -A- TA- --- G-- -T- T-T --- --- --- -C- --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- ---
BLV11F3 T-G -CG --- GCG --- T-- CTC --T T-- -C- --- --- --- --T --- --- --- --G --- --- --- --- --- --C --- ---
```

Fig. 4.1 A (Continued)

```
            80       82  a   b   c                                  90                  95                  100  a
BLV1H12 TCT CTG TCA GTG AGC AGC GTG ACA ACT GAG GAC TCG GCC ACA TAC TAC TGT ACT TCT GTG CAC CAG GAA ACA AAA AAA
BLV5B8  --- --- --- --- --- --- --- --G --G --- --- --- --- --- --- --- --- --- A-- --- --- --A --- --C -G- ---
BLV5D3  --- --- --- --- --- -A- --- G-- --G --- --- --- --- --- --- --- --- -G- AG- --A ACT --- AG- --C C-C GT-
BLV8C11 --- --- --- --- --- --- --- G-- G-- --- --- --- --- --- --- --- --- --- A-- --A --- --A A-- --A -C- -G-

BLV1F3  --- --- --- --- --- --- --- --- C-- --- --- A-- --- --- --T --- --- G-A A-G TAC GC- ... ... ... ... ...
BLV1G9  --- --- --- --- --- --- --- T-- --- --- --- A-- --- --- --- --- --- G-G AAG TCA A-T AGT CCT TAC TGC TTT
BLC3D7  --- --- --- --- --- --- --- --- C-- --- --- A-- --- --- --T --- --- G-A A-G TAC GC- ... ... ... ... ...
BLV3D11 --- --- --- --A --- GA- --- --- C-- --C --- G-- --- --- --- --- --- G-C AAA AAT ACG G-T -GC CAT TCT CTT
BLV4B8  --- --- --- --A -AT --- --- --- C-- --- --- A-- --- --- --- --- --- G-A AAA AGT TGT GCT A-T GAT C-T CCT
BLV6D10 --- --C --- --- --- --- --A G-- --- --- --- A-- --- --- --T --- --- G-G AAG TGC AGT GTT AC- CAT GGT G-T
BLV7A8  -TC --- --- C-- -A- --- --- --- C-- --- --- A-- --- --- --T --- --C -TA AGA TG- ACT GGA -GT GGC G-T --C
BLV7D7  --- --C --- --- --T G-A --- --- C-- --- --- A-- --- --- --- --- --- T-A AAA --C ACT GGT -GG --T T-T GGT
BLV7F8  --- --- --- --- --- --- --- --- --- --- --- A-- --- --- --- --- --C G-G AAG TGT -TG TCT -GT GGT GGC -CG
BLV9B8  --- --- --- C-- --- --- --- --- --- --- --- A-- --- --- --- --- --- G-G AAA AAT ACA --C ACT GGT TGT -CG
BLV9C12 --- --- --- C-- --- --- --- --- --- --- --- A-- --- --- --- --- --- G-G AAG -GT AGT AGT CGC --C GCT GCG
BLV9H3  --- --- --- --- --- --- --- --- C-- --- --- AT- --- --- --- --- --- G-A AAA AAT GGT GGT CCG CGT TGT C-G
BLV10H2 --- --A --- --- --- --- --- --- --- --- --- A-- --- --- --- --- --- G-G AAA AG- A-A -CT -G- TAT G-T G-T
BLV11F3 --- --- --- C-- --- --- --- -TG -G- --- --- A-- --- --- --- --- --- GGA AGA ACA GGT GGA -GT GGT TGG GCG


         b   c   d   e   f   g   h   i   j   j1  j2  j3  j4  j5  j6  j7  j8  j9 j10 j11 j12 j13 j14 j15 j16 j17
BLV1H12 TAC CAA AGT TGT CCT GAT GGG TAT AGA GAA CGT TCG GAT TGT AGT AAC AGA CCT GCT TGT GGT ACT AGT GAT TGT TGT
BLV5B8  AC- TGT TC- GA- GG- T-- ATT GC- GT- --T A-- -GT -G- C-- G-- C-G --T GA- -G- --- -TC -A- GA- TGC AA- -CA
BLV5D3  -CA -GT --- --- --- --- --T -G- --T --T G-- GAT -G- --- GT- G-T G-T TG- TG- --- A-- G-A TA- CG- --- -A-
BLV8C11 AAA ACT T-- --- T-- --- -CT --- --- T-T GA- AGT -G- --- G-- -GT G-T TG- -A- --- T-- GG- GCC --- --- -A-

BLV1F3  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV1G9  CGT TGT GT- A-- AG- TC- TAC CC- GAT CGC ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV3D7  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV3D11 -TT A-T GAG GAG TA- AG- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV4B8  --T G-T CA- CT- TG- -G- -AT GG- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV6D10 ATT GGT --- ATG GGA T-- ATA A-- GCT -G- --- -AT -GG -A- -A- -GT G-T GAG A-C GCG ... ... ... ... ... ...
```

Fig. 4.1 A (Continued)

```
BLV7A8  A-T G-T T-G CTG ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV7D7  --T AT- GAA A-- ATC ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV7F8  --T TT- T-- -A- --C AGT --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV9B8  --T AGT TA- GT- GAC ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV9C12 ATT TGT --- A-- GG- A-- TAT ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV9H3  -TT ATT TA- CC- TA- --- ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV10H2 --T G-T TA- G-- GA- T-- -AC -G- TAT AGT TA- GGT --- CAC TAC TTT ... ... ... ... ... ... ... ... ... ...
BLV11F3 G-G TGC -CG G-- A-A TG- -C- -G- TAC TAT GCC ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

        j18 j19 j20 j21 j22 j23 j24 j25 j26 j27 j28 j29 j30 j31 j32 j33 j34 j35 j36 j37 j38 j39 j40 j41 j42 k
BLV1H12 CGT GTT AGT GTT TTT GGT AAT TGT CTT ACT ACT CTT CCT GTG AGT TAT AGT TAT ACT TAC AAT TAC GAA TGG CAC GTC
BLV5B8  T-- TA- TA- -G- -GG C-G --C --- -GC -GG CAG -C- G-A ... ... ... ... ... -T- C-- -G- --- --- -TT --- ---
BLV5D3  AC- CC- G-- --- CG- -A- CT- A-- TG- --C ... ... ... ... --- --- --- AT- --- --T -C- --- --- --- A-- ---
BLV8C11 GT- T-- G-- -C- -G- AC- TT- G-- --- GA- -G- ... ... ... --- --- --- --- -T- --T -T- --- C-- --- T-- ---

BLV1F3  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV1G9  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... T-- A--
BLV3D7  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV3D11 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... T-- C--
BLV4B8  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV6D10 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... A-- ---
BLV7A8  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV7D7  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... T-- ---
BLV7F8  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ---
BLV9B8  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... G-- A--
BLV9C12 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... --G
BLV9H3  ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... T-- A--
BLV10H2 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
BLV11F3 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... T-- ---
```

Fig. 4.1 A (Continued)

										110			
BLV1H12	**GAT**	**GTC**	TGG	GGC	CAA	GGA	CTC	CTG	GTC	ACC	GTC	TCC	TCA
BLV5B8	---	**-C-**	---	---	-G-	---	---	---	---	---	---	---	---
BLV5D3	---	**-C-**	---	---	---	---	---	---	---	---	---	---	---
BLV8C11	---	**-C-**	---	---	---	---	---	---	---	---	---	---	---
BLV1F3	**AG-**	**-C-**	---	---	---	---	---	---	---	---	---	---	---
BLV1G9	---	**-C-**	---	---	---	---	---	---	---	---	---	---	---
BLV3D7	**AG-**	**-C-**	---	---	---	---	---	---	---	---	---	---	---
BLV3D11	---	**-C-**	-AC	---	---	---	---	---	--T	--T	--G	---	---
BLV4B8	**AG-**	**-C-**	---	---	---	---	---	---	---	---	---	---	---
BLV6D10	---	**-C-**	---	---	---	---	---	---	---	---	---	---	---
BLV7A8	**T--**	**ACT**	---	---	---	---	---	---	---	---	---	---	---
BLV7D7	---	**-C-**	---	---	---	---	---	---	---	---	---	---	A--
BLV7F8	---	**-C-**	---	---	---	---	---	---	---	---	---	---	---
BLV9B8	---	**-C-**	---	---	--G	---	--A	---	---	---	---	---	---
BLV9C12	---	**TCT**	---	---	---	---	---	---	---	---	---	---	---
BLV9H3	---	**-C-**	---	---	---	---	---	---	---	--A	---	---	---
BLV10H2	---	**-C-**	---	---	---	---	---	A-T	---	---	---	---	---
BLV11F3	---	**TC-**	---	---	---	---	---	---	---	---	---	---	---

Fig. 4.1 A

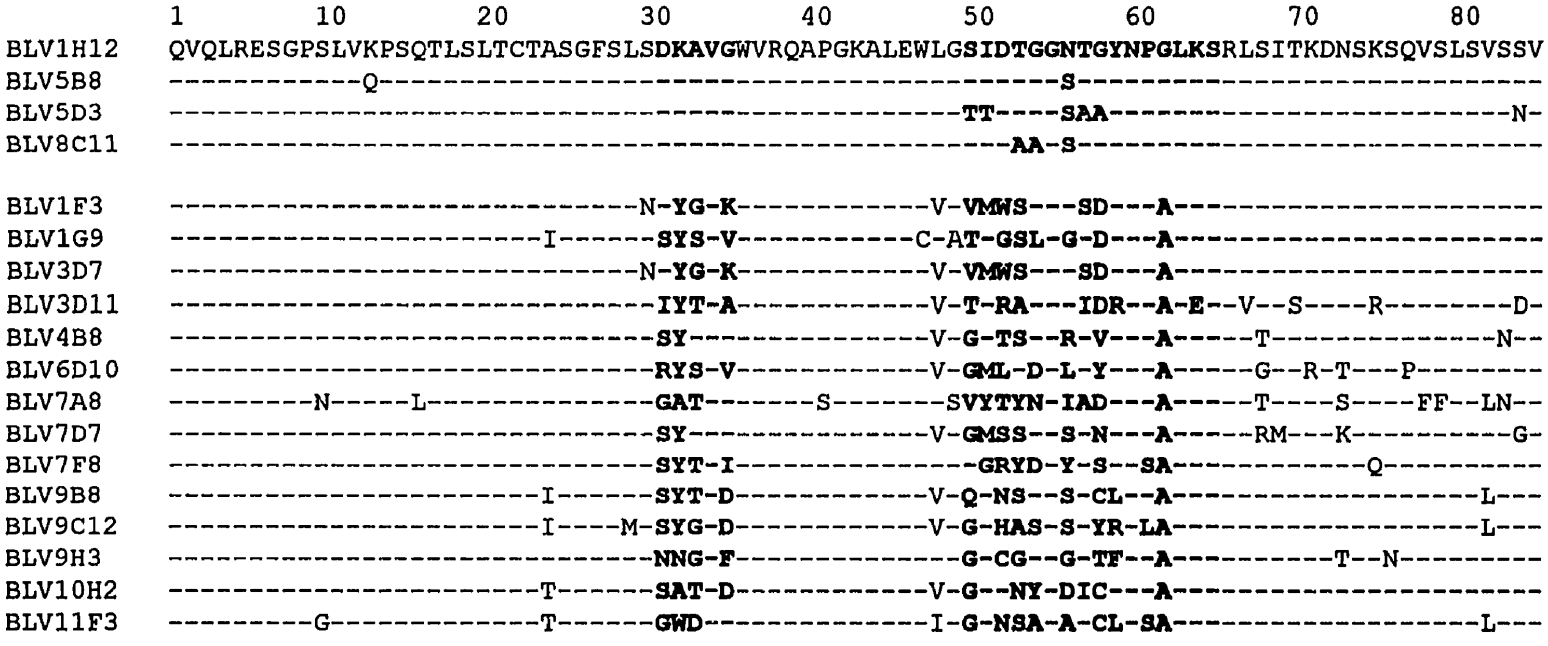


Fig. 4.1 B (Continued)

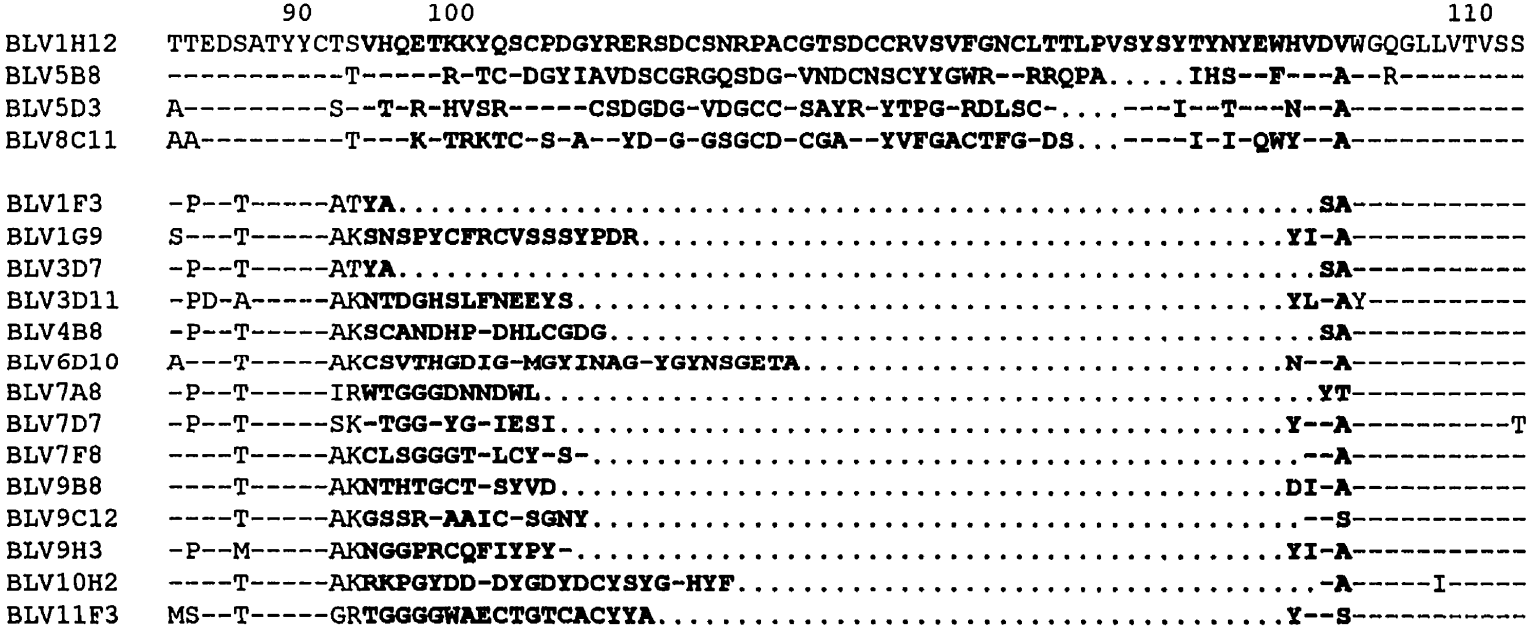

90
100
110
BLV1H12 TTEDSATYYCTSVHQETKKYQSCPDGYRERSDCSNRPACGTSDCCRVSVFGNCLTTLPVSYSYTYNYEWHVDVWGQGLLVTVSS
BLV5B8 ----------T-----R-TC-DGYIAVDSCGRGQSDG-VNDCNSCYYGWR--RRQPA.....IHS--F---A--R--------
BLV5D3 A---------S--T-R-HVSR-----CSDGDG-VDGCC-SAYR-YTPG-RDLSC-....---I--T---N--A----------
BLV8C11 AA---------T---K-TRKTC-S-A--YD-G-GSGCD-CGA--YVFGACTFG-DS...----I-I-QWY--A----------
BLV1F3 -P--T-----ATYA.....SA----------
BLV1G9 S---T-----AKSNSPYCFRCVSSSYPDR.....YI-A----------
BLV3D7 -P--T-----ATYA.....SA----------
BLV3D11 -PD-A-----AKNTDGHSLFNEEYS.....YL-AY----------
BLV4B8 -P--T-----AKSCANDHP-DHLCGDG.....SA----------
BLV6D10 A---T-----AKCSVTHGDIG-MGYINAG-YGYNSGETA.....N--A----------
BLV7A8 -P--T-----IRWTGGGDNNDWL.....YT----------
BLV7D7 -P--T-----SK-TGG-YG-IESI.....Y--A----------T
BLV7F8 ----T-----AKCLSGGGT-LCY-S-.....--A----------
BLV9B8 ----T-----AKNTHTGCT-SYVD.....DI-A----------
BLV9C12 ----T-----AKGSSR-AAIC-SGNY.....--S----------
BLV9H3 -P--M-----AKNGGPRCQFIYPY-.....YI-A----------
BLV10H2 ----T-----AKRKPGYDD-DYGDYDCYSYG-HYF.....-A-----I-----
BLV11F3 MS--T-----GRTGGGGWAECTGTCACYYA.....Y--S----------

Fig. 4.1 B

Fig. 4.2. Comparison of nucleotide sequence of V_H gene-encoded CDR1 and CDR2 regions of bovine VDJ rearrangements with closest available germline bovine V_H genes (*Dr. R.A. Goldsby, Amherst College, Amherst, MA, USA and ** GenBank).

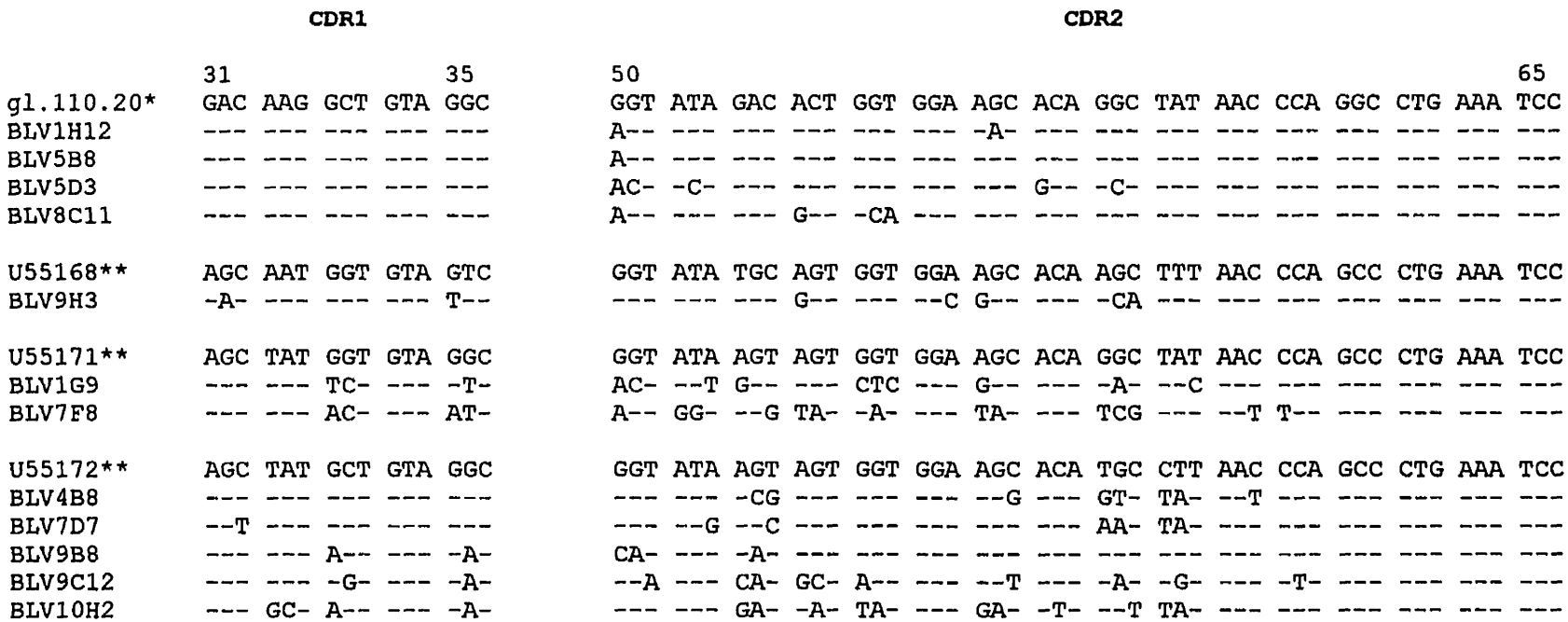

CDR1
CDR2
31
35
50
65
gl.110.20* GAC AAG GCT GTA GGC GGT ATA GAC ACT GGT GGA AGC ACA GGC TAT AAC CCA GGC CTG AAA TCC
BLV1H12 --- --- --- --- --- A-- --- --- -A- --- --- --- --- --- --- --- ---
BLV5B8 --- --- --- --- --- A-- --- --- --- --- --- --- --- --- --- --- ---
BLV5D3 --- --- --- --- --- AC- -C- --- --- --- --- --- G-- -C- --- --- --- --- --- --- ---
BLV8C11 --- --- --- --- --- A-- --- --- G-- -CA --- --- --- --- --- --- --- --- --- --- ---
U55168** AGC AAT GGT GTA GTC GGT ATA TGC AGT GGT GGA AGC ACA AGC TTT AAC CCA GCC CTG AAA TCC
BLV9H3 -A- --- --- --- T-- --- --- --- G-- --- --C G-- --- -CA --- --- --- --- --- --- ---
U55171** AGC TAT GGT GTA GGC GGT ATA AGT AGT GGT GGA AGC ACA GGC TAT AAC CCA GCC CTG AAA TCC
BLV1G9 --- --- TC- --- -T- AC- --T G-- --- CTC --- G-- --- -A- --C --- --- --- --- --- ---
BLV7F8 --- --- AC- --- AT- A-- GG- --G TA- -A- --- TA- --- TCG --- --T T-- --- --- --- ---
U55172** AGC TAT GCT GTA GGC GGT ATA AGT AGT GGT GGA AGC ACA TGC CTT AAC CCA GCC CTG AAA TCC
BLV4B8 --- --- --- --- --- --- --- -CG --- --- --- --G --- GT- TA- --T --- --- --- --- ---
BLV7D7 --T --- --- --- --- --- --G --C --- --- --- --- --- AA- TA- --- --- --- --- --- ---
BLV9B8 --- --- A-- --- -A- CA- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV9C12 --- --- -G- --- -A- --A --- CA- GC- A-- --- --T --- -A- -G- --- -T- --- --- --- ---
BLV10H2 --- GC- A-- --- -A- --- --- GA- -A- TA- --- GA- -T- --T TA- --- --- --- --- --- ---

Fig. 4.2.

Table 4.1 Characteristics of bovine IgM-secreting hetero-hybridomas from a BLV infected cow.

Clone number	Presence of *env* gene of BLV		CDR3H Length-Amino acids (cysteine residues)	%Homology (closest germline V_H gene-codon 1 to 92)	V_λ1 sub-family (% homology with germline gene-codon 1 to 81)*	PI Value		Antibody** specificity
	Genomic DNA	cDNA				VDJ	VJ	
BLV1H12	+	-	61(6)	97.7[a]	V_λ1x (99.2)	7.47	4.28	Polyspecific
BLV5B8	+	+	56(6)	97.3[a]	V_λ1x (99.2)	7.48	4.14	Polyspecific
BLV5D3	+	+	57(8)	95.9[a]	V_λ1x (96.8)	5.66	4.05	Unknown
BLV8C11	+	-	58(8)	96.7[a]	V_λ1***	6.64	9.18	Unknown
BLV1F3	-	-	4	90.5[b]	V_λ1a (98.4)	8.70	8.43	Unknown
BLV1G9	-	-	21(2)	93.3[c]	V_λ1a (95.6)	8.36	6.41	Unknown
BLV3D7	-	-	4	90.5[b]	V_λ1a (97.2)	8.70	8.43	Unknown
BLV3D11	-	-	17	89.1[d]	V_λ1a (94.8)	5.10	6.41	Unknown
BLV4B8	-	-	17(2)	94.0[d]	V_λ1a (97.2)	8.20	6.41	Unknown
BLV6D10	-	-	31(1)	90.2[d]	V_λ1a (97.2)	8.39	6.41	Unknown
BLV7A8	-	-	13	85.6[c]	V_λ1a (96.8)	8.14	8.14	Unknown
BLV7D7	+	-	16	93.3[d]	V_λ1b (96.4)	9.35	6.98	Unknown
BLV7F8	-	-	17(2)	91.2[c]	V_λ1a (96.8)	8.20	6.41	Unknown
BLV9B8	-	-	16(1)	97.2[d]	V_λ1b (95.9)	6.84	6.41	Unknown
BLV9C12	+	-	17(1)	94.4[d]	V_λ1a (96.4)	9.18	4.50	Unknown
BLV9H3	+	+	17(1)	95.4[b]	V_λ1a (97.2)	8.60	6.41	Unknown
BLV10H2	+	-	25(1)	93.3[d]	V_λ1b (99.2)	4.77	6.41	Unknown
BLV11F3	-	-	22(3)	90.5[d]	V_λ1b (96.8)	7.58	5.47	Unknown

[a]g1.110.20 (from codon 1 to 73; R.A. Goldsby, personal communication), [b]clone '5' (GenBank BTU55168), [c]clone '10' (GenBank BTU55171) and [d]clone '6' (GenBank BTU55172).
*Germline bovine V_λ1a (clone 8), V_λ1b (clone 17) and V_λ1x (clone 27) genes are from Sinclair *et al.* (1995).
**The specificity of all IgM antibodies was determined against a panel of antigens (BSA, thyroglobulin, ubiquitin, TNP, TMA, actin, mouse IgG1, r-Hsp 65 and r-Hsp 70) in an ELISA. Polyspecific antibodies recognized all test antigens whereas remaining antibodies did not recognize any of the test antigens.
***Unclassified novel bovine V_λ1 gene.

with distinct VJ rearrangements. The J_H encoded region of all the VDJ rearrangements could be classified into either of the two J_H genes identified earlier (Saini *et al.*, 1997). It could be argued that generation of exceptionally long CDR3H may be associated with BLV transcription and leukemiagenesis. The detection of BLV *env* gene in the genomic DNA and its transcription in several clones (Table 4.1) confirmed their origin from BLV-infected cattle, however, the transcription of *env* gene was not confined to B cells expressing VDJ rearrangements resulting in exceptionally long CDR3H. Further, CDR3H length > 50 amino acids was also observed in two hetero-hybridomas (BF1H1 and BF4E9) originating from the splenic B-cells of a 125-day-old bovine fetus (Chapter 5). Thus, it is likely that generation of long CDR3H is not related to transcriptional events associated with BLV leukemiagenesis.

A comparison of V_H encoded region of the VDJ rearrangements with the available germline V_H gene sequences, especially CDR1H and CDR2H (Fig. 4.2), confirms our previous observations that single point hypermutations contribute to the diversification of IgM antibody in cattle (Saini *et al.*, 1997). The consensus hot-spot triplet AGC/AGT occurs at a higher frequency in the CDR1H (AGPy : TCN = 2.5) and FR3 (AGPy : TCN =1.15) as compared to CDR2H (AGPy : TCN = 0.8). There is no evidence for gene conversion in the V_H encoded region of the cDNAs analysed. As for the light-chain, few single point mutations were evident in the $V_\lambda 1$ gene encoded region. Similar to V_H gene, the consensus hot-spot triplet occurs at a higher frequency in CDR1 (AGPy : TCN = 3.8) as compared to CDR2 (AGPy : TCN = 1.3) of the $V_\lambda 1$ gene. Overall, these findings suggest that generation of exceptionally long CDR3H length and single point hypermutations contribute to the diversification of the IgM antibody repertoire in cattle.

4.4.2 Multiple cysteine amino acid residues are characteristic of long CDR3H

A majority of the bovine antibodies analysed had, at least, one cysteine residue in the CDR3H region. Those with exceptionally long CDR3H had 6 to 8 cysteine residues with one of these being remarkably conserved in the middle (position 100j11) of the CDR3H region (Fig. 4.1). It seems that even numbered cysteine residues form intra-CDR3H disulfide bridges that provide rigidity and an unique configurational ability to the antigen combining site for interaction with the ligand. An analysis of amino acid distribution in the FRs and CDRs encoded by V_H genes revealed that the hydrophilic amino acid serine is used at a higher frequency in the FRs (18.4%) of bovine antibodies as compared to the FRs of murine (12.1%) and camel (11.4%) antibodies (Padlan, 1990; Muylderman *et al.*, 1994). The average PI values for variable regions of heavy and light chains are 7.63±1.3 and 6.38±1.47, respectively. An unique hydrophilic region in the heavy chain FR3 is common to all the bovine IgM antibodies studied.

4.4.3 VDJ rearrangements resulting in long CDR3H pair with V_λ1x or novel V_λ1 genes

All VJ rearrangements analysed were encoded by bovine V_λ1 gene family, predominantly by V_λ1a and V_λ 1b subfamilies (Fig. 4.3; Table 4.1). None of the VDJ rearrangements with unusually long CDR3H paired with VJ rearrangements encoded by the members of the V_λ1a and V_λ1b subfamilies. But three such clones, BLV1H12, BLV5B8 and BLV5D3, paired with VJ rearrangements encoded by V_λ1x gene, earlier noted to be not expressed (Sinclair *et al.*, 1995). Another VDJ rearrangement, BLV8C11, paired with VJ rearrangement encoded by a novel V_λ1 gene that did not share ≥91% nucleotide homology with any of the existing V_λ1 subfamilies (Sinclair *et al.*, 1995). Thus VDJ rearrangements

Fig. 4.3 The nucleotide (A) and the deduced amino acid (B) sequences of bovine VJ rearrangements from IgM secreting mouse x cattle hetero-hybridomas. The codons are numbered according to Kabat *et al.* (1991) and the CDRs are shown in bold letters. The sequences are available in the GenBank (Accession numbers: AF015791 to AF015795, AF01598 to AF015801, AF015804, AF015805 to AF015809, AF023841, AF023843 and AF109697). The $V_{\lambda}1$ genes are grouped into subfamilies and the closest germline gene of each subfamily is included for comparison (Sinclair *et al.*, 1995). The dash (-) indicates identical nucleotide base or amino acid and dot (.) indicates a lack of nucleotide base or amino acid at the particular position.

```
          1                                   10                                                  20
BLV1F3   CAG GCT GTG CTG ACT CAG CCG TCC TCC ... GTG TCC GGG TCC CTG GGC CAG AGG GTC TCC ATC ACC TGC TCT GGA AGC
BLV1G9   --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --G --- ---
BLV3D7   --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV3D11  --- --- --- AA- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- -G- --- ---
BLV4B8   --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV6D10  --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV7A8   --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV7F8   --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV9C12  --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV9H3   --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Vλ1a(8)  --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

BLV7D7   --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV9B8   --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV1OH2  --- --- --- --- --- --- --- --- --- ... --- --- --C --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV11F3  --- --- --- --- --- --- --- --- --- ... --- --- --C --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Vλ1b(17) --- --- --- --- --- --- --- --- CT- ... --- --- --C --- --- --- --- --- --- --- --- --- --- --- --- --- ---

BLV1H12  --- --- --- --- -A- --- --A --A --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV5B8   --- --- --- --- -C- --- --A --A --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV5D3   --- --- --- --- -C- --- --A G-A --- ... --- --- --- --- --- --- --- --T --- --- --- --- --- --- --- --- ---
Vλ1x(27) --- --- --- --- -C- --- --A --A --- ... --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---


         27  a   b   C                                                          40
BLV1F3   AGC AGC AAC GTT GGA TAT GGC AGT TAT GTG AAC TGG TTC CAA CAG ATC CCA GGA TCG GCC CCC AGA ACC CTC ATC TAT
BLV1G9   --- --- --- --- --G CG- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --T ---
BLV3D7   --- --- --- --- --G CG- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV3D11  --- --- --- --- --G CG- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --T ---
BLV4B8   --- --- --- --- --G CG- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV6D10  --- --- --- --- --G CG- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
```

Fig. 4.3 A (Continued)

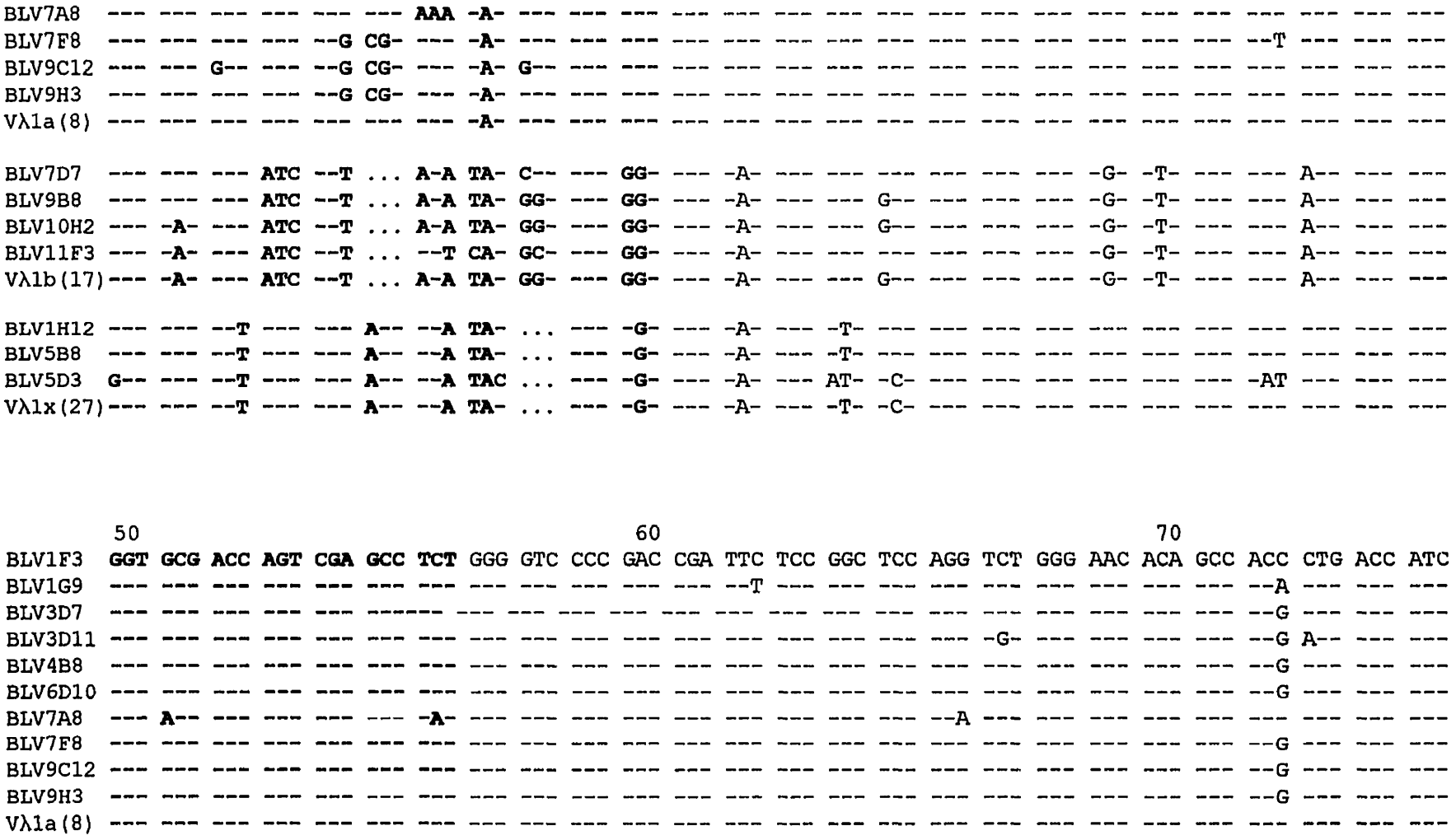


Fig. 4.3 A (Continued)

```
BLV7D7   --- AGT -G- -C- --- C-- --G --- --- --- --- --- --- --- --- --- -A- --- --C --- --- --- --- --T --- ---
BLV9B8   AA- AGT -G- --- --- --- --- --- --- --- --- --- --- --- --- --- -G- --- --G --- --- --- --- --- --- ---
BLV10H2  --- AGT -G- --- --- C-- --G --- --- --- --- --- --- --- --- --- -A- --- --C --- --- --- --- --- --- ---
BLV11F3  --- AGT GG- --- --- C-- --G --- --- --- --- --- --- --- --- --- -A- --- --C --- --- --- --- --- --- ---
Vλ1b(17)--- AGT -G- --- --- C-- --G --- --- --- --- --- --- --- --- --- -A- --- --C --- --- --- --- --- --- ---

BLV1H12  --- -AC --- --- --- --- --G --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV5B8   -A- -AC --- --- --- --- --G --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BLV5D3   --- -AC --- --- --- --- --G --- --- --- --- --- --- --- --- --- --- --- --C --- --- --- --- --- --- ---
Vλ1x(27)--- -AC --- --- --- --- --G --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                        80                                          90                  95  a   b   c
BLV1F3   AAC TCG CTT CAG GCC GAG GAC GAG GCG GAT TAT TTC TGT ACA TCT TAT CAC AGT AGT AGC AAA AAT ... AGA GTG TGC
BLV1G9   -C- --- --C --- --- --- --- --- --- --- --- --- --- G-- A-- --- G-- --C --- --T --T --- ... G-T --T -T-
BLV3D7   -G- --- --C --- --- --- --- --- --- -C- --- --- --- G-- GT- --- G-- --C --- --- --T --- ... GCT --T -T-
BLV3D11  -G- --- --C --- --- --- --- --- --- --- --- --- A-- G-- GT- --- G-- --C --- --- --T --- ... GCT -CC -T-
BLV4B8   -G- --- --C --- --- --- --- --- --- --- --- --- --- G-- G-- --- G-- --C --- -CA --T --- ... GCT --T -T-
BLV6D10  -G- --- --C --- --- --- --- --- --- --- --- --- --- G-- G-- --- G-- --C --- --- --T --- ... GCT --T -T-
BLV7A8   -G- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- GG- G-- --- GT- -GT --- TAT GCT --T -T-
BLV7F8   -G- --- --C --- --- --- --- --- --- --- --- --- --- G-- G-- --- G-- --C --- --- --T --- ... GCT --T -T-
BLV9C12  -G- --- --C --- --- --- --- --- --- --- --- --- --- GT- G-- --- G-- --C --- --- -TT --- ... GC- A-T -T-
BLV9H3   -G- --- --C --- --- --- --- --- --- --- --- --- --- G-- G-G --- G-- --C --- --- --T --- ... GCT --T -T-
Vλ1a(8)  -G- --- --C --- --T --- --

BLV7D7   -G- --- --C --- --T --- --- --- --A --- --- --- --- G-- G-A GG- G-- --C --- --- -TG -C- ... GCT --T -T-
BLV9B8   -G- --- --C --- --T --- --- --- --A --- --- --- --- G-- G-- GG- G-- --C --- --- -GT -C- ... GCT --T -T-
BLV10H2  -G- --- --C --- --T --- --- --- --- --- --- --- --- G-- A-- GC- G-- TAC --- --- -GT -C- ... GCT --T -T-
BLV11F3  -G- --- --C --- --T --- --- --- --- --- --- --- --- G-- G-- GG- G-- --T G-- --- -GT -C- ... G-T -CT -T-
Vλ1b(17)-G- --- --C --- --T --- --
```

Fig. 4.3 A (Continued)

```
BLV1H12 -G- --- --C --- --T --- --- --- --A --- --- --- --- G-- --- GC- G-G GA- --- --- -GT --- ... GCT --T -T-
BLV5B8  -G- --- --C --- --T --- --- --- --A --- --C --- --- G-- --- GC- G-G GA- --- --- -GT --- ... GCT --T -T-
BLV5D3  -G- --- --C --- --T --- --- --- --A --- --- --- --- G-- --- GC- G-G GA- --- --- -GT --- ... GCT --T -T-
Vλ1x(27)-G- --- --C --- --T --- --
```

```
          100
BLV1F3  GGC AGC GGG ACC ACA CTG ACC GTC CTG
BLV1G9  --- --- --- --- --- --- --- --- ---
BLV3D7  --- --- --- --- --- --- --- --- ---
BLV3D11 --- --- --- --- --- --- --- --- ---
BLV4B8  --- --- --- --- --- --- --- --- ---
BLV6D10 --- --- --- --- --- --- --- --- ---
BLV7A8  --- --- --- --- --- --- -TT --- ---
BLV7F8  --- --- --- --- --- --- --- --- ---
BLV9C12 --- --- --- --- --- --- --- --- ---
BLV9H3  --- --- --- --- --- --- --- --- ---

BLV7D7  --- --- --- --- --- --- --- --- ---
BLV9B8  --- --- --- --- --- --- --- --- ---
BLV10H2 --- --- --- --- --- --- --- --- ---
BLV11F3 --- --- --- --- --- --- --- --- ---

BLV1H12 --- --- --- --- --- --- --- --- ---
BLV5B8  --- --- --- --- --- --- --- --- ---
BLV5D3  --- --- --- --- --- --- --- --- ---
```

Fig. 4.3 A

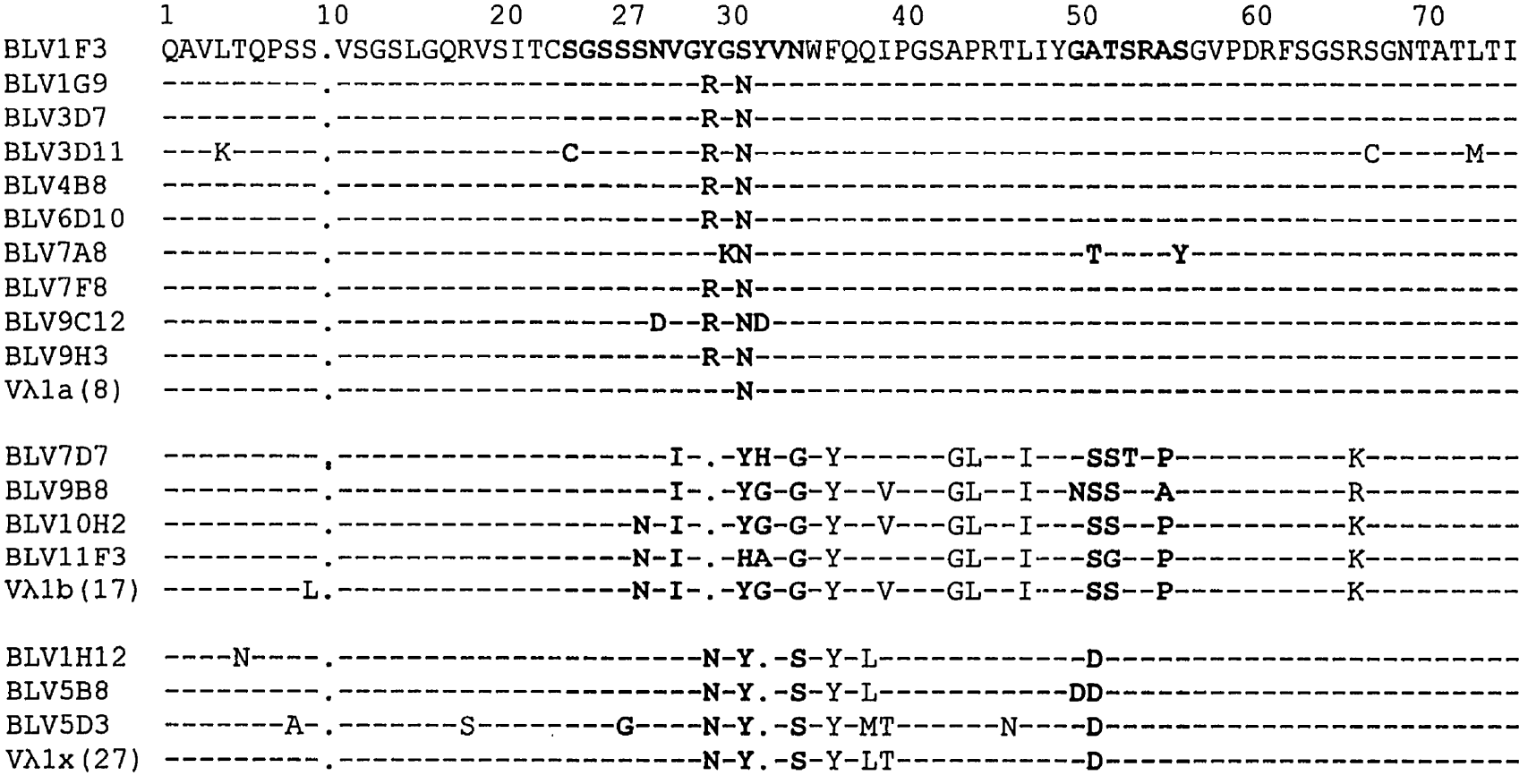


Fig. 4.3 B (Continued)

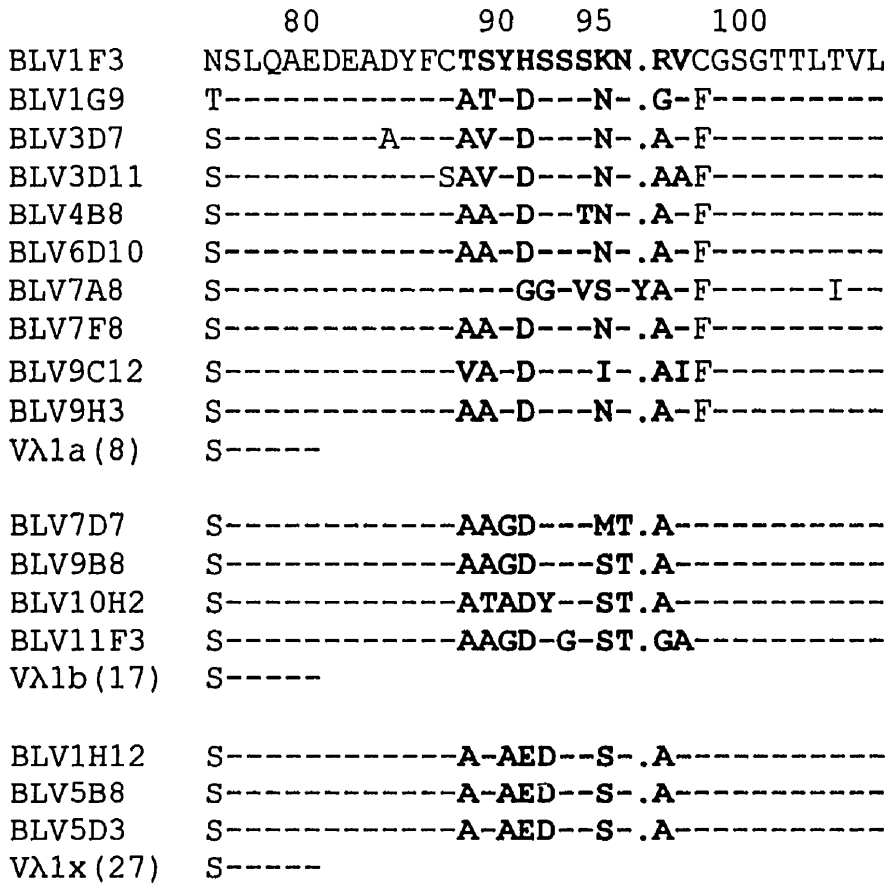

80 90 95 100
BLV1F3 NSLQAEDEADYFCTSYHSSSKN.RVCGSGTTLTVL
BLV1G9 T----------AT-D---N-.G-F---------
BLV3D7 S--------A---AV-D---N-.A-F---------
BLV3D11 S----------SAV-D---N-.AAF---------
BLV4B8 S----------AA-D--TN-.A-F---------
BLV6D10 S----------AA-D---N-.A-F---------
BLV7A8 S----------GG-VS-YA-F------I--
BLV7F8 S----------AA-D---N-.A-F---------
BLV9C12 S----------VA-D---I-.AIF---------
BLV9H3 S----------AA-D---N-.A-F---------
Vλ1a(8) S-----
BLV7D7 S----------AAGD---MT.A----------
BLV9B8 S----------AAGD---ST.A----------
BLV10H2 S----------ATADY--ST.A----------
BLV11F3 S----------AAGD-G-ST.GA----------
Vλ1b(17) S-----
BLV1H12 S----------A-AED--S-.A----------
BLV5B8 S----------A-AED--S-.A----------
BLV5D3 S----------A-AED--S-.A----------
Vλ1x(27) S-----

Fig. 4.3 B

resulting in exceptionally long CDR3H exclusively pair with either $V_\lambda 1x$ or novel $V_\lambda 1$ genes. Few somatic hypermutations were noted in the $V_\lambda 1x$ gene encoded region upon comparison with the closest germline gene $V_\lambda 1x$ 27 (Sinclair *et al.*, 1995). All of the three clones expressing $V_\lambda 1x$ genes (BLV1H12, BLV5B8 and BLV5D3) had identical CDR3 regions comprising 11 codons (Fig. 4.3).

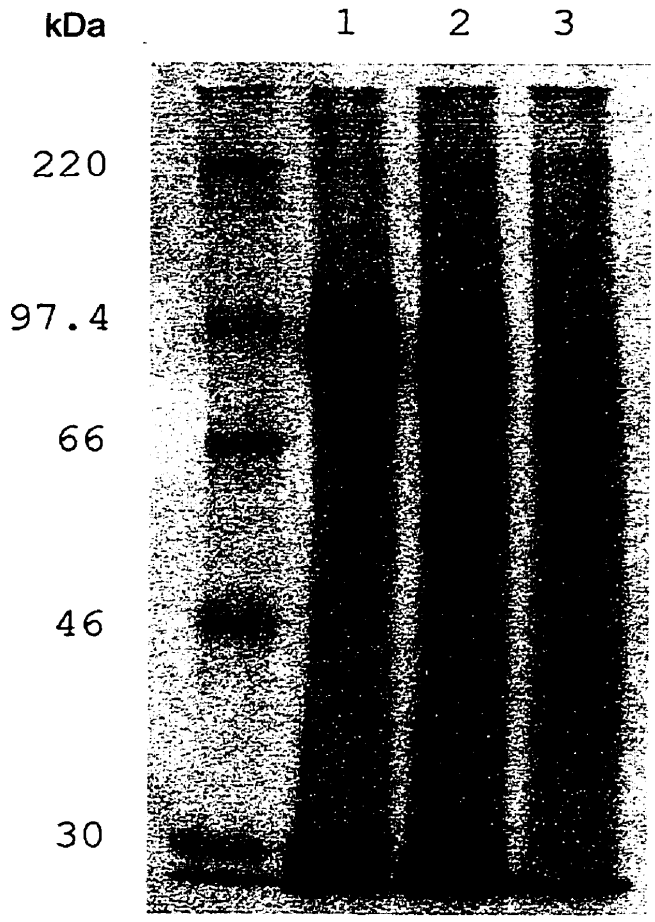
4.4.4 VDJ rearrangements with long CDR3H encode functional IgM antibodies

The VDJ rearrangements resulting in unusually long CDR3H are not aberrant indeed encode functional IgM antibodies. The SDS-PAGE analysis of internally ^{35}S-cysteine-radiolabeled IgM secreted by clones BLV1H12 (CDR3H of 61 amino acids) and BLV2C7 (CDR3H of 19 amino acids) showed μ-heavy chain of 90 and 84 kDa, respectively, and λ-light chain of 30 kDa (Fig. 4.4). Further, two clones with exceptionally long CDR3H (BLV1H12 and BLV5B8) secreted polyreactive IgM antibodies (Table 4.1) as these recognize all naturally conserved antigens (BSA, thyroglobulin, ubiquitin, actin, mouse IgG1, Hsp65, Hsp70) and synthetic haptens (TMA and TNP coupled to BSA). IgM antibodies secreted by other clones did not recognize any of these antigens (Table 4.1).

4.5 Discussion

In order to understand antibody diversification mechanisms in cattle, a species with limited germline pool both at V_H and V_λ loci, the structural features of bovine IgM antibodies originating from the PBLs of a BLV-infected cow were analysed. The BLV is known to cause generalized transformation and expansion of $CD5^+$ and IgM^+ B lymphocytes regardless of antigen specificity (Meirom *et al.*, 1993). The experiments outlined here demonstrate that

Fig. 4.4 SDS-PAGE of internally ^{35}S-cysteine radiolabeled bovine IgM antibodies. A μ-heavy chain of 90 kDa (1) in clone BLV1H12 (CDR3H of 61 amino acids) and of 84 kDa (2) in clone BLV2C7 (CDR3H of 19 amino acids; Saini *et al.*, 1997) associated with a λ-light chain of 30 kDa. The X63.Ag8.653 mouse myeloma provided the negative control (3)..


kDa
1
2
3
220
97.4
66
46
30

functional IgM antibodies with cysteine rich exceptionally long CDR3H (56 to 61 amino acids) occur in cattle. Such a long CDR3H appears to provide a novel mechanism of antibody diversification since probable intra-CDR3H disulfide bridging between even numbered cysteine residues would provide a hitherto unknown configurational ability to the antigen combining site. The X-ray crystallographic studies with these antibodies would help advance our understanding of the three-dimensional structure of such an antigen combining site. The VDJ rearrangements with unusually long CDR3H indeed result in generation of functional IgM antibodies as some of these (BLV1H12 and BLV5B8) recognize multiple natural antigens and are, thus, polyspecific. The processes leading to the generation of such a long CDR3H are not understood at present since bovine germline D_H genes remain to be identified. An unusually long CDR3H may be generated as a result of long D_H genes in the bovine genome. Alternatively, such a long CDR3H may be generated as a result of mechanisms intrinsic to the recombination processes, e.g., multiple D-D fusions and rearrangement processes, that break 12/23 rule. This is supported by the observations that the junctional modifications are developmentally regulated and complex recombinations with multiple D-D fusions are indeed involved in the generation of CDR3H region (Vandyk and Meek, 1992). In chicken, the D-D junctions are known to exist among 25% of DJ alleles as well as functional VDJ rearrangements (Reynaud *et al.*, 1991) that probably contribute to length heterogeneity of CDR3H. The fact that all the hybridomas that secrete IgM antibodies with long CDR3H are clonally unrelated, suggests that these did not originate as a result of selection by an antigen. The occurrence of B lymphocytes capable of secreting IgM antibodies with an exceptionally long CDR3H at approximately 9% frequency *in vivo* has important

practical implications for genetic engineering strategies of the antibodies.

A comparison of the V_H encoded nucleotide sequences with the closest germline V_H genes clearly shows occurrence of single point mutations, both in CDR1 and CDR2, of IgM antibodies of cattle and, thus, confirms previous observations (Saini *et al.*, 1997). Evidence for gene conversion was not noted at the V_H locus based on an analysis of these VDJ rearrangements. The consensus hot-spot triplet AGC/AGT occurs at a higher frequency in CDR1H and FR3 as compared to CDR2H. Therefore, a higher degree of intrinsic hypermutability also exists in the FR3 region of the heavy chain of bovine antibody. Overall, these finding suggest that generation of an exceptionally long CDR3H and somatic hypermutations contribute to antibody diversification in cattle.

Several unique features of IgM antibodies with long CDR3H are noted: (i) these are encoded by a single germline V_H gene (gl.110.20) and relatively few somatic hypermutations occur in the V_H gene encoded region, (ii) three of the four VDJ rearrangements with long CDR3H paired with V_λ1x gene, previously reported to be not expressed (Sinclair *et al.*, 1995), while another associated with a novel V_λ1 gene; and (iii) two of the four IgM antibodies with long CDR3H showed polyreactive specificity. In humans afflicted with B-cell chronic lymphocytic leukemia (CLL), B-cell clones express 51P1 gene of V_H1 gene family with none or few somatic hypermutations and exhibit autoreactive antibody specificity (Johnson *et al.*, 1997). The CDR3H length observed in bovine IgM antibodies (56 to 61 amino acids) is much longer than that observed in CLL patients (26 amino acids). Such a long CDR3H is unlikely to be generated as a result of an antigen selection since BLV is known to transform B-cells regardless of antigen specificity (Meirom *et al.*, 1993). The possibility that

such a long CDR3H is related to the transcriptional events of BLV induced leukemiagenesis is excluded because *env* gene transcription is noted among B-cells with varying CDR3H length. The VDJ rearrangements encoding such a long CDR3H, with repeat glycine and tyrosine residues, are also noted in bovine fetal B-lymphocytes (Chapter 5). None of the VJ rearrangements encoded long CDR3 for the light-chain. In addition, BLV does not infect germinal cells (Ferrer, 1979; Piper *et al.*, 1979). Therefore, it is unlikely that such VDJ rearrangements originated as a result of BLV leukemiagenesis. It appears, therefore, that occurrence of IgM antibodies with exceptionally long CDR3H reflects a novel mechanism of antibody diversification via processes intrinsic to VDJ rearrangements in cattle. Nevertheless, further analysis of VDJ rearrangements in the B lymphocytes of BLV-free animals would confirm these observation.

In conclusion, the experiments outlined here demonstrate that single point mutations significantly contribute to antibody diversification in cattle. The VDJ rearrangements leading to generation of exceptionally long CDR3H encode functional IgM antibodies and preferentially pair with $V_{\lambda}1x$ or novel $V_{\lambda}1$genes.

Chapter 5.

OCCURRENCE OF REPETITIVE CODONS, GGT AND TAT, CHARACTERIZES THE CDR3H REGION OF BOVINE FETAL VDJ REARRANGEMENTS

5.1 Abstract

In order to understand antibody diversification during B-cell ontogeny, VDJ rearrangements were analysed in xenogenic hetero-hybridomas originating from splenic B lymphocytes of a 125-day-old bovine fetus. Similar to adult cattle, two of the seven fetal VDJ rearrangements expressed exceptionally long CDR3H comprising 55 (BF1H1) and 56 (BF4E9) codons. The fetal VDJ rearrangements lacked somatic hypermutations in the V_H gene encoded CDR1 and CDR2 regions but were remarkable for mutational hot-spots predisposing them to somatic hypermutations. Most striking, however, is the occurrence of repetitive codons GGT (glycine) and TAT (tyrosine) in the D_H gene-encoded CDR3H region of all fetal VDJ rearrangements that may favor the recruitment of somatic hypermutations. The bovine D_H genes appear to be composed of cryptically simple repetitive DNA sequences and, thus, may provide a major source of genetic variation both in sequence and length diversity of the CDR3H. The bovine D_H genes are preferentially used in hydrophilic reading frame. Overall, these findings suggest that diversification of IgM antibody in cattle results from generation of exceptionally long CDR3H, junctional flexibility and inherent high predisposition to somatic hypermutations.

5.2 Introduction

Studies on the generation of primary antibody repertoire across species have provided useful insight into the acquired acquisition of humoral immunity. Significant differences exist among species with regard to the use of κ- and λ-light chain isotypes. The κ-light chains are used at ≥90% frequency in rabbit and mouse, whereas λ-light chains are exclusively used in chicken antibodies. The κ- and λ-light chains are expressed in 60% and 40% of human antibodies, respectively (Butler, 1998). Interestingly, some camel (Hamers-Casterman *et al.*, 1993) and nurse shark (Greenberg *et al.*, 1995) antibodies are expressed as homodimers of heavy chains only. The germline sequence diversity, both at V_H and V_L loci, also differs significantly across species and, therefore, potential for combinatorial diversity via V(D)J rearrangements is highly variable. For example, about 100 germline V_H genes, classified into seven V_H gene families, in humans (Berman *et al.*, 1988; Van Dijk *et al.*, 1993) and 100-500 germline V_H genes, grouped into 15 V_H gene families, in mice (Brodeur and Riblet, 1984; Kofler *et al.*, 1992; Mainville *et al.*, 1996) contribute significantly to combinatorial diversity. In contrast, a limited number of functional V_H genes grouped into a single V_H gene family exist in chicken (Reynaud *et al.*, 1989, 1994), rabbit (Becker and Knight, 1990; Knight, 1992) and swine (Sun *et al.*, 1994). Studies with the ruminant species, like sheep (Dufour *et al.*, 1996) and cattle (Berens *et al.*, 1997; Saini *et al.*, 1997; Sinclair *et al.*, 1997), have also led to identification of a single predominantly expressed V_H gene family, corresponding to human V_H4 and murine Q-52 genes related to evolutionarily less conserved mammalian group I (Tutter and Riblet, 1989) or clan II (Kirkham *et al.*, 1992) V_H genes. The presence of few germline V_H genes results in limited combinatorial diversity in these species. The antibody

diversification processes like gene conversion in chickens (Reynaud *et al.*, 1989, 1994), gene conversion and somatic hypermutations in rabbit (Weinstein *et al.*, 1994) and non-antigen-dependent somatic hypermutations in sheep (Reynaud *et al.*, 1991, 1995) have been noted. Previous studies have shown that the generation of an exceptionally long CDR3H (up to 61 codons) provided an additional component contributing to antibody diversity in the functional IgM antibodies of cattle (Chapter 4).

The immune system of the bovine fetus develops in the absence of possible influences from maternal antibodies (Kearney and Vakil, 1986) because the syndesmochorial type of placenta prevents in-utero transfer of maternal immunoglobulins to fetus (Tizard, 1996). In order to understand antibody diversification during B-cell ontogeny, the VDJ rearrangements were analysed in mouse x bovine hetero-hybridomas originating from splenic B-cells of a 125-day-old bovine fetus. The VDJ rearrangements were studied in the splenic B-cells of 125-day-old fetus because IgM is detectable in the circulating blood of bovine fetuses at 130 days of gestation (Schultz *et al.*, 1971), and also antibodies to certain pathogens are detected in the bovine fetus as early as 120 days of gestation (Osburn *et al.*, 1982). The experiments outlined here demonstrate that B-cells producing IgM could be rescued from a 125-day-old fetus by somatic cell fusion. As in adults, VDJ rearrangements resulting in exceptionally long CDR3H were noted in the developing bovine fetal splenic B-cells. However, in contrast to adult cattle, the D_H encoded CDR3H regions of all fetal B-cell clones show extensive repetition of GGT (glycine) and TAT (tyrosine) codons that appear to be involved in the recruitment of somatic hypermutations. A comparison of V_H encoded sequences with available bovine germline V_H genes revealed few, if any, single point mutations in fetal VDJ rearrangements. These

experiments suggest that generation of exceptionally long CDR3H is an important component contributing to the diversification of antibody repertoire during B cell ontogeny in cattle.

5.3 Materials and Methods

5.3.1 B-cell hetero-hybridomas

Seventy-five mouse x cattle hetero-hybridomas were developed by somatically fusing splenic B-cells from a 125-day-old bovine fetus (Evans and Sack, 1973), stimulated *in-vitro* for 48 hours with 25 μg/ml of lipopolysaccharide from *Escherichia coli* serotype 0127:B8 (Sigma Chemical Co., St. Louis, MO, USA) and 300 ng/ml of phorbol 12-myristate 13-acetate (Sigma Chemical Co.), with non-immunoglobulin secreting X63.Ag8.653 mouse myeloma cells under limiting dilution conditions (Saini *et al.*, 1997). Of these hetero-hybridomas two (BF2D12 and BF5F10) tested positive for bovine IgM secretion in ELISA (Saini *et al.*, 1997; Chapter 3) using unlabeled murine monoclonal anti-bovine IgM (Sigma Chemical Co.) as capture antibody and biotin-conjugated murine monoclonal anti-bovine IgM (Sigma Chemical Co.) and avidin-conjugated alkaline phosphatase (Pierce, Rockford, IL) as secondary reagents.

5.3.2 PCR amplification of rearranged bovine VDJ and VJ genes

Total RNA extracted from all the hetero-hybridomas was reverse transcribed using polydT primer (Pharmacia LKB Biotechnology, Uppsala, Sweden). The rearranged bovine VDJ genes were PCR amplified with 5' primer (5'AGCTCGAGATGAACCCACTGTG 3') from the V_H leader sequence (Jackson *et al.*, 1992) and 3' primer (5' AGACTAGTGAAGACTCTCGGGTGTG 3') from the C_H1 region of the bovine Cμ gene

(Chapter 7). The PCR steps involved a hot start at 95°C for 2 min, denaturation at 95 °C for 1 min and combined annealing and extension steps at 72°C for 2.5 min up to a total of 30 cycles. The rearranged bovine λ-light chain genes were amplified using 5' primer (5' AGGTCGACTCTGCACAGGATCCTG 3') from the bovine $V_{\lambda}1$ leader sequence and 3' primer (5' AGTCTAGAACAGGGTGACCGAG 3') from the bovine C_{λ} gene (Jackson *et al.*, 1992). The PCR conditions included denaturation at 95°C for 1 min, annealing at 68°C for 1 min and extension at 72°C for 1 min up to a total of 30 cycles. A 295 bp fragment of the bovine λ-light chain constant region was amplified using primers 5'-ACCCTCGGTCACCCTGTT-3' and 5'-CTCTGAGGGCTTCACT-3', that corresponded to nucleotides 436-453 and 715-730, respectively (Jackson *et al*, 1992). The PCR steps included denaturation at 95 °C (1 min), annealing at 55°C (1 min) and extension at 72°C (1 min) up to 30 cycles. The PCR conditions for all reactions were, 1.5 mM $MgCl_2$ concentration, 0.8 μM of each primer and 2.5 U of Taq polymerase (Perkin Elmer, Branchburg, NJ, USA) in 100 μl volume.

5.3.3 DNA sequencing and structural analysis of rearranged bovine VDJ genes

The PCR amplified products were purified with GeneClean II (Bio 101, Vista, CA, USA). The nucleotide sequence of the VDJ rearrangements was determined by direct sequencing of the PCR product using bovine Cμ primer by automated DNA sequencing (MOBIX, McMaster University, Hamilton, Ontario, Canada). The codons are numbered according to Kabat *et al.* (1991).

5.3.4 ^{35}S-cysteine internal radiolabeling and SDS-polyacrylamide gel electrophoresis

Seven fetal hetero-hybridomas, positive for VDJ rearrangements, bovine IgM

secreting BLV2C7 (Saini *et al.*, 1997) hetero-hybridoma (positive control) and X63.Ag8.653 mouse myeloma cells (negative control) were grown overnight in RPMI-1640 Select-Amine medium (GIBCO, BRL, Gaithersburg, MD, USA) containing 100 μCi of ^{35}S-labeled L-cysteine (Du Pont Canada Inc., Markham, ON, Canada). The supernates were dialysed extensively in PBS containing cold 10^{-2} M cysteine. Fifty microlitres of 2-mercaptoethanol-denatured supernates were run in 15% reducing SDS-PAGE at 100 volts for 4 h. The gel was fixed (40% methanol and 10% acetic acid) for 30 min, soaked in Amplify solution (Amersham Canada Ltd., Oakville, ON, Canada) for 30 min, dried in a gel dryer (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada) at 80°C for 2 h and exposed to XAR-5 film (Eastman Kodak Company, Rochester, NY, USA).

5.3.5 Antibody specificity

The specificity of IgM antibodies, secreted by hetero-hybridomas BF2D12 and BF5F10, was determined in a solid-phase enzyme immunoassay against a panel of test antigens including bovine serum albumin (BSA) fraction V (Boehringer Mannheim, Germany), bovine thyroglobulin, ubiquitin, trimethyl (p-aminophenyl)-ammonium chloride (TMA) conjugated to BSA (substitution of 10 TMA molecules/molecule of BSA), TNP (2,4,6-trinitrobenzenesulfonic acid) conjugated to BSA (substitution of 26 TNP molecules/molecule of BSA), actin (all from Sigma Chemical Co.), mouse IgG1 (Chemicon International Inc., Temecula, CA, USA), recombinant mycobacterial heat shock protein hsp65 and hsp70 (courtesy of Dr. J.D.A. van Embden, UNDP/World Bank/ WHO program) as described (Chapter 4).

5.4 Results

5.4.1 B lymphocytes secreting IgM or light chain alone are detected in 125-day-old bovine fetal spleen

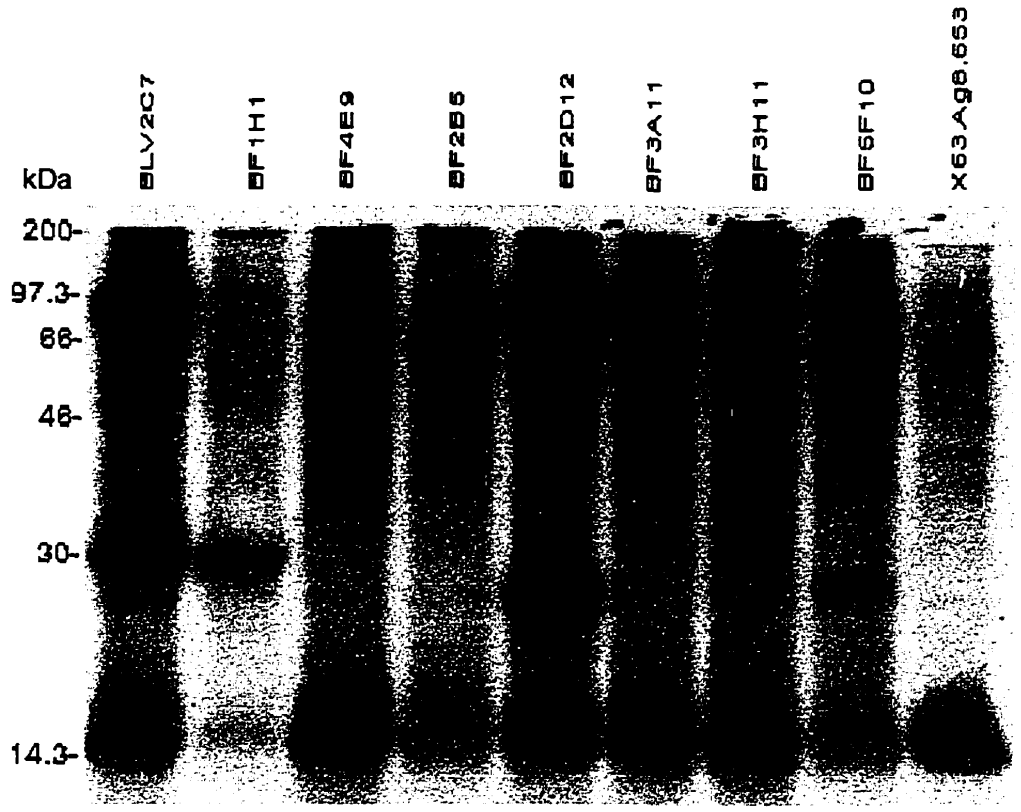
Seven out of seventy-five hetero-hybridomas, originating from splenic B lymphocytes of a 125-day-old bovine fetus, tested positive for VDJ rearrangements. However, only two clones (BF2D12 and BF5F10) secreted bovine IgM (Table 5.1). The SDS-PAGE analysis of internally ^{35}S-cysteine radiolabeled immunoglobulins revealed that clones BF2D12 and BF5F10 secreted μ-heavy chain of 83 kDa and 84 kDa, and light chain of 27 kDa and 28 kDa, respectively. The clone BF1H1, though had undergone VDJ rearrangement at the heavy chain locus, secreted only a λ-light chain of 29 kDa (Fig. 5.1). While a 295 bp fragment of λ-constant region, which shared >98% nucleotide homology with a previously published bovine C_λ sequence (Jackson *et al.*, 1992), could be amplified from all the fetal cDNAs, except clone BF2B5, VJ rearrangement was evident only in clone BF1H1 using primers specific for bovine $V_\lambda 1$ leader and λ-constant region sequences. Thus, it is likely that clones BF2D12 and BF5F10 secrete λ-light chains encoded by a V_λ gene other than $V_\lambda 1$. The clone, BF2B5, has either not undergone rearrangement at λ-light chain locus or is encoded by a κ-light chain. None of the fetal λ-light chains were encoded by the members of $V_\lambda 1a$ and $V_\lambda 1b$ subfamilies predominantly expressed in adult cattle (Sinclair *et al.*, 1995). The clone BF1H1, though a member of $V_\lambda 1$ gene family, is encoded by a novel $_\lambda$V 1 subfamily because of nucleotide homology <91% with the prototype members of $V_\lambda 1a$, $V_\lambda 1b$, $V_\lambda 1c$ and $V_\lambda 1x$ subfamilies (Sinclair *et al.*, 1995). None of the IgM antibodies secreted by two fetal clones (BF2D12 and BF5F10) showed binding to any of the test antigens comprising BSA, thyroglobulin,

Table 5.1. Characteristics of mouse x bovine hetero-hybridomas originating from the splenic B-cells of a 125-day-old bovine fetus.

Clone number	IgM secretion[1]	Secretory property[2]		Immunoglobulin gene transcript[3]			CDR3H Length (codons)
		Heavy chain	Light chain	V_HDJ_H	$V_\lambda1J_\lambda$	C_λ	
BF1H1	-	-	+	+	+	+	55
BF4E9	-	-	-	+	-	+	56
BF2B5	-	-	-	+	-	-	22
BF2D12	+	+	+	+	-	+	19
BF3A11	-	-	-	+	-	+	9
BF3H11	-	-	-	+	-	+	22
BF5F10	+	+	+	+	-	+	23

[1]Solid phase ELISA; [2]SDS-PAGE (15%) analysis of internally ^{35}S cysteine radiolabeled Igs; and [3]RT-PCR standardized for amplification of bovine V_HDJ_H and $V_\lambda1J_\lambda$ rearrangements; and a 295 bp fragment of bovine C_λ gene.

Fig. 5.1. SDS-PAGE of internally ^{35}S-cysteine radiolabeled hybridoma supernates. The clones BF2D12 and BF5F10 secreted μ-heavy chain of 83 kDa and 84 kDa and light chain of 27 kDa and 28 kDa, respectively. The clone BF1H1 secreted only λ-light chain of (29 kDa). The bovine IgM-secreting clone BLV2C7 (Saini *et al.*, 1997) and the X63.Ag8.653 mouse myeloma cells provided positive and negative controls, respectively.


kDa
BLV2C7
BF1H1
BF4E9
BF2B5
BF2D12
BF3A11
BF3H11
BF6F10
X63Ag8.653
200-
97.3-
66-
46-
30-
14.3-

ubiquitin, actin, mouse IgG1, Hsp 65, Hsp 70, TMA and TNP. Therefore, these fetal antibodies recognize an unknown antigen.

5.4.2 Repetitive codons, GGT and TAT, characterize the CDR3H of bovine fetal VDJ rearrangements

Similar to adult cattle (Chapter 4), VDJ rearrangements from a 125-day-old fetus also had exceptionally long CDR3Hs comprising 55 (clone BF1H1) and 56 (clone BF4E9) codons (Table 5.1; Fig. 5.2).The CDR3H length of the remaining five clones ranged between 9 and 23 codons. The VDJ rearrangements resulting in exceptionally long CDR3H were encoded by a single germline V_H gene gl.110.20, similar to adult cattle (Chapter 4). Indeed, a comparison of V_H gene encoded CDR1 and CDR2 regions of all fetal VDJ rearrangements with available germline V_H genes showed a lack of single point somatic hypermutations (Fig. 5.3). All the fetal VDJ rearrangements appear to be encoded by four different V_H genes, members of the Bov V_H1 gene family. The most striking observation, in contrast to the CDR3H region of adult cattle, is the occurrence of repetitive codons GGT (glycine) and TAT (tyrosine) in the D_H gene encoded CDR3H region of all fetal VDJ rearrangements. The occurrence of repetitive GGT and TAT codons probably reflects cryptic simplicity in the bovine D_H gene(s) that encode the CDR3H region. Six of the seven VDJ rearrangements showed that the D_H gene is expressed in hydrophilic reading frame similar to mouse (Kaartinen and Makela, 1985), chicken (Reynaud *et al.*, 1994) and rabbit (Knight and Crane, 1994). There was no evidence for N-region insertion either at V_H-D_H or D_H-J_H junctions. The clone BF3A11 had a deletion of base 'A' in the codon at position 100f (Fig. 5.2A) that resulted in a shift in the reading frame of the D_H gene. These observations suggest that

Fig. 5.2. The nucleotide (A) and the deduced amino acid (B) sequences of VDJ rearrangements from mouse x cattle hetero-hybridomas originating from the splenocytes of a 125-day-old bovine fetus. The codons are numbered according to Kabat *et al.* (1991) and the CDRs are shown in bold letters. The sequences are available in GenBank (Accession numbers: AF097203 to AF097209). The dash (-) indicates identical nucleotide or amino acid and dot (.) indicates lack of a nucleotide or an amino acid at the position.

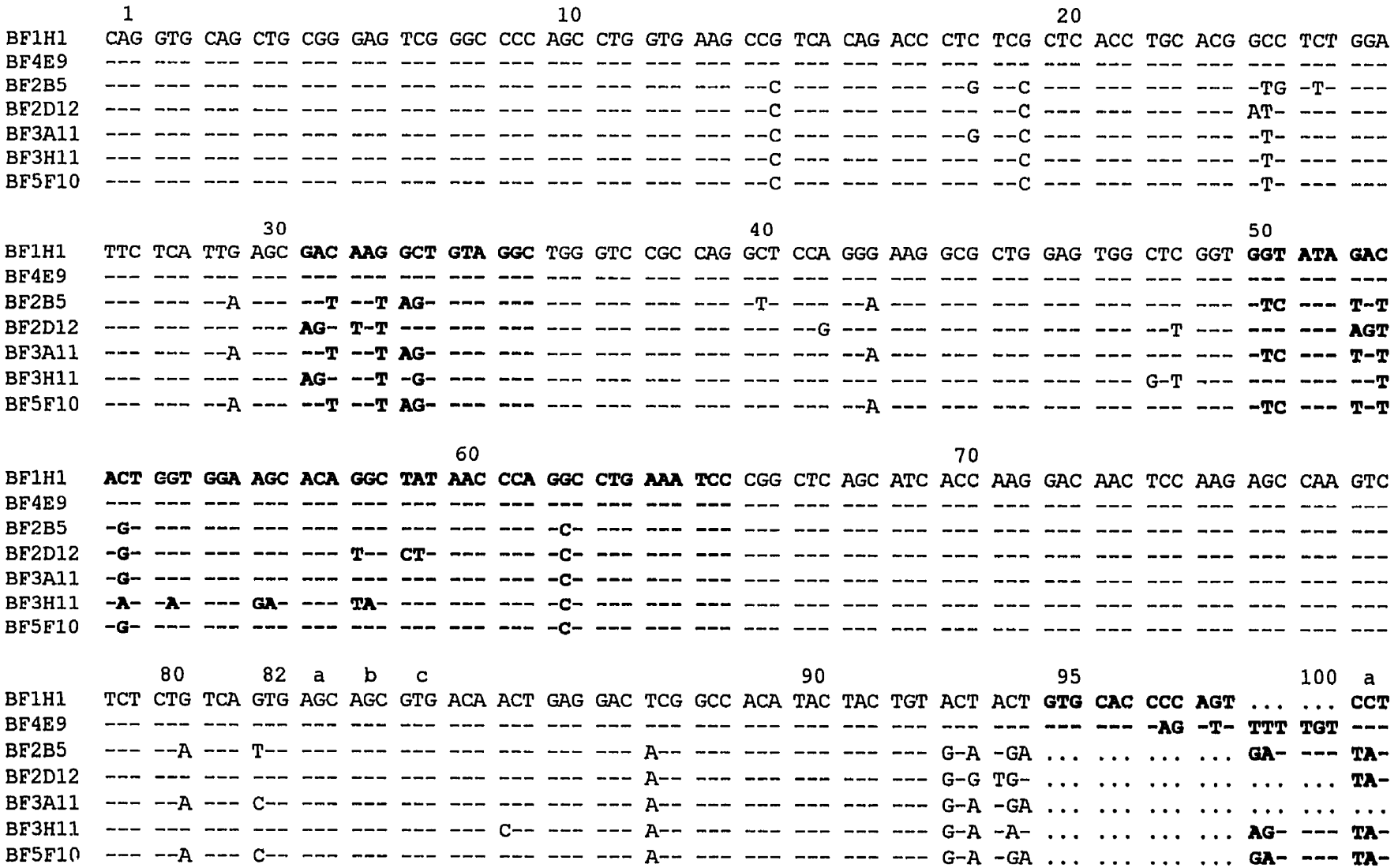


Fig. 5.2A (Continued)

	b	c	d	e	f	g	h	i	j	j1	j2	j3	j4	j5	j6	j7	j8	j9	j10	j11	j12	j13	j14	j15	j16	j17
BF1H1	GAT	GGT	TAT	AGT	TAT	GGT	TAT	GGT	TGT	GGT	TAT	GGT	TAT	GGT	TGT	AGT	GGT	TAT	GAT	TGT	TAT	GGT	TAT	GGT	GGT	TAT
BF4E9	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
BF2B5	AG-	-A-	---	G--	A--	---	---	---	---	---	---	---	---	---	---	...	...	...	...	...	...	...	...	...	...	...
BF2D12	AG-	---	---	G--	---	---	---	---	-A-	-C-	---	---	---	...	...	...	...	...	...	...	...	...	...	...	...	...
BF3A11	...	...	...	G--	-TG	-T-	ATG	-T-	ATG	-T-	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...
BF3H11	AG-	---	---	G--	---	---	-G-	---	-A-	---	---	---	---	...	...	...	...	...	...	...	...	...	...	...	...	...
BF5F10	AG-	---	---	G--	---	---	---	---	---	---	---	---	---	---	...	...	...	...	...	...	...	...	...	...	...	...

	j18	j19	j20	j21	j22	j23	j24	j25	j26	j27	j28	j29	j30	j31	j32	j33	j34	j35	j36	j37	j38	k			
BF1H1	GGT	TAT	GGT	GGT	TAT	GGT	GGT	TAT	AGT	AGT	TAT	AGT	TAT	AGT	TAT	AGT	CCA	AGG	AAA	TAC	TAC	GTC	GAT	GCC	TGG	GGC
BF4E9	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	...	TAC	G--	---	---	-G-	---	---	---	---
BF2B5	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-C-	---	---	---	---	---	---
BF2D12	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	G--	---	---	---	---	---	---
BF3A11	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-TG	ACA	---	---
BF3H11	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	G--	---	---	---	---	---	---
BF5F10	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	-C-	---	---	---	---	---	---

						110			
BF1H1	CAA	GGA	CTC	CTG	GTC	ACC	GTC	TCC	TCA
BF4E9	---	---	---	---	---	---	---	---	---
BF3B5	---	---	---	---	---	---	---	---	---
BF2D12	---	---	---	---	---	---	---	---	---
BF3A11	---	---	---	---	---	---	---	---	---
BF3H11	---	---	---	---	---	---	---	---	---
BF5F10	---	---	---	---	---	---	---	---	---

Fig. 5.2 A

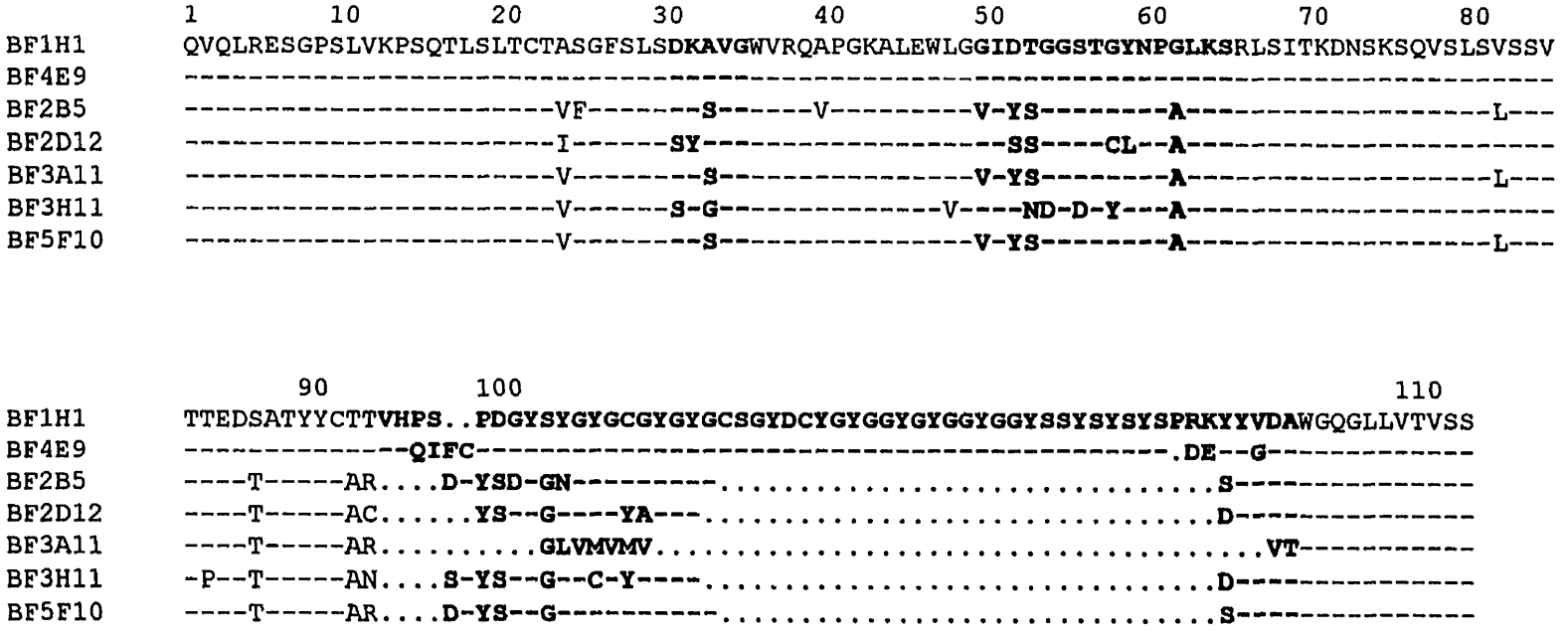

1 10 20 30 40 50 60 70 80
BF1H1 QVQLRESGPSLVKPSQTLSLTCTASGFSLSDKAVGWVRQAPGKALEWLGGIDTGGSTGYNPGLKSRLSITKDNSKSQVSLSVSSV
BF4E9 ----------
BF2B5 ----------VF-------S------V---------V-YS--------A----------L---
BF2D12 ----------I------SY----------SS----CL--A----------
BF3A11 ----------V--------S----------V-YS--------A----------L---
BF3H11 ----------V------S-G----------V----ND-D-Y---A----------
BF5F10 ----------V--------S----------V-YS--------A----------L---
90 100 110
BF1H1 TTEDSATYYCTTVHPS..PDGYSYGYGCGYGYGCSGYDCYGYGGYGYGGYGGYSSYSYSYSPRKYYVDAWGQGLLVTVSS
BF4E9 ----------QIFC----------.DE--G----------
BF2B5 ----T-----AR....D-YSD-GN---------.....S----------
BF2D12 ----T-----AC......YS--G----YA---.....D----------
BF3A11 ----T-----AR.....GLVMVMV.....VT----------
BF3H11 -P--T-----AN....S-YS--G--C-Y----.....D----------
BF5F10 ----T-----AR....D-YS--G----------.....S----------

Fig. 5.2 B

Fig. 5.3. Comparison of nucleotide sequence of V_H gene-encoded CDR1 and CDR2 regions of bovine fetal VDJ rearrangements with closest available germline bovine V_H genes (*Dr. R.A. Goldsby, personal communication and ** GenBank).

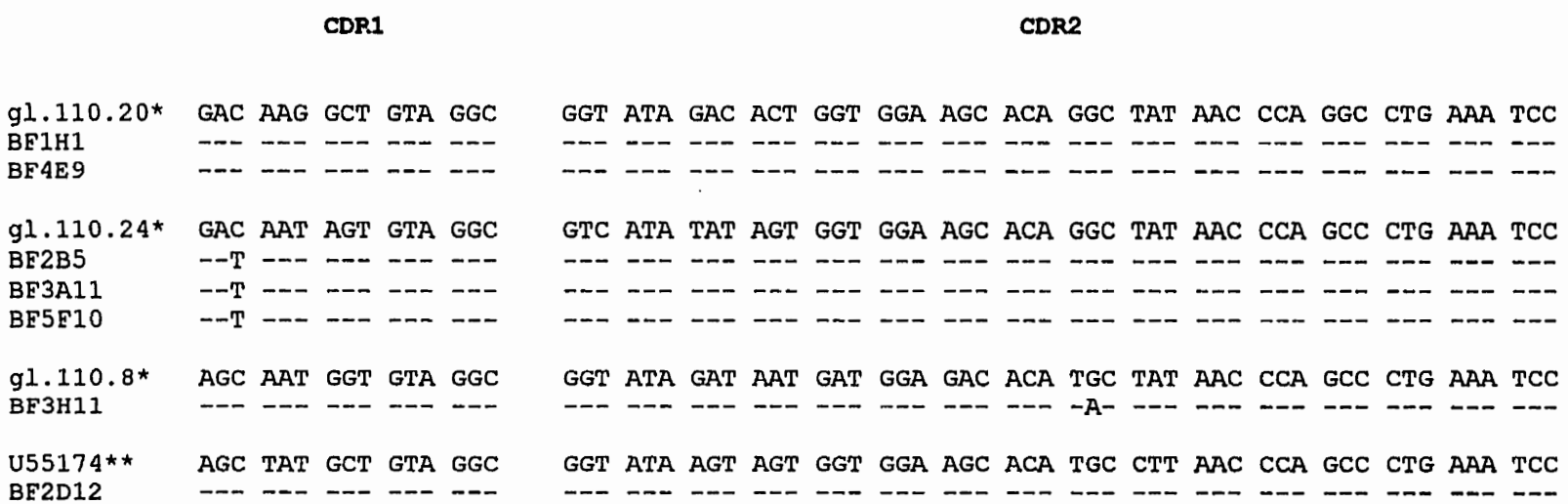

CDR1
CDR2
gl.110.20* GAC AAG GCT GTA GGC GGT ATA GAC ACT GGT GGA AGC ACA GGC TAT AAC CCA GGC CTG AAA TCC
BF1H1 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BF4E9 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
gl.110.24* GAC AAT AGT GTA GGC GTC ATA TAT AGT GGT GGA AGC ACA GGC TAT AAC CCA GCC CTG AAA TCC
BF2B5 --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BF3A11 --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BF5F10 --T --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
gl.110.8* AGC AAT GGT GTA GGC GGT ATA GAT AAT GAT GGA GAC ACA TGC TAT AAC CCA GCC CTG AAA TCC
BF3H11 --- --- --- --- --- --- --- --- --- --- -A- --- --- --- --- --- ---
U55174** AGC TAT GCT GTA GGC GGT ATA AGT AGT GGT GGA AGC ACA TGC CTT AAC CCA GCC CTG AAA TCC
BF2D12 --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

Fig. 5.3

junctional flexibility and/or deletional mutation may contribute to variation in the CDR3H length at this stage of VDJ rearrangements during ontogeny.

A comparison of the J_H gene encoded FR4 region showed that all fetal clones, except clone BF3A11, expressed J_H genes having highest nucleotide homology to sheep J_H2 (94.8%); (Dufour and Nau, 1997) and human J_H5 (89.7%); (Kabat *et al.*, 1991) gene segments. Therefore, the fetal VDJ rearrangements, similar to adult cattle, express J_H genes that are homologs of human J_H5 gene. The J_H gene expressed in the clone BF3A11 shared relatively low homology with sheep J_H2 (84.6%) and human J_H 5 (82.1%) genes and, probably represents a novel bovine J_H gene.

5.4.3 Consensus hot-spot triplet AGC/AGT occurs at a high frequency in the CDRs of fetal VDJ rearrangements

The frequency of hot-spot triplet AGPy (AGC/AGT) in V_H gene encoded CDR1 and CDR2 of seven fetal VDJ rearrangements is two times higher as compared to 23 adult VDJ rearrangements (Table 5.2). However, the hot-spot triplet occurs at a comparable frequency in the FR regions in both fetal and adult VDJ rearrangements. Thus, selective codon usage in fetal CDR1 and CDR2 predisposes them to higher hypermutability and, obviously, is evident as single point mutations in the IgM antibodies of adult cattle (Saini *et al.*, 1997; Chapter 4). The AGPy :TCN ratio was even higher in the D_H gene encoded CDR3H region of fetal VDJ rearrangements (10.0) as compared to adult VDJ rearrangements (2.8). An analysis of A+T and C+G nucleotides revealed remarkably high (A+T): (C+G) ratios in the CDR3H regions of fetal (1.71) and adult (1.62) VDJ rearrangements as compared to the human CDR3H (0.98) region (Padlan, 1997). The (A+T) : (C+G) ratios in the FR regions,

Table 5.2. AGPy : TCN and (A+T) : (C+G) ratios in the bovine VDJ rearrangements encoding IgM antibodies.*

Region	AGPy : TCN ratio		(A+T) : (C+G) ratio	
	Fetal clones (n=7)	Adult clones (n=23)**	Fetal clones (n=7)	Adult clones (n=23)
FR1	0.41	0.35	0.56	0.57
FR2	ND	1.00	0.43	0.45
FR3	1.21	1.14	0.86	0.83
FR4	ND	ND	0.56	0.59
CDR1	5.00	2.50	1.01	1.10
CDR2	1.57	0.79	1.06	1.06
CDR3	10.00	2.80	1.71	1.62

*Py: pyrimidine (G or T); N: A/T/C/G; **(Saini *et al.*, 1997; Chapter 4); ND: not done due to lack of either AGPy or TCN.

CDR1 and CDR2 regions were, however, comparable with the corresponding regions of human VDJ rearrangements (Table 5.2). Such biases in the nucleotide base usage may contribute to higher frequency of mutations in the CDR3H region of bovine antibodies. Significant differences with regard to amino acid composition of the CDR3H region, most likely as a result of somatic hypermutations, are clearly evident between fetal and adult VDJ rearrangements. For example, hydrophilic glycine (27.45%) and tyrosine (30.39%) residues are most remarkable in the fetal B-cells as compared to adult B-cells (11.8 and 11.62%, respectively). Nevertheless, a cysteine residue is remarkably conserved in the centre of the CDR3H (position 100j11) among VDJ rearrangements resulting in exceptionally long CDR3H both in fetal and adult B-cells. The retention of cysteine in the middle of CDR3H is probably essential to maintain the CDR3H configuration in the antigen combining site.

5.5 Discussion

The experiments outlined here demonstrate that B lymphocytes capable of secreting IgM antibodies are present in the spleen of 125-day-old bovine fetus. These results are consistent with the observation that intra-cytoplasmic IgM is present in the lymph node B lymphocytes of a 90-day-old bovine fetus (Ishino *et al.*, 1991) and that IgM antibodies appear in the serum of bovine fetus at 130 days of gestation (Schultz *et al.*, 1971). Nevertheless, VDJ and VJ rearrangements may not always be expressed as secreted IgM molecules since one clone (BF1H1) had undergone complete VDJ rearrangement but secreted only the light chain. The secretion of light chain alone has been noted to occur in murine neonatal B-cells (Kaushik *et al.*, 1988). Despite the fact that C_λ gene was amplified from all the fetal cDNAs, except

clone BF2B5, the VJ rearrangements were evident only in BF1H1 clone. None of the fetal clones secreting light-chain appears to be encoded by the $V_\lambda 1a$ and $V_\lambda 1b$ genes and, therefore, their predominant expression in adult cattle is likely to be a consequence of peripheral selection. Detection of C_λ gene, but not the VJ rearrangements, in the cDNAs from BF4E9, BF3A11 and BF3H11 suggests that these clones might express V_λ gene other than the $V_\lambda 1$ family. Alternatively bovine C_λ gene is transcriptionally competent prior to its rearrangement as noted for murine C_κ gene (Perry *et al.*, 1981). As in adults, two types of VDJ rearrangements, that have remarkable difference in CDR3H length (55-56 and 9-23 codons), are evident in the fetal B-cells. However, the most striking observation with regard to fetal VDJ rearrangements is the occurrence of repetitive codons GGT (glycine) and TAT (tyrosine) in the CDR3H. Obviously, the bovine D_H genes encoding the CDR3H region are composed of cryptically simple DNA sequences. Such cryptically simple regions are known to be biased in nucleotide composition and are suggested to be involved in several functions like gene regulation, signals for gene conversion and recombination probably via ubiquitous DNA slippage and mispairing during recombination (Tautz *et al.*, 1986). These observations raise several questions with regard to the CDR3H sequence and length diversity in cattle that may perhaps arise from intra-helical DNA slippage mechanisms that take place within DNA sequences that are internally repetitive and are, therefore, prone to mis-alignment during DNA synthesis. Indeed, it is known that sequences made up of repeats of short (2-5 bp) motifs are prone to replication slippage (Levinson and Gutman, 1987). It is possible that the repetitive bases GGT and TAT present in bovine D_H genes may generate variable CDR3H length as a result of replication slippage mechanisms. However, the possibility of existence of bovine

germline D_H genes of varying length is not excluded. As in mouse (Kaartinen and Makela, 1985), chicken (Reynaud *et al.*, 1994) and rabbit (Knight and Crane, 1994), the bovine D_H gene segments are used in hydrophilic reading frame which frequently encodes glycine, tyrosine and serine amino acids (Corbett *et al.*, 1997). These amino acids are also known to occur in the antigen binding loops suggesting that these residues are particularly good at antigen binding or recruiting somatic hypermutations (Corbett *et al.*, 1997). However, deletion of a base 'A' in the codon at position 100f (clone BF3A11) either as a result of junctional flexibility or deletional mutation led to a shift in the reading frame of D_H gene but this is unlikely to be the prefered D_H reading frame in cattle.

The fetal VDJ rearrangements appear to be mainly encoded by germline V_H genes because of a lack of somatic hypermutations in CDR1 and CDR2 regions. Nevertheless, the intrinsic hypermutability of CDR1 and CDR2 regions of fetal VDJ rearrangements is most remarkable. In addition, the (A+T) : (C+G) ratio is remarkably high in CDR3H of both fetal and adult VDJ rearrangements as compared to humans (Padlan, 1997). Such biases in the occurrence of codons AGC/AGT and high (A+T) : (C+G) ratio contribute to both intrinsic as well as antigen selected mutations in the CDR regions (Padlan, 1997). Overall, these observations suggest that replication slippage mechanisms and/or varying length of germline D_H genes, junctional flexibility and somatic hypermutations contribute to sequence diversity as well as length variation of the bovine CDR3H.

The VDJ rearrangements resulting in exceptionally long CDR3H, both in fetal and adult B-cells, are encoded by a single germline V_H gene, gl.110.20. It remains unclear how VDJ rearrangements involving a single germline V_H gene generate long CDR3H but

preferential V_H-D_H-J_H rearrangements may occur as a result of particular recombinational signal sequences or positional proximity between the V_H and D_H gene segments. It is unlikely that such VDJ rearrangements occur as a result of selection by an antigen in the periphery as these are detected early during B cell ontogeny.

In conclusion, the experiments outlined here provide evidence that fetal VDJ rearrangements, similar to adult cattle, result in generation of exceptionally long CDR3Hs. The D_H gene encoded CDR3H region of the bovine fetus is characterized by repetitive codons GGT (glycine) and TAT (tyrosine) that may predispose to sequence and length diversity of the CDR3H. The bovine D_H genes are used preferentially in hydrophilic reading frame. The fetal VDJ rearrangements lack point mutations in the V_H gene encoded CDR1 and CDR2 but the mutational hot-spots are remarkable in all the CDRs including the D_H encoded CDR3H region. Noticeable differences in the AGPy : TCN ratios in the fetal VDJ rearrangements as compared to adult support the proposal that somatic hypermutations contribute significantly to the diversification of adult IgM antibody repertoire.

Chapter 6

NOVEL BOVINE $V_\lambda 1$ GENES PREFERENTIALLY PAIR WITH VDJ REARRANGEMENTS THAT ENCODE EXCEPTIONALLY LONG CDR3H

6.1 Abstract

The bovine λ-light chain VJ rearrangements were analysed among hetero-hybridomas with the objective to understand the structural properties of bovine λ-light chains associated with VDJ rearrangements resulting in exceptionally long CDR3H. These experiments led to identification of two novel bovine $V_\lambda 1$ genes, designated as $V_\lambda 1d$ and $V_\lambda 1e$ subfamilies, that, similar to the $V_\lambda 1x$ gene, preferentially paired with VDJ rearrangements resulting in exceptionally long CDR3H. Such preferential H- and L-chain pairings among IgM antibodies originating from polyclonally activated B-cells are unique and, probably relate to structural requirements associated with the generation of the antigen combining site with long CDR3H. In contrast to predominantly expressed $V_\lambda 1a$ and $V_\lambda 1b$ genes, the V_λ 1x, V_λ 1d and V_λ 1e genes share alanine-serine-alanine residues in the V_λ gene encoded region of the CDR3 which may provide a structural basis for these restricted pairings. Further, the genomic complexity of the bovine $V_\lambda 1$ gene family ranges from 9 to 15 members and allelic polymorphism is evident at the bovine $V_\lambda 1$-locus. The known bovine V_λ genes are classified into three V_λ gene families ($V_\lambda 1$, $V_\lambda 2$ and $V_\lambda 3$) based upon ≥80% nucleotide homology. Overall, these experiments provide evidence for preferential H- and L-chain pairings in IgM secreting polyclonally activated bovine B cells probably as a result of intrinsic affinity and/or structural

requirements associated with exceptionally long CDR3H.

6.2 Introduction

In mice and humans, remarkable germline sequence divergence generates enormous antibody diversity via combinatorial V(D)J rearrangements occurring in the bone marrow throughout life (Brodeur and Riblet, 1984; Berman *et al.*, 1988; Kofler *et al.*, 1992; Van Dijk *et al.*, 1993). The process of V(D)J rearrangements alone is not sufficient to generate a comparable antibody diversity in species with a limited germline gene pool, but alternative post-V(D)J rearrangement processes contribute to antibody diversification. For example, the pre-immune antibody repertoire in chickens is generated from a single functional V_H and V_λ gene, each via gene conversion in the bursa of Fabricius (Reynaud *et al.*, 1987, 1989; Thompson and Neiman, 1987). A large number of V_H and V_λ pseudogenes, located upstream of the functional genes, serve as donors during non-reciprocal intra-chromosomal exchange of genetic material. Although many functional V_H genes exist in the rabbit genome, few D_H proximal V_H genes undergo VDJ rearrangement and antibody diversification is subsequently generated via gene conversion and somatic hypermutations in the appendix of neonatal animals (Knight, 1992; Weinstein *et al.*, 1994). The λ-light chain antibody repertoire of sheep is diversified via non-antigen-dependent somatic hypermutations in the ileal Peyer's patches (IPP) during fetal and neonatal life (Reynaud *et al.*, 1991, 1995). Somatic hypermutations and generation of exceptionally long CDR3H contribute to the diversification of the IgM antibody repertoire in cattle (Saini *et al.*, 1997, Chapter 4). The process of gene conversion has been suggested to contribute to bovine λ-light chain diversity (Parng *et al.*, 1996).

Remarkable species-related differences exist with regard to the use of κ- and λ-light chain antibody isotypes. For example, human and swine express κ- and λ-light chains at 60% and 40% frequencies, respectively. The κ-light chain contributes most to the antibody repertoire of mouse (95%) and rabbit (90%), whereas λ-light chains are expressed predominantly (>90%) in the antibody repertoire of cattle, sheep and horse. Chicken antibodies exclusively use λ-light chains (Butler, 1998). By contrast, camel (Hamers-Casterman *et al.*, 1993) and nurse shark (Greenberg *et al.*, 1995) antibodies are also secreted as homodimers of the heavy chains, without light chains. Studies related to the generation of the primary antibody repertoire across species are, therefore, likely to provide useful insight into the construction of the humoral immune system as species differ in genetic composition and antibody diversification strategies.

Two bovine V_λ gene families, V_λ 1 and V_λ 2, have been identified (Sinclair *et al.*, 1995). On the basis of nucleotide homology ≥91%, members of the bovine $V_\lambda 1$ gene family are grouped into $V_\lambda 1a$, $V_\lambda 1b$, $V_\lambda 1c$ and $V_\lambda 1x$ sub-families. The V_λ 1a and V_λ 1b genes are predominantly expressed in the primary antibody repertoire. Though $V_\lambda 1x$ genes constitute about 50% of the bovine V_λ germline repertoire, these are reported to be not expressed in the primary antibody repertoire (Sinclair *et al.*, 1995). The V_λ genes associated with the VDJ rearrangements resulting in unusually long CDR3H originating from polyclonally activated bovine B cells have been studied here. Interestingly, the bovine $V_\lambda 1x$ genes were noted to preferentially pair with the VDJ rearrangements resulting in exceptionally long CDR3H (Chapter 4). Analysis of VJ rearrangements from two hetero-hybridomas BLV8C11 (CDR3H length of 58 codons) and BF1H1 (CDR3H length of 55 codons) led to identification of two

novel $V_\lambda 1$ subfamilies, designated as $V_\lambda 1d$ and $V_\lambda 1e$, respectively. Such preferential H- and L-chain pairings in polyclonally activated bovine B cells secreting IgM antibody are most likely related to intrinsic affinity and/or structural requirements associated with the exceptionally long CDR3H. In addition, the available bovine V_λ genes are grouped into three V_λ gene families ($V_\lambda 1$, $V_\lambda 2$ and $V_\lambda 3$) based upon $\geq 80\%$ nucleotide homology. The genomic complexity of the bovine $V_\lambda 1$ gene family comprises 9 to 15 members and, similar to other species, polymorphism exists at the bovine $V_\lambda 1$ locus.

6.3 Materials and Methods

6.3.1 Hybridomas

Mouse x cattle hetero-hybridomas were developed by somatically fusing bovine blood B-cells, stimulated *in vitro* with 25 μg/ml of lipopolysaccharide from *Escherichia coli* (serotype 0127:B8) and 300 ng/ml of phorbol 12-myristate 13-acetate (Sigma Chemical Co., St. Louis. MO. USA) for 48 hours, with X63.Ag8.653 mouse myeloma cells (Saini *et al.*, 1997). The clone BF1H1 originated from splenic B cells of a 125-day-old bovine fetus while other clones originated from the peripheral blood B cells of an adult Holstein cow. The supernates from all the clones were tested for bovine IgM secretion in an ELISA (Saini *et al.* 1997; Chapter 3) using unlabeled murine monoclonal anti-bovine IgM (Sigma Chemical Co.) as a capture antibody and biotin-conjugated murine monoclonal anti-bovine IgM (Sigma Chemical Co.) and avidin-conjugated alkaline phosphatase (Pierce, Rockford, IL, USA) as secondary reagents. With the exception of clone BF1H1, all the hybridomas secreted bovine IgM.

6.3.2 PCR amplification, cloning and sequencing of VJ rearrangements

Total RNA was extracted from all hetero-hybridomas using Trizol reagent (GIBCO BRL Gaithersburg, MD, USA). The cDNA was synthesized from 7 μg of total RNA using polydT primer (Pharmacia LKB Biotechnology, Uppsala, Sweden). The rearranged bovine λ-light chain genes were amplified using a 5' primer (5' AGGTCGACTCTGCACAGGATCCTG 3') from the bovine V_λ leader sequence and a 3' primer (5' AGTCTAGAACAGGGTGACCGAG 3') from the bovine C_λ gene (Jackson *et al.*, 1992) with built-in restriction sites for *Sal* I and *Xba* I endonuclease, respectively. The PCR conditions included denaturation at 95°C for 1 min, annealing at 68°C for 1 min and extension at 72°C for 1 min up to a total of 30 cycles. The PCR products from clone BLV8C11 and BF1H1 were cloned in the Bluescript KS +/- vector (Stratagene, La Jolla, CA, USA) as described (Saini *et al.*, 1997). The recombinant plasmids extracted from, at least, two colonies were purified using mini-affinity column (Qiagen Inc., Stanford, CA, USA) and sequenced in both directions with T7 and T3 primers by automated DNA sequencing (MOBIX, McMaster University, Hamilton, Ontario, Canada). The PCR products from five clones were sequenced directly using 3' C_λ primer. The codons were numbered according to Kabat *et al.* (1991) and analysed using the GenBank BLAST program (Altschul *et al.*, 1990). The phylogenetic relationships between nucleotide sequences of cattle, sheep and human V_λ genes were determined using MEGALIGN program (DNA star, South Park St., Madison, USA).

6.3.3 Southern blot hybridization

The genomic DNA was extracted from the blood cells of Holstein Friesian, Jersey and Hereford cattle using a DNA extraction kit (Stratagene, LaJolla, CA, USA). Approximately

10-15 µg of DNA, digested to completion with *Bam*H I, *Eco*R I and *Hin*d III restriction enzymes, was electrophoresed and transferred onto maximum strength nytran membrane (Schleicher and Schuell Inc., Keene, NH, USA) using VacuGene (Pharmacia LKB Biotechnology, Uppsala, Sweden) as described (Saini *et al.*, 1997). The membranes were pre-hybridized overnight at 42°C followed by hybridization (50% formamide, 5X Denhardt's solution, 5X SSPE, 0.5% SDS, 100 µg/ml sonicated and denatured salmon sperm DNA and 2.5-3.0 x10^6 dpm of [α-^{32}P]dCTP radiolabeled cattle V_λ1 gene specific pBLV8C11 DNA probe/ml). The DNA probe was prepared from pBLV8C11 recombinant plasmid by double restriction digestion. First, the whole VJ insert was excised by restriction digestion with *Sal* I and *Xba* I enzymes. Following purification, it was digested with *Hinf* I enzyme to obtain a 176 bp V_λ1-specific DNA probe spanning nucleotides 12 to 188. The probe was radiolabeled with [α-^{32}P]dCTP (Amersham Canada Ltd., Oakville, ON, Canada) by random priming (Boehringer Mannheim, Germany). Following overnight hybridization, the membranes were washed three times in 2X SSC, 0.1% SDS solution at room temperature; once in 1X SSC, 0.1% SDS for 10-15 min at 65°C; and finally in 0.1X SSC, 0.1% SDS at 65°C for 10-15 min. The membranes were exposed to XAR-5 films (Eastman Kodak Co., Rochester, NY, USA) for 2 to 5 days at -70°C.

6.4 Results

6.4.1 Novel V_λ1 subfamilies, designated as V_λ1d and V_λ1e, pair with VDJ rearrangements resulting in exceptionally long CDR3H

The structural analysis of VJ rearrangements from seven hetero-hybridomas revealed

that two clones, BLV4H9 and BLV5H4, expressed V_λ1a genes while three clones, BLV2C7, BLV7B7 and BLV7G1, expressed V_λ1b genes (Fig. 6.1). The VJ rearrangements of two clones, BLV8C11 and BF1H1, expressed V_λ1 genes that could not be grouped into the known bovine V_λ1a, V_λ1b, V_λ1c and V_λ1x subfamilies. The distinct molecular features of bovine V_λ1 subfamilies (Sinclair *et al.*, 1995) were not evident in BLV8C11 and BF1H1 cDNA sequences. For example, codon TCC/TCA at position 8 was replaced with CCC; codon TCC at position 20 was replaced with ACC; codon GGT/CGT at position 50 was replaced with AAT; codon ACC/AGC at position 52 was replaced with AAC; codon TCC at position 65 was replaced with ACC; codon AGC at position 76 was replaced with codon GCT (Fig. 6.1a). The nucleotide sequence comparison revealed that BLV8C11 and BF1H1 V_λ genes shared >80% (except clone 15 and U31897) but <91% nucleotide homology with the members of V_λ1a, V_λ1b, V_λ1c and V_λ1x sub-families. Further, both the cDNAs shared <80% homology with clone 13, the only member of V_λ2 gene family (Fig. 6.2). Thus, based on the criteria of ≥80% nucleotide homology, BLV8C11 and BF1H1 V_λ genes belong to bovine V_λ1 gene family. However, these could not be grouped into any of the existing V_λ1 sub-families because of ≤91% nucleotide homology (Sinclair *et al.*, 1995). Although the V_λ gene encoded region of the BLV8C11 and BF1H1 VJ rearrangements shares high nucleotide identity (88.8%), the CDR1 region of BF1H1 is one codon shorter in length (Fig. 6.1). Therefore, the novel BLV8C11 and BF1H1 V_λ1 genes have been designated as V_λ 1d and V_λ1e subfamilies, respectively. Similar to other V_λ 1 genes, the consensus hot-spot triplet AGC/AGT occurs at a higher frequency in the CDR1 as compared to the CDR2 region (Fig. 6.1a). Few somatic hypermutations were noted in the V_λ1d and V_λ1e gene encoded region

Fig. 6.1 The nucleotide (a) and deduced amino acid (b) sequence of bovine VJ rearrangements. The codons are numbered according to Kabat *et al.* (1991) and the CDRs are shown in bold letters. The sequences are available in GenBank (Accession numbers: AF015796, AF015797, AF015802, AF015803, AF015805, AF023842 and AF097214). The most homologous germline gene sequences are included for comparison (Sinclair *et al.*, 1995; Parng *et al.*, 1996). The dash (-) indicates identical nucleotide/amino acid and the dot (.) indicates a lack of a nucleotide/amino acid at the particular position. The slash (/) indicates nucleotide/amino acid not known.

```
                1                                   10                                                  20
BLV8C11(λ1d)   CAG GCT GTG CTG ACT CAG CCG CCC TCC ... GTG TCC GGG TCC CTG GGC CAG AGG GTG ACC ATC ACC TGC ACC
Clone-R3       --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- -C- --- --- --- --- --- ---

BF1H1(λ1e)     --- --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- -C- --C --- --- T-- --- ---
Clone-R7       --A --- --- --- --- --- --- --- --- ... --- --- --- --- --- --- --- -C- --C --- --- T-- --- ---

BLV4H9         --- --- --- --- --- --- --- T-- --- ... --- --- --- --- --- --- --- --- --C T-- --- --- --- T-T
BLV5H4         --- --- --- --A --- --- --- T-- --- ... --- --- --- --- --- --- --- --- --A T-- --- --- --- T-T
Vλ1a(8)        --- --- --- --- --- --- --- T-- --- ... --- --- --- --- --- --- --- --- --C T-- --- --- --- T-T

BLV2C7         --- --- --- --- --- --- --- T-- --- ... --- --- --C --- --- --- --- --- --C T-- --- --- --- T-T
BLV7B7         --- --- --- --- --- --- --- T-- --- ... --- --- --- --- --- --- --- --- --C T-- --- --- --- T-T
BLV7G1         --- --- --- --- --- --- --- T-- --- ... --- --- --- --- --- --- --- --- --C T-- --A --- --- T-T
Vλ1b(17)       --- --- --- --- --- --- --- T-- CT- ... --- --- --C --- --- --- --- --- --C T-- --- --- --- T-T
                         27   a   b   C                                                      40
BLV8C11(λ1d)   GGA AGC AGC AGC TAC GTT TCG CGT GGC AAT CAT GTG AGC TGG TAC CAA CTG ATC CCA GGA TTG GCC CCC AAA
Clone-R3       --- --- --- --- --- --- --- --G --- --- T-- --- --- --- --- --- --- --- --- --- --- --- --- ---

BF1H1(λ1e)     --- --- --- -A- A-- A-C GG- ... ATT TTG GG- --- --- --- --- --- -A- --- --- --- -C- --- --- -G-
Clone-R7       --- --- --- -A- A-- A-C GG- ... ATT TTG GG- --- --- --- --- --- -A- --- --- --- -C- --- --- -G-

BLV4H9         --- --- --- --- A-- --- GGA TA- --- --- A-- --- -A- --- -T- --- -A- --- --- --- -C- --- --- -G-
BLV5H4         --- --- --- --- A-- --- GGA AC- --- --- T-- --- -G- --- -T- --- -A- --- --- --- -C- --- --- -G-
Vλ1a(8)        --- --- --- --- A-- --- GGA TA- --- --- T-- --- -A- --- -T- --- -A- --- --- --- -C- --- --- -G-

BLV2C7         --- --- --- -A- A-- A-C GGT ... A-A T-- AG- --- -AC --- --- --- -A- G-- --- --- -C- -G- -T- -G-
BLV7B7         --- --- --- -G- A-- A-C GGT ... ACT T-- GG- --- G-- --- --- --- -A- G-- --- --- -C- -G- -T- -G-
BLV7G1         --- --- --- -G- A-- A-C GGT ... A-A T-- GG- --- G-- --- --- --- -A- G-- --- --- -C- -G- -T- -G-
Vλ1b(17)       --- --- --- -A- A-- A-C GGT ... A-A T-- GG- --- G-- --- --- --- -A- G-- --- --- -C- -G- -T- -G-
                                 50                                                  60
BLV8C11(λ1d)   ACC CTG ATC TAT AAT AGT AAC AAA CGA CCC TCG GGG GTC CCT GAC CGA TTC TCT GGC ACC AAG TCC GGC AAC
Clone-R3       --- --- --- --- --- --- --- G-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- G--
BF1H1-λ1e      --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- --- --- --- --- --- --- --- --- ---
Clone-R7       --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- --- --- --- --- --- --- --- --- ---
```

Fig. 6.1a (Continued)

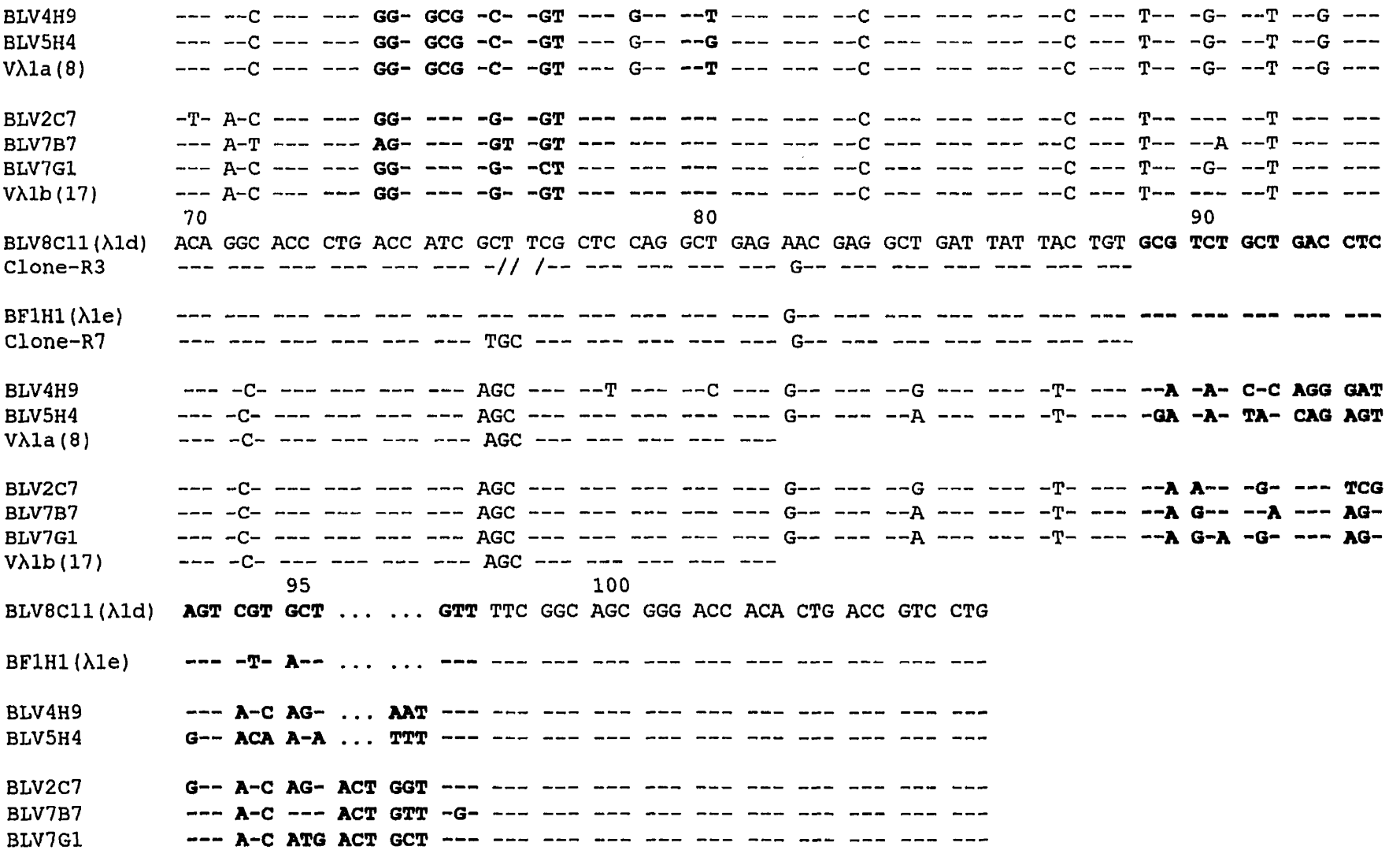

BLV4H9
BLV5H4
Vλ1a(8)
BLV2C7
BLV7B7
BLV7G1
Vλ1b(17)
70
80
90
BLV8C11(λ1d)
Clone-R3
BF1H1(λ1e)
Clone-R7
BLV4H9
BLV5H4
Vλ1a(8)
BLV2C7
BLV7B7
BLV7G1
Vλ1b(17)
95
100
BLV8C11(λ1d)
BF1H1(λ1e)
BLV4H9
BLV5H4
BLV2C7
BLV7B7
BLV7G1

Fig. 6.1a

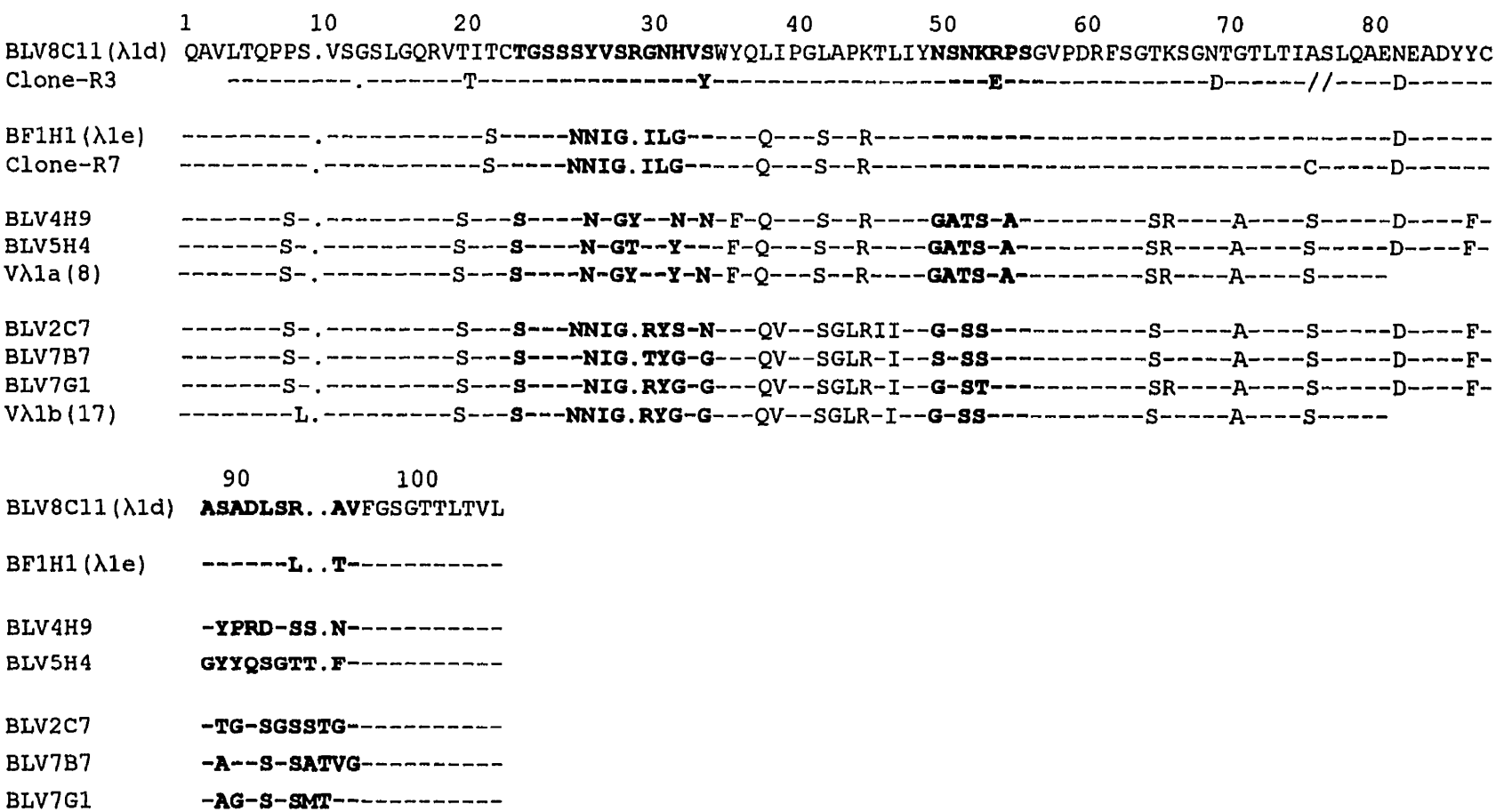

1 10 20 30 40 50 60 70 80
BLV8C11(λ1d) QAVLTQPPS.VSGSLGQRVTITCTGSSSYVSRGNHVSWYQLIPGLAPKTLIYNSNKRPSGVPDRFSGTKSGNTGTLTIASLQAENEADYYC
Clone-R3 ---------.-------T----------Y----------E----------D------//----D------
BF1H1(λ1e) ---------.----------S-----NNIG.ILG-----Q---S--R----------D------
Clone-R7 ---------.----------S-----NNIG.ILG-----Q---S--R----------C-----D------
BLV4H9 -------S-.---------S---S----N-GY--N-N-F-Q---S--R----GATS-A---------SR----A----S-----D----F-
BLV5H4 -------S-.---------S---S----N-GT--Y---F-Q---S--R----GATS-A---------SR----A----S-----D----F-
Vλ1a(8) -------S-.---------S---S----N-GY--Y-N-F-Q---S--R----GATS-A---------SR----A----S-----
BLV2C7 -------S-.---------S---S---NNIG.RYS-N---QV--SGLRII--G-SS----------S-----A----S-----D----F-
BLV7B7 -------S-.---------S---S----NIG.TYG-G---QV--SGLR-I--S-SS----------S-----A----S-----D----F-
BLV7G1 -------S-.---------S---S----NIG.RYG-G---QV--SGLR-I--G-ST----------SR----A----S-----D----F-
Vλ1b(17) --------L.---------S---S---NNIG.RYG-G---QV--SGLR-I--G-SS----------S-----A----S-----
90 100
BLV8C11(λ1d) ASADLSR..AVFGSGTTLTVL
BF1H1(λ1e) ------L..T----------
BLV4H9 -YPRD-SS.N----------
BLV5H4 GYYQSGTT.F----------
BLV2C7 -TG-SGSSTG----------
BLV7B7 -A--S-SATVG----------
BLV7G1 -AG-S-SMT----------

Fig. 6.1b

Fig. 6.2 Percent nucleotide homology between the members of bovine V_λ gene families.

Percent nucleotide homology between bovine V_λ gene families

1	2	3	4	5	6	7	8	9	10	11	Clone	Sub-family	V_λ Family
	96.3	85.4	86.9	86.5	91.8	91.5	84.1	80.5	73.3	63.5	**1 BLV4H9**	V_λ1a	V_λ1
		87.6	88.0	87.3	93.3	92.7	84.1	80.9	74.1	64.2	**2 BLV5H4**	V_λ1a	V_λ1
			96.3	80.9	86.5	85.5	80.9	86.5	71.2	66.9	**3 BLV7B7**	V_λ1b	V_λ1
				83.5	88.0	87.1	80.9	85.8	70.4	67.3	**4 BLV7G1**	V_λ1b	V_λ1
					91.8	91.5	78.3	79.8	70.4	62.3	**5 Clone 15**	V_λ1c	V_λ1
						99.2	80.5	81.6	72.7	65.8	**6 BLV1H12**	V_λ1x	V_λ1
							79.4	81.0	71.8	62.9	**7 U31897**	V_λ1x	V_λ1
								88.8	76.7	63.8	**8 BLV8C11**	V_λ1d	V_λ1
									76.0	65.8	**9 BF1H1**	V_λ1e	V_λ1
										63.5	**10 Clone 13**	-	V_λ2
											11 Clone C4	-	V_λ3

Figure 6.2

based on comparison with the closest R3 and R7 germline genes (Parng *et al.*, 1996), respectively. Hydrophobicity indices indicated that CDR2 is more hydrophilic as compared to CDR1. Interestingly, the novel V_λ1d and V_λ1e genes paired with the VDJ rearrangements that encode exceptionally long CDR3H (58 and 55 codons). Similar to bovine V_λ1x genes, that also pair with the VDJ rearrangements resulting in exceptionally long CDR3H (Chapter 4), the alanine-serine-alanine residues are conserved in the V_λ1d and V_λ1e encoded CDR3 region of the VJ rearrangements. The conservation of these amino acids, in contrast to other bovine V_λ genes, may provide the required configurational support to the antigen combining site of IgM antibodies with exceptionally long CDR3H.

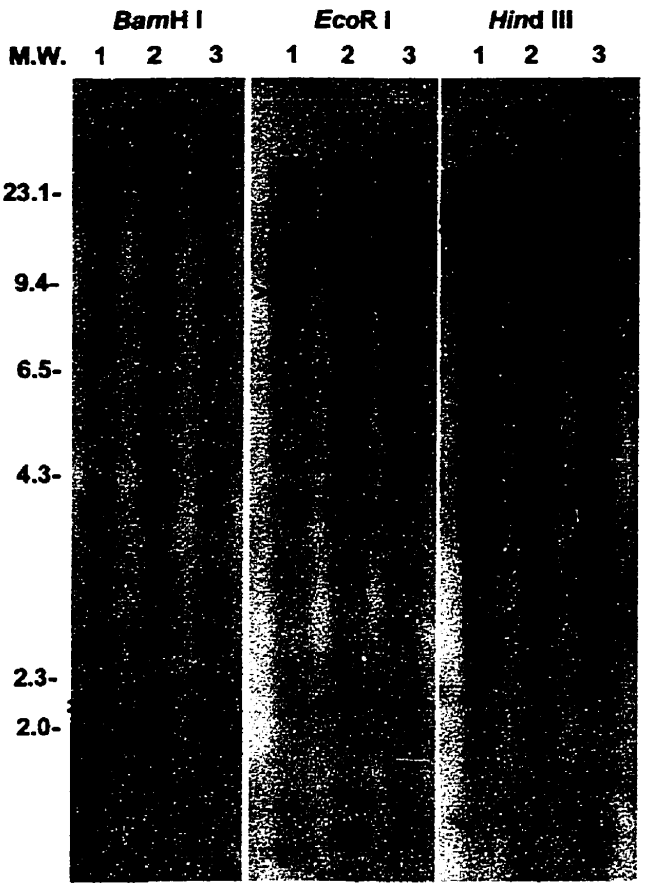
6.4.2 Bovine V_λ1 gene family comprises 9-15 genes

In a Southern blot, two distinct RFLP patterns were noted in the genomic DNA from Holstein Friesian, Jersey and Hereford cattle following restriction digestion with *Bam*H I, *Eco*R I and *Hin*d III and hybridization with a bovine V_λ1-specific DNA probe (pBLV8C11). Approximately, 9 to 15 bovine V_λ1-hybridizing restriction fragments were noted among cattle of the three breeds (Fig. 6.3). Thus, the genomic complexity of the predominantly expressed bovine V_λ1 gene family comprises 9 to 15 members and polymorphism, similar to other species, exists at the bovine V_λ1-locus.

6.4.3 Three V_λ gene families exist in cattle

While comparing novel BLV8C11 and BF1H1 V_λ1genes with eighty available germline and expressed bovine V_λ gene sequences (Sinclair *et al.*, 1995; Parng *et al.*, 1996; Chapter 4), three bovine V_λ gene families, V_λ1, V_λ2 and V_λ3, were defined on the basis of nucleotide homology ≥80% (Fig. 6.2). A majority of the bovine V_λ genes were grouped into

Fig. 6.3 The RFLP analysis of *Bam*H I, *Eco*R I and *Hind* III restriction digested genomic DNA from Holstein Friesian (1), Jersey (2) and Hereford (3) cattle hybridized with [α-^{32}P]dCTP radiolabeled cattle $V_{\lambda}1$-specific DNA probe (pBLV8C11). The arrows indicate polymorphic bands.


BamH I
EcoR I
Hind III
M.W.
1 2 3
1 2 3
1 2 3
23.1-
9.4-
6.5-
4.3-
2.3-
2.0-

$V_\lambda 1$ gene family, while $_\lambda V$ 2 and$_\lambda$ V 3 gene families comprised one and two members, respectively (Table 6.1). The bovine $V_\lambda 1$ and $V_\lambda 2$ gene families shared highest nucleotide homology with sheep V_λI (92.6%) and V_λII (88.9%), and human V_λ I (74.8%) and V_λ II (79.3%) gene families, respectively. The bovine $V_\lambda 3$ gene family, however, showed highest nucleotide homology to an as yet unclassified sheep V_λ 6b gene (75.0%), distinct from other sheep V_λ families, and also with the human LBR1104 V_λ gene (73.8%), a member of human V_λ III gene family (Table 6.2). The representative members of cattle, sheep and human V_λ gene families clustered together within the corresponding V_λ gene groups with the exception of sheep clone 3.1, a member of sheep V_λIII gene family that clustered with the group I genes, and sheep clone 6b, an unclassified sheep V_λ gene that clustered with the group III genes (Fig. 6.4). Although the members of $V_\lambda 1$ x subfamily (BLV1H12 and U31897) shared nucleotide homology ≥91% with the members of $V_\lambda 1$a (BLV4H9 and BLV5H4) and $V_\lambda 1$c (Clone 15) subfamilies (Fig. 6.2), the $V_\lambda 1$a genes showed a CDR1 length of 14 codons while $V_\lambda 1$c and $V_\lambda 1$x genes had a CDR1 length of 13 codons. In addition, $V_\lambda 1$c and $V_\lambda 1$x genes expressed identical codons at positions 27b (AAT), 30 (GGA), 34 (AGC) and 51 (GAC), and thus differ from other $V_\lambda 1$ subfamilies in which codons AAC, GGC/AGA, AAC/GGC and GCG/AGT are noted at these positions, respectively. It appears, therefore, that $V_\lambda 1$c and $V_\lambda 1$x genes constitute a single V_λ 1 subgroup. Overall, the available bovine V_λ genes are grouped into three distinct V_λ families based on the criteria of ≥80% nucleotide homology.

6.5 Discussion

A remarkable preferential pairing of bovine $V_\lambda 1$x genes with the VDJ rearrangements,

Fig. 6.4 Phylogenetic relationship based upon nucleotide homology between the members of bovine (B) $V_\lambda 1$, $V_\lambda 2$ and $V_\lambda 3$ gene families and the V_λ genes of sheep (S) and human (H). Clones 10 and 13, Sinclair *et al.* (1995); Clone C4, Parng *et al.*, (1996); Clones 5.3, 6.1 and 3.1, Reynaud *et al.*,(1997); Clone V_λ 6b, (GenBank: AF038146); Clone DPL 8, Williams and Winter (1993); Clone HSLV 2066, Brockly *et al.*, (1989); and Clone LBR 1104 , Fang *et al.*, (1994).

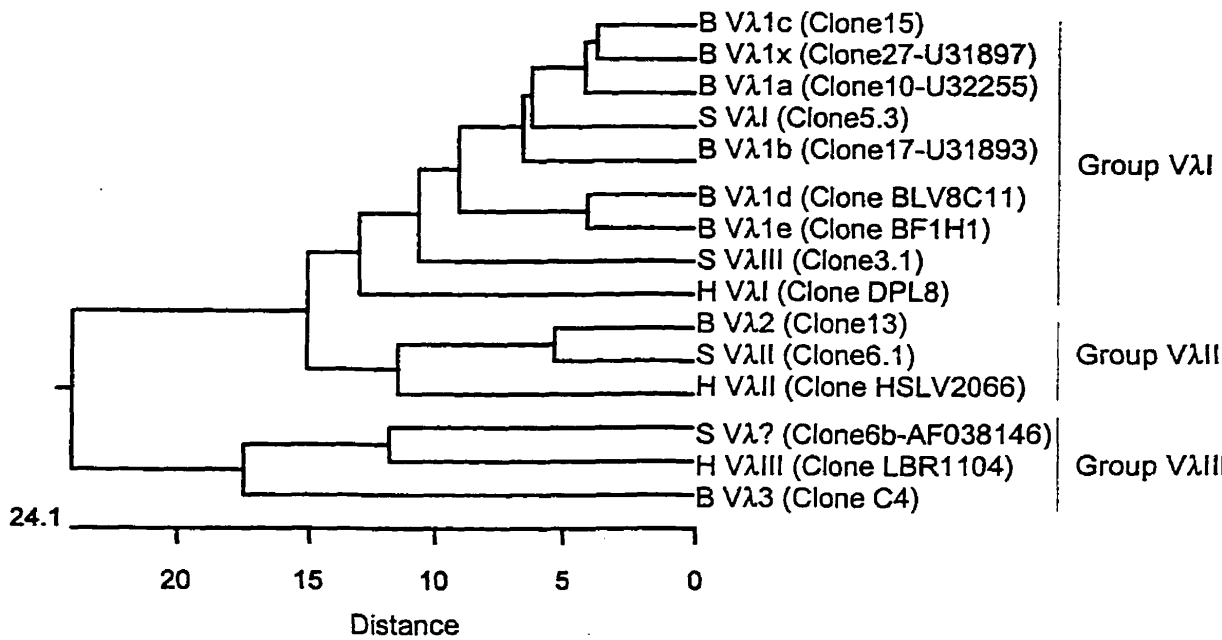

B Vλ1c (Clone15)
B Vλ1x (Clone27-U31897)
B Vλ1a (Clone10-U32255)
S VλI (Clone5.3)
B Vλ1b (Clone17-U31893)
B Vλ1d (Clone BLV8C11)
B Vλ1e (Clone BF1H1)
S VλIII (Clone3.1)
H VλI (Clone DPL8)
B Vλ2 (Clone13)
S VλII (Clone6.1)
H VλII (Clone HSLV2066)
S Vλ? (Clone6b-AF038146)
H VλIII (Clone LBR1104)
B Vλ3 (Clone C4)
Group VλI
Group VλII
Group VλIII
24.1
20
15
10
5
0
Distance

Table 6.1. Classification of bovine V_λ genes.

Bovine Vλ genes		CDR1 Length (codons)	Prototype gene	Members	
Families	Subfamilies			Germline gene(s)	cDNA(s)
$V_\lambda1$	$V_\lambda1a$	14	U31887 (Clone 8)	U31891(Clone 15),U31894 (Clone 20), U31898(Clone 28), Clone R11.	BLV1F3, BLV3D11, BLV4B8, BLV6D10, BLV9C12, BLV9H3, BLV7F8, BLV4H9, BLV3D7, BLV1G9, BLV7A8, BLV5H4, U32250 (Clone3), U32255 (Clone10), U32261 (Clone16), U32254 (Clone8), U31106, U32249 (Clone2), Clone C6, Clone V15.
	$V_\lambda1b$	13	U31893 (Clone 17)	U31888(Clone 8), U31892.	U32263 (Clone4), U32264 (Clone20), BLV10H2, U32259 (Clone14), CloneC7, Clone V8, Clone C2, Clone C3, U32257 (Clone5), U32251 (Clone9), U32253 (Clone7), BLV2C7, Clone 32.5, Clone 32.6, Clone 32.7, Clone 32.16, Clone B4, Clone 32.2, BLV11F3, BLV7B7, BLV 7G1, BLV9B8, BLV7D7, U32256 (Clone11), U32258 (Clone15), U32260 (Clone15), U32262 (Clone17), Clone V2, U32252 (Clone 6).
	$V_\lambda1c$	13	Clone 15*	-	-
	$V_\lambda1d$	14	BLV8C11	Clone R3	-
	$V_\lambda1e$	13	BF1H1	Clone R7	-
	$V_\lambda1x$	13	U31897 (Clone 27)	U31895(Clone 21), U31899 (Clone 29), U31889(Clone 10), U31890 (Clone 11), U31896(Clone 22), U31886(Clone 7).	BLV1H12, BLV5B8, BLV5D3
	$V_\lambda1$(Others)		-	Clone R2, Clone R20, Clone R18	Clone 32.12, Clone 32.13, Clone 32.1, Clone 32.4, Clone 32.14.
$V_\lambda2$	-	14	Clone13*	-	-
$V_\lambda3$	-	11	Clone C4	-	Clone V6

The sequences with prefix BLV and BF are from this laboratory (Chapters4 and 5) and those with prefix U and * are from Sinclair *et al.* (1995). Other sequences are from Parng *et al.* (1996).

Table 6.2 Percent nucleotide homology of bovine V_λ genes with the corresponding sheep and human V_λ gene families.

Bovine V_λ gene family (Gene)	Sheep V_λ genes				Human V_λ genes		
	V_λI (Clone 5.3)	V_λII (Clone 6.1)	V_λIII (Clone 3.1)	V_λ(?) (Clone V_λ 6b)	V_λ I (DPL 8)	V_λ II (HSLV 2066)	V_λ III (LBR 1104)
V_λ1(Clone10)	**92.6**	69.3	77.2	56.3	**74.8**	68.1	60.5
V_λ2(Clone 13)	75.2	**88.9**	68.5	58.2	72.6	**79.3**	63.6
V_λ3(Clone C4)	64.2	60.0	60.8	**75.0**	62.3	63.5	**73.8**

Clone 10 and Clone 13, Sinclair *et al.* (1995); Clone C4, Parng *et al.*, (1996); Clone 5.3, Clone 6.1, and Clone 3.1, Reynaud *et al.*,(1997); Clone V_λ 6b, (GenBank: AF038146); Clone DPL 8, Williams and Winter (1993); Clone HSLV 2066, Brockly *et al.*, (1989); and Clone LBR 1104 , Fang *et al.*, (1994). Maximum nucleotide homologies are shown in bold.

resulting in an exceptionally long CDR3H length (56 to 61 amino acids) was previously observed. These VDJ rearrangements originated from clonally unrelated bovine B cells that secreted IgM (Chapter 4). These observations suggested that bovine $V_\lambda 1x$ germline genes are functional and are indeed expressed in the bovine primary antibody repertoire. Two novel bovine $V_\lambda 1$ genes, $V_\lambda 1d$ (BLV8C11) and $V_\lambda 1e$ (BF1H1), are described here which also pair with the VDJ rearrangements resulting in exceptionally long CDR3H (58 and 55 codons). Clone BLV8C11 originated from an adult cow and secreted functional IgM antibody (Chapter 4) whereas clone BF1H1 originated from the splenic B-cells of a 125-day-old bovine fetus and secreted λ-light chain only, despite VDJ rearrangement at the V_H-locus (Chapter 5). The BLV8C11 and BF1H1 $V_\lambda 1$ genes could not be grouped into existing $V_\lambda 1$ subfamilies because of nucleotide homology <91%; therefore, we have designated these as $V_\lambda 1d$ and $V_\lambda 1e$ gene subfamilies, respectively. The preferential pairing of $V_\lambda 1x$, $V_\lambda 1d$ and $V_\lambda 1e$ genes with VDJ rearrangements resulting in exceptionally long CDR3H in IgM antibodies appears to be unique to cattle. These observations provide the evidence for intrinsic affinity of particular V_H+V_λ pairings in the primary antibody repertoire of a species. In fact, all the VDJ rearrangements with exceptionally long CDR3H are encoded by a single germline V_H gene (gl.110.20) with few somatic hypermutations (> 95% nucleotide homology with the germline gene) (Chapters 4 and 5) and these pair preferentially with $V_\lambda 1x$, $V_\lambda 1d$ and $V_\lambda 1e$ genes with few somatic hypermutations (>96% nucleotide homology with the corresponding germline gene). None of the VDJ rearrangements resulting in exceptionally long CDR3H pair with predominantly expressed $V_\lambda 1a$ or $V_\lambda 1b$ genes. Possibly, the amino acid residues alanine-serine-alanine in the CDR3 region encoded by $V_\lambda 1x$, $V_\lambda 1d$ and V_λ 1e genes provide the

required configurational support to the combining site generated as a result of an exceptionally long CDR3H region. These observations, thus, provide evidence for restricted V_H+V_L gene pairings in polyclonally activated B-cells possibly as a result of intrinsic structural requirements associated with the generation of a combining site with unusually long CDR3H. In mouse, V_H+V_L pairings mostly occur stoichastically (Kaushik et al., 1990), though some V_H+V_λ pairings may be non-random (Nadel *et al.*, 1992; Yurovsky and Kelsoe, 1993; Knott *et al.*, 1998).

Based upon the criteria of ≥80% nucleotide homology, the available bovine V_λ genes (Sinclair *et al.*, 1995; Parng *et al.*, 1996; Chapter 4) are grouped into three families, V_λ1, V_λ2 and V_λ3. Bovine V_λ1 and V_λ2 gene families are closest to sheep V_λI and V_λII (Reynaud *et al.*, 1997) and human V_λI (Williams and Winter, 1993) and V_λ II (Brockly *et al.*, 1989) gene families, respectively. However, the bovine V_λ3 gene family shares highest homology to the unclassified but distinct sheep V_λ gene family (Genbank AF038146; clone 6b) and also to the human V_λIII gene family (Fang *et al.*, 1994). The bovine V_λ1, V_λ2 and V_λ3 genes cluster with the human V_λI, V_λII and V_λIII gene families and with sheep V_λI, V_λII families and unclassified clone 6b gene, respectively. However, the clone 3.1, a member of sheep V_λIII gene family, clusters with the bovine V_λ1, sheep V_λI and human V_λI genes. Southern blot analysis of genomic DNA revealed 9 to 15 V_λ1 hybridizing fragments in Holstein Friesian, Jersey and Hereford cattle suggesting that the bovine V_λ1 gene family comprises 9 to 15 members. The differences noted in the RFLP patterns suggest allelic polymorphism at the bovine V_λ1-locus, similar to human and sheep V_λ-loci (Taub *et al.*, 1983; Foley and Beh, 1992).

In conclusion, the experiments outlined here provide evidence for the existence of two novel bovine $V_\lambda 1$ genes designated as $V_\lambda 1d$ and $V_\lambda 1e$ subfamilies. Similar to $V_\lambda 1x$ genes, the $V_\lambda 1d$ and $V_\lambda 1e$ genes pair with VDJ rearrangements resulting in exceptionally long CDR3H. The occurrence of such restricted V_λ ($V_\lambda 1x$, V_λ 1d and V_λ 1e) gene pairings with VDJ rearrangements in polyclonally activated B-cells are unique and might be related to the structural requirements associated with the generation of an antigen combining site with a long CDR3H. The genomic complexity of $V_\lambda 1$ genes comprises 9 to 15 members and polymorphism exists at the bovine $V_\lambda 1$-locus. The available bovine V_λ gene are classified into three bovine V_λ gene families $V_\lambda 1$, $V_\lambda 2$ and $V_\lambda 3$.

Chapter 7

MOLECULAR EVIDENCE FOR BOVINE IgM ALLOTYPES

7.1 Abstract

The complete nucleotide sequence of bovine Cμ gene, encoding the four domains of the IgM antibody, was determined from two IgM-secreting mouse x cattle hetero-hybridomas, BLV7G1 (Holstein) and B5D8 (Angus x Hereford cross-bred). Both the cDNAs shared 98.7% nucleotide homology but the clone B5D8 had nine additional bases between the CH1 and CH2 domain as a result of cleavage of pre-mRNA at an alternate cryptic 5' splice donor site in the intervening intron. Sporadic nucleotide substitutions resulting in amino replacements in all the four domains provide a structural basis of bovine IgM allotypes. Bovine IgM has fewer proline residues at the junction of CH1-CH2 domains and this would result in relatively low flexibility in this region as compared to other species. Bovine IgM showed an overall maximum amino acid homology with sheep IgM (89.4%) followed by pig (64.5%), rabbit (59.7%), human (59.2%), horse (58.3%) and mouse (54.8%). The highest amino acid homology was noted in the CH4 domain. The RFLP analysis of *Bam*H I, *Eco*R I and *Hin*d III digested genomic DNA from Holstein, Jersey, Hereford and Charolais cattle, upon hybridization with bovine Cμ-specific DNA probe, revealed that, at least, four allelic forms of Cμ gene exist in the bovine genome.

7.2 Introduction

IgM antibody appeared first in phylogeny of vertebrates and is also the first molecule to be expressed as an antigen receptor on the surface of B-cells during the development of the immune system. IgM antibodies exist either in secreted or membrane form bound to the surface of the B-cell membrane and these differ in their carboxyl terminal sequences, located downstream of the Cμ4 exon. The differential splicing of the primary mRNA transcript generates either the secreted or membrane form of IgM (Alt *et al.*, 1980; Early *et al.*, 1980; Roger *et al.*, 1980). Secreted IgM occurs as a pentamer in association with J chain (Mestecky *et al.*, 1971) or as a hexamer in the absence of J chain (Cattaneo and Neuberger, 1987).

The immunoglobulin isotypes known to exist in cattle include IgM, IgG (IgG1, IgG2, IgG3), IgE and IgA (Butler, 1998). Similar to sheep and swine, bovine B-cells do not express surface-IgD (Butler *et al.*, 1996; Naessens, 1997), co-expressed with IgM on the surface of mature B-cells in other species like mice and humans. Constant region of bovine Ig genes have been characterized for IgG1 (Symons *et al.*, 1989), IgG2 (Heyermann *et al.*, 1992; Kacskovics and Butler, 1996; Symons *et al.*, 1989), IgG3 (Rabbani *et al.*, 1997), IgE (Mousavi *et al.*, 1997) and IgA (Brown *et al.*, 1997) antibodies. The allotypic variants have been recognized for all bovine IgG sub-isotypes (Blakeslee *et al.*, 1971; Butler and Heyermann, 1986; Heyermann and Butler, 1987; Heyermann *et al.*, 1992; Kacskovics and Butler, 1996; Rabbani *et al.*, 1997). Both serological and RFLP studies have provided evidence for allelic polymorphism at the bovine Cα-locus (DeBenedictis *et al.*, 1984; Brown *et al.*, 1997). The bovine Cγ, Cε and Cα genes have also been expressed as transfectomas to produce bovine-murine chimeric antibodies (Knight *et al.*, 1988). The structural features

of the Cμ gene have been determined in sheep (Patri and Nau, 1992), horse (Schrenzel *et al.*, 1997), pig (Bosch *et al.*, 1992) and rabbit (Bernstein *et al.*, 1984). Two allotypes of the sheep Cμ gene have been identified which differ significantly at the 3' end of the CH1 domain (Hein and Dudler, 1993). The existence of bovine IgM allotypes has been reported with isotype-specific monoclonal anti-IgM antibodies that recognized different epitopes on bovine IgM antibody (Naessens *et al.*, 1988; Williams *et al.*, 1990) but these have not been fully characterized. In addition, structural basis of IgM allotypes remains to be defined. More recently, germline Cμ exons have been characterized from a Brown Swiss cow and, as in other mammalian species, the TM1 exon is spliced into the Cμ4 exon in the membrane form of bovine IgM antibody (Mousavi *et al.*, 1998). To understand the molecular basis of bovine IgM allotypes, we analysed cDNAs from IgM-secreting mouse x bovine hetero-hybridomas originating from a Holstein and an Angus x Hereford cross-bred cow. The experiments outlined here demonstrate that allelic polymorphism in the bovine IgM results from sporadic nucleotide substitutions leading to amino acid replacements in all the domains of IgM. The retention of nine nucleotides at the Cμ1 and Cμ2 junction from the intervening intron due to the cleavage of pre-mRNA at an alternate 5' splice donor site provides an additional source of IgM structural variants. The RFLP analysis showed existence of, at least, four allelic variants of Cμ gene in the bovine genome.

7.3 Materials and Methods

7.3.1 B-cell hetero-hybridomas

Two mouse x cattle hetero-hybridomas, BLV7G1 and B5D8, were developed by

somatic cell fusion of bovine PBLs, stimulated *in vitro* with 25 μg/ml of lipopolysaccharide from *Escherichia coli* (serotype 0127:B8) and 300 ng/ml of phorbol 12-myristate 13-acetate (Sigma Chemical Co., St. Louis. MO. USA) for 48 hours, with X63.Ag8.653 mouse myeloma cells as described (Saini *et al.*, 1997). The clones BLV7G1 and B5D8 originated from a Holstein Friesian and Angus x Hereford cross-bred cow, respectively. The supernates from both the clones tested positive for bovine IgM secretion in ELISA (Saini *et al.*, 1997; Chapter 3) using murine monoclonal anti-bovine IgM as a capture antibody (Sigma Chemical Co.) and biotin-conjugated murine monoclonal anti-bovine IgM (Sigma Chemical Co.), and avidin-conjugated alkaline phosphatase (Pierce, Rockford, IL) as secondary reagents.

7.3.2 PCR amplification, cloning and sequencing of bovine Cμ genes

Total RNA was extracted from the hetero-hybridomas using Trizol reagent (GIBCO BRL Gaithersburg, MD,USA). The cDNA was synthesized from 7 μg of total RNA using polydT primer (Pharmacia LKB Biotechnology, Uppsala, Sweden) and PCR amplified using 5' primer (5' AGCTCGAGTCACCGTCTCCTCA 3') from the bovine J_H gene sequence (Saini *et al.*, 1997) and 3' primer (5' AGACTAGTTTACCGGTGGACTTGTCC 3') from the 3' sequence of the Cμ4 exon conserved across species. The 5' and 3' primers had built-in restriction sites for *Xho* I and *Spe* I endonuclease, respectively. The PCR steps involved a hot start at 95°C for 2 min, denaturation at 95 °C for 1 min and combined annealing and extension steps at 72°C for 2.5 min up to a total of 30 cycles. The PCR conditions for all reactions included 1.5 mM $MgCl_2$, 0.8 μM of each primer and 2.5 U of Taq polymerase (Perkin Elmer, Branchburg, NJ, USA) in a 100 μl volume.

The PCR product (approximately 1.3 kb) from clone BLV7G1 was digested with *Xho*

I and *Spe* I, fractionated by gel electophoresis, purified with GeneClean II (Bio 101, Vista, CA, USA) and ligated into the dephosphorylated (calf intestinal alkaline phosphatase from Boehringer Mannheim, Germany) multiple cloning site of Bluescript KS +/- vector (Stratagene, La Jolla, CA, USA) as described (Saini *et al.*, 1997). The recombinant plasmids extracted from at least two colonies originating form clone BLV7G1 and the PCR product from clone B5D8 were sequenced in both directions with T3/J_H and T7/Cμ4 primers using an automated DNA sequencing facility at MOBIX, McMaster University, Hamilton, Ontario, Canada. In addition, internal Cμ sense primers; BMS-I (5' GTGTGCGAAGTCCAGCA 3') and BMS-II (5' AGCTGTCCTGTCTGGTCA 3') corresponding to nucleotides 262-278 and 740-757, respectively, and anti-sense primers; BMR-I (5' TGCTGGACTTCGCACAC 3') and BMR-II (5' TGACCAGACAGGACAGCT 3') also corresponding to nucleotides 262-278 and 740-757, respectively, were synthesized according to the determined sequences of cattle Cμ gene and used to extend the sequence in both directions. The codons are numbered according to Kabat *et al.* (1991) following the Ou index (Putnam *et al.*, 1973).

In addition, genomic DNA extracted from both hetero-hybridomas and peripheral blood and/or sperms from Holstein (2), Brown Swiss (2), Angus (1) and Hereford (1) breeds was PCR amplified with BMS-I and Cμ4 primers. The PCR was performed for 30 cycles, each consisting of denaturation at 95°C for 1 min, annealing at 65°C for 1 min and extension at 72°C for 1 min. The PCR products were purified with GeneClean II (Bio 101, Vista, CA, USA) and sequenced with either BMS-I or Cμ4 primer to analyse the structural features at the CH1-CH2 junction and the CH4 domain.

7.3.3 Southern blot hybridization

Genomic DNA was extracted from the blood cells of Holstein Friesian, Jersey, Brown Swiss, Angus, Hereford and Charolais cattle using DNA extraction kit (Stratagene, La Jolla, CA, USA). Sperm genomic DNA was also extracted from fresh semen samples collected from seven Holstein bulls. About 50 µl of washed sperm were resuspended in 580 µl of 0.01 M Tris (pH 7.2) containing 50 mM EDTA and digested at 37°C for 24 h in 60 µl of proteinase K (20 mg/ml), 80 µl of SDS (20%) and 80 µl of dithiothreitol (0.39 M). The DNA was extracted twice with phenol:chloroform (1:1) and twice with chloroform:isoamylalcohol (24:1) and precipitated with 1/10th volume of 5 M NaCl and two volumes of chilled 100% ethanol. About 10-15 µg of genomic DNA samples, digested to completion with *Bam*H I, *Eco*R I and *Hin*d III restriction enzymes, were electrophoresed at 18 V for 36 h in a 0.8% agarose gel. The DNA was depurinated in 0.2 N HCl for 30 minutes and transferred onto maximum strength nytran membrane (Schleicher and Schuell Inc., Keene, NH, USA) using an alkaline transfer method (1 M NaOH) by VacuGene (Pharmacia LKB Biotechnology). The membranes were pre-hybridized overnight at 42°C followed by hybridization (50% formamide, 5X Denhardt's solution, 5X SSPE, 0.5% SDS, 100 µg/ml sonicated and denatured salmon sperm DNA and 2.5-3.0 x10^6 dpm of [α-^{32}P]dCTP radiolabeled bovine Cµ DNA probe/ml.).

The bovine Cµ specific DNA probe was prepared by PCR amplification of a 900 bp fragment (corresponding to nucleotides 419 to 1323) of rearranged Cµ gene from clone BLV7G1. The DNA probe was purified with GeneClean II (Bio 101, Vista, CA, USA) and radiolabeled with [α-^{32}P]dCTP (Amersham Canada Ltd., Oakville, ON) by random priming (Boehringer Mannheim, Germany). Following overnight hybridization, the membranes were

washed three times in 2X SSC, 0.1% SDS solution at room temperature, once in 1X SSC, 0.1% SDS for 10-15 min at 65°C, and finally in 0.1X SSC, 0.1% SDS at 65°C for 10-15 min. The membranes were exposed to XAR-5 films (Eastman Kodak Co., Rochester, NY, USA) for 2 to 5 days at -70°C.

7.4 Results

7.4.1 Evidence for allelic polymorphism in bovine Cμ gene

The complete nucleotide sequence of Cμ exons, encoding the four domains of bovine IgM antibody, was obtained after cloning of cDNA from bovine IgM-secreting hetero-hybridoma, BLV7G1, originating from a Holstein cow and was compared with the cDNA sequence of Cμ exons from hetero-hybridoma B5D8, originating from an Angus x Hereford cross-bred cow (Fig. 7.1). Both the cDNAs shared high nucleotide (98.7%) and amino acid (97.9%) homology, but the Cμ exons from the Angus x Hereford cross-bred cow had seven nucleotide substitutions that resulted in five amino acid replacements in the four Cμ exons. Most remarkable, however, is the insertion of nine extra bases (GTGAGTCAG) at the junction of Cμ1 and Cμ2 exons. Such an insertion at the junction of Cμ1 and Cμ2 exons has neither been noted in Holstein and Brown Swiss cattle nor in other species like sheep, pig, rabbit, horse, mice and humans. Since these observations were consistently made in four different sets of experiments, a comparison of germline Cμ gene sequence, spanning 3' end of Cμ1 exon and 5' end of Cμ2 exon, including the intervening intron, was made with the corresponding region of the cDNA sequence. The nine bases retained at the junction of Cμ1 and Cμ2 exons of the Angus x Hereford cross-bred cow originated from the intervening intron DNA sequence. A comparison of the genomic Cμ sequences revealed that the clone

Fig. 7.1 The cDNA sequences of cattle Cμ gene from hetero-hybridomas BLV7G1 and B5D8 originating from a Holstein and an Angus x Hereford cross-bred cow, respectively. The deduced amino acid sequence of BLV7G1 cDNA is indicated above the nucleotide sequence. The codons are numbered according to Kabat *et al.* (1991). The cDNA sequence from clone B5D8 shows nine additional bases between CH1 and CH2 domains retained from the intervening intron sequence. The potential N-linked glycosylation sites are underlined. The germline Cμ sequence of a Brown Swiss (BS) cow is included for comparison (Mousavi *et al.*, 1998). The dash (-) indicates identical nucleotide and dot (.) indicates a lack of nucleotide. The sequences presented are available from GenBank accession numbers AF005274 (clone BLV7G1) and AF097213 (clone B5D8).

```
        114                     120                                     130
         E   G   E   S   H   P   R   V   F   P   L   V   S   C   V   S   S   P   S   D   E   S   T   V   A
BLV7G1  GAA GGT GAA TCA CAC CCG AGA GTC TTC CCC CTG GTG TCC TGC GTG AGC TCG CCA TCC GAT GAG AGC ACG GTG GCC 75
B5D8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS      --- --- --- --- --- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

            140                                     150                                     160
         L   G   C   L   A   R   D   F   V   P   N   S   V   S   F   S   W   K   F   N   N   S   T   V   N
BLV7G1  CTG GGC TGC CTG GCC CGG GAC TTC GTG CCC AAT TCA GTC AGC TTC TCC TGG AAG TTC AAC AAC AGC ACA GTC AAC 150
B5D8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -G-
BS      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -G-

                                170                                     180
         S   E   R   F   W   T   F   P   E   V   L   R   D   G   L   W   S   A   S   S   Q   V   V   L   P
BLV7G1  AGC GAG AGA TTC TGG ACC TTC CCC GAA GTC CTG AGG GAC GGC TTG TGG TCG GCC TCC TCT CAG GTG GTC CTG CCC 225
B5D8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- T--
BS      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

            190                                     200                                     210
         S   S   S   A   F   Q   G   P   D   D   Y   L   V   C   E   V   Q   H   P   K   G   G   K   T   V
BLV7G1  TCC TCA AGC GCC TTT CAA GGG CCG GAT GAC TAC CTG GTG TGC GAA GTC CAG CAC CCC AAG GGA GGA AAG ACC GTC 300
B5D8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                220     ⌈CH2                                    230
         G   T   V   R   V   I   A   T                   K   A   E   V   L   S   P   V   V   S   V   F   V   P
BLV7G1  GGC ACC GTG AGG GTG ATC GCT ACA A.. ... ... .AG GCG GAA GTG CTG TCC CCA GTC GTG AGT GTC TTT GTC CCG 375
B5D8    --- --- --- --- --- --- --- --- -GT GAG TCA G-- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS      --- --- --- --- --- --- --- --- -.. ... ... .-- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                        240                                     250                                     260
         P   R   N   S   L   S   G   D   G   N   S   K   S   S   L   I   C   Q   A   T   D   F   S   P   K
BLV7G1  CCT CGC AAC AGC CTC TCT GGT GAC GGC AAT AGC AAG TCC AGC CTC ATC TGC CAG GCC ACG GAC TTC AGC CCC AAA 450
B5D8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
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Fig. 7.1 (Continued)

```
                                                  270                                                  280
         Q   I   S   L   S   W   F   R   D   G   K   R   I   V   S   G   I   S   E   G   Q   V   E   T   V
BLV7G1  CAG ATC TCC TTG TCC TGG TTT CGT GAT GGA AAG CGG ATA GTG TCT GGA ATT TCT GAA GGC CAG GTG GAG ACT GTG 525
B5D8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- --- --- --- --- --- --- --- ---
BS      --- --- --G --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                        290                                                 300                                                 310
         Q   S   S   P   V   T   F   R   A   Y   S   M   L   T   I   T   E   R   D   W   L   S   Q   N   V
BLV7G1  CAG TCC TCA CCC GTA ACT TTC AGG GCC TAC AGC ATG CTG ACC ATC ACG GAG AGA GAC TGG CTC AGC CAG AAC GTG 600
B5D8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS      --- --- --- --- A-- --C --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -C-

                                                  320                                                 330         ⌈CH3
         Y   T   C   Q   V   E   H   N   K   E   T   F   Q   K   N   V   S   S   S   C   D   V   A   P   P
BLV7G1  TAC ACC TGC CAG GTA GAA CAC AAC AAG GAA ACC TTC CAG AAG AAC GTG TCC TCC TCA TGT GAT GTT GCA CCA CCA 675
B5D8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                        340                                                 350                                                 360
         S   P   I   G   V   F   T   I   P   P   S   F   A   D   I   F   L   T   K   S   A   K   L   S   C
BLV7G1  TCT CCC ATC GGG GTC TTC ACC ATC CCC CCA TCC TTC GCC GAC ATC TTC CTC ACG AAG TCA GCC AAG CTG TCC TGT 750
B5D8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS      --- --G --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                                          370                                                 380
         L   V   T   N   L   A   S   Y   D   G   L   N   I   S   W   S   R   Q   N   G   K   A   L   E   T
BLV7G1  CTG GTC ACA AAC CTG GCC TCC TAT GAT GGC CTG AAC ATC AGC TGG TCC CGT CAG AAC GGC AAG GCC CTG GAG ACC 825
B5D8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                        390                                                 400                                                 410
         H   T   Y   F   E   R   H   L   N   D   T   F   S   A   R   G   E   A   S   V   C   S   E   D   W
BLV7G1  CAC ACG TAT TTT GAG CGA CAC CTC AAC GAC ACC TTC AGC GCC CGG GGT GAG GCC TCG GTC TGC TCG GAG GAC TGG 900
B5D8    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
```

Fig. 7.1 (Continued)

```
                                                                  420                                                                  430
          E   S   G   E   E   F   T   C   T   V   A   H   S   D   L   P   F   P   K   K   N   S   V   S   K
BLV7G1 GAG TCC GGA GAG GAG TTC ACG TGC ACA GTG GCC CAC TCG GAC CTG CCC TTC CCA AAA AAG AAC AGC GTC TCC AAG 975
B5D8   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- G-- --- --- -C- --- --- ---
BS     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- G-- --- --- GC- --- --- ---

                 ⌈CH4      440                                                                  450                                                                  460
          P   K   D   V   A   M   K   P   P   S   V   Y   L   L   P   P   T   R   E   Q   L   S   L   R   E
BLV7G1 CCC AAA GAC GTC GCC ATG AAA CCG CCG TCC GTG TAC CTG CTG CCT CCA ACG CGG GAA CAG CTG AGC CTG CGG GAG1050
B5D8   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --C ---

                                                                  470                                                                  480
          S   A   S   V   T   C   L   V   K   G   F   A   P   A   D   V   F   V   Q   W   L   Q   R   G   E
BLV7G1 TCG GCC TCC GTC ACC TGC CTG GTG AAG GGC TTC GCG CCC GCG GAC GTG TTC GTG CAG TGG CTG CAG AGG GGG GAG1125
B5D8   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS     --- --- --- --- --- --- --- --- --- -CG --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

                          490                                                                  500
          P   V   T   K   S   K   Y   V   T   S   S   P   A   P   E   P   Q   D   P   S   V       Y   F   V
BLV7G1 CCC GTG ACC AAG AGC AAG TAC GTG ACC AGC AGC CCG GCG CCC GAG CCT CAG GAC CCC AGC GTG ... TAC TTT GTG1200
B5D8   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --C --- --- --- --- --- ... --- --- ---
BS     --- --- --- --- --- --- --- --- --- --- GC- -GC --- --- --- --C --- --- --- --- --- GTG --- --- ---

          510                                                                  520                                                                  530
          H   S   I   P   T   V   A   E   E   D   W   S   K   G   E   T   Y   T   C   V   V   G   H   E   A
BLV7G1 CAC AGC ATC CCG ACG GTG GCC GAG GAG GAC TGG AGC AAA GGG GAG ACC TAC ACC TGC GTC GTG GGC CAC GAG GCC1275
B5D8   --- --- --- -T- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS     --- --- --- -T- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ... --- --A ---

                                  540                                                                  550
          L   P   H   M   V   T   E   R   T   V   D   K   S   T   G   K
BLV7G1 CTG CCC CAC ATG GTC ACC GAG CGG ACC GTG GAC AAG TCC ACC GGT AAA  1323
B5D8   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
BS     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
```

Fig. 6.1

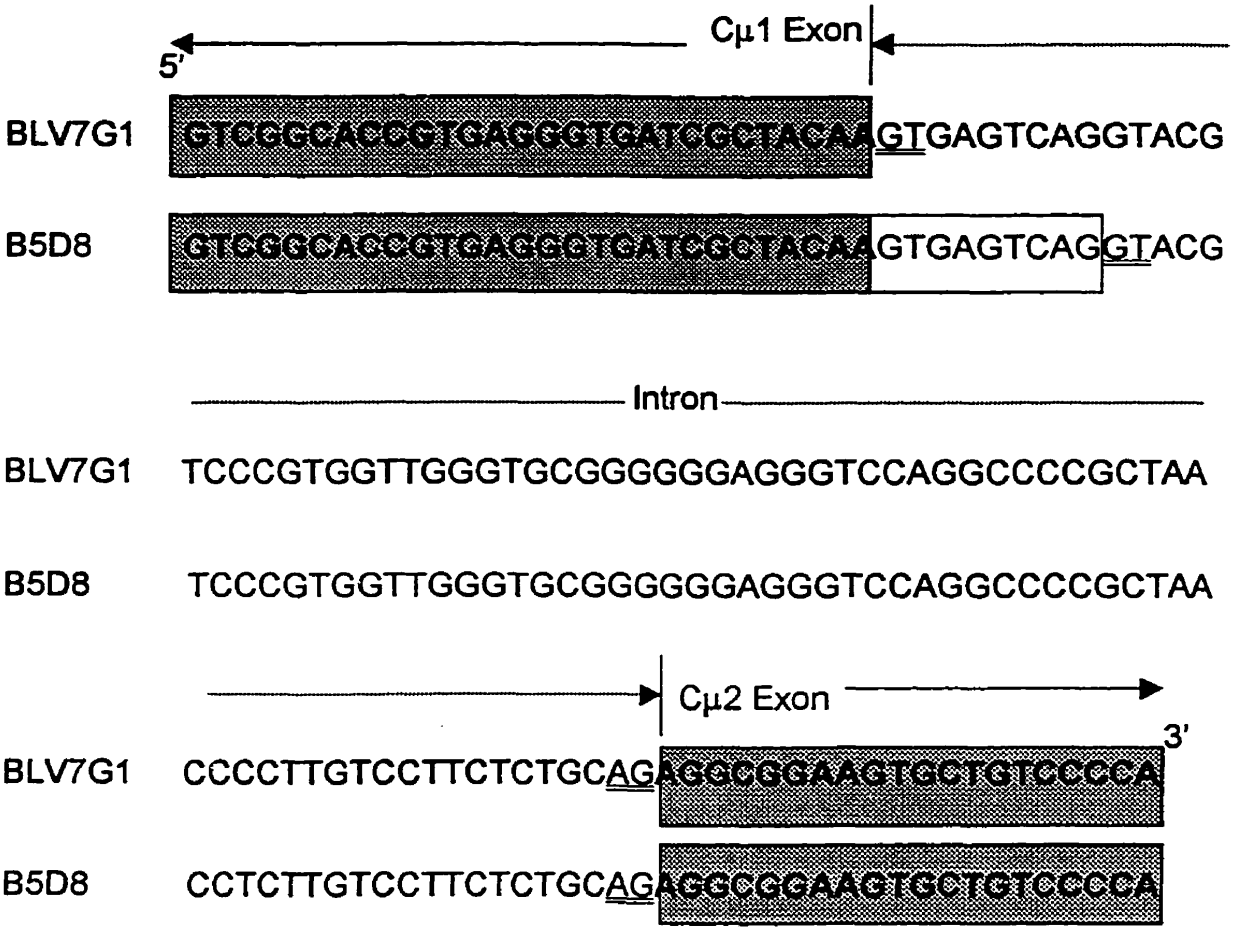
BVL7G1 utilized a 5' splice donor site located immediately after the Cμ1 exon while clone B5D8 employed an alternate cryptic 5' splice donor site located downstream in the intron (Fig. 7.2). The GT dinucleotides were noted at both the sites which are usually conserved at 5' splice donor sequences (Mount, 1982). Both clones, however, utilized identical 3' splice acceptor sites located at the intron-Cμ2 exon junction and are characterized by highly conserved AG residues preceded by nine pyrimidines, a characteristic of consensus 3' splice acceptor site (Mount, 1982). Thus, cleavage of pre-mRNA at two alternate 5' splice donor sites provides the molecular basis for additional IgM structural variants.

A comparison with the available germline Cμ gene sequence from Brown Swiss cattle (Mousavi *et al.*, 1998) showed high nucleotide (97.8%) and amino acid (97.2%) homology with the Holstein Cμ gene. The alignment of the three bovine Cμ sequences revealed an extra codon GTG inserted between positions 506 and 507 in the Cμ4 exon while codon GGC was deleted from position 531 in the Brown Swiss cattle (Fig. 7.1). Such additions and deletions in the most conserved CH4 domain of IgM (Matsunaga *et al.*, 1994) were, however, not detected in six additional bovine germline Cμ gene sequences from Holstein (2), Brown Swiss (2), Angus (1) and Hereford (1) cattle (not shown). Overall, nucleotide substitutions resulting in amino acid replacements in all the Cμ domains provide molecular evidence for allelic variants of bovine IgM.

7.4.2 Few proline residues occur at CH1-CH2 junction of bovine IgM

The bovine IgM showed high amino acid homology with the sheep IgM (89.4%), but not with pig (64.5%), rabbit (59.7%), human (59.2%), horse (58.3%) and mouse (54.8%) (Table 7.1). Both cattle and sheep IgM antibodies showed unique amino acid substitutions

Fig. 7.2 The partial genomic Cμ gene sequence including 5' portion of Cμ1 and 3' portion of Cμ2 exon (shaded boxes) and the intervening intron (unshaded letters) from hetero-hybridomas BLV7G1 and B5D8. The 5' splice donor (GT) and 3' splice acceptor (AG) sites are doubly underlined. The letters in unshaded box depict the nucleotides retained between the CH1 and CH2 domains of B5D8 cDNA as a result of cleavage of pre-mRNA at an alternate cryptic 5' splice donor site.


Cμ1 Exon
5'
BLV7G1
GTCGGCACCGTGAGGGTGATCGCTACAAGTGAGTCAGGTACG
B5D8
GTCGGCACCGTGAGGGTGATCGCTACAAGTGAGTCAGGTACG
Intron
BLV7G1
TCCCGTGGTTGGGTGCGGGGGGAGGGTCCAGGCCCCGCTAA
B5D8
TCCCGTGGTTGGGTGCGGGGGGAGGGTCCAGGCCCCGCTAA
Cμ2 Exon
3'
BLV7G1
CCCCTTGTCCTTCTCTGCAGAGGCGGAAGTGCTGTCCCCA
B5D8
CCTCTTGTCCTTCTCTGCAGAGGCGGAAGTGCTGTCCCCA

at 12 positions, distinct from other species (Fig. 7.3). The cattle IgM has 11 cysteine residues which are involved either in intra-domain disulfide bridging (positions 141-202, 252-313, 360-418 and 466-518) or inter-domain disulfide bridging with the light chain (position 127) and corresponding heavy chain (positions 330 and 406) (Putnam *et al.*, 1973). While seven tryptophan residues are noted in the IgM antibodies of other species, cattle and sheep IgM antibodies have nine tryptophan residues. The additional tryptophans are located in the CH1 domain at position 168 and 179 (Fig. 7.1 and 7.3). Surprisingly, the bovine IgM has five proline amino acids between positions 200 and 260. This is the lowest number of proline residues in this region as compared to other species such as sheep, rabbit and horse (6 each), pig (7), mouse (8) and human (9). Similar to other species, bovine IgM has four potential sites for N-linked glycosylation (Asn-X-Thr/Ser) located between positions 158-160, 325-327, 372-374 and 394-396 (Fig. 7.1).

7.4.3 RFLP analysis reveals polymorphism at bovine Cμ-locus

Restriction digestion of genomic DNA with *Bam*H I generated either one (Jersey and Hereford), two (Holstein) or three (Charolais) fragments hybridizing with the bovine Cμ-specific DNA probe (Fig. 7.4). The *Eco*R I restriction digestion revealed a 24.8 kb prominent band in all the breeds tested. However, a 23 kb band observed in other breeds was not seen in Charolais DNA. Three to four Cμ-specific hybridizing bands, in the range of 8.5 to 23.1 kb, were detected in the genomic DNA digested with *Hin*d III. The 15.4 kb band was common to all the breeds, whereas, a 8.5 kb fragment was seen only in Hereford cattle. A less prominent 6.2 kb band was detected in all the samples that corresponded to the IGHML (immunoglobulin M-like 1) gene previously reported to exist in the bovine genome (Tobin-

Table 7.1 Comparison of nucleotide sequence of Holstein Cμ gene (BLV7G1) with Angus x Hereford cow (B5D8) and other species*.

CH Domain	Per cent homologies with													
	Angus x Hereford		Sheep		Pig		Rabbit		Human		Horse		Mouse	
	Nucleot-ides	Amino acids	Nucleot-ides	Amino acids	Nucleot-ides	Amino acids	Nucleot-ides	Amino acids	Nucleot-ides	Amino acids	Nucleot-ides	Amni acids	Nucleot-ides	Amino acids
CH1	96.7	95.5	88,3	82.4	-	-	63.3	44.0	61.8	51.4	52.7	44.5	62.3	44.4
CH2	99.4	99.1	92.7	88.2	69.1	58.2	64.6	53.9	64.6	50.9	66.1	52.7	55.9	40.2
CH3	99.3	98.1	93.3	92.4	72.3	63.2	76.4	64.1	73.9	62.3	73.4	64.1	66.7	60.4
CH4	99.4	99.1	96.1	94.6	82.7	71.9	82.7	76.8	80.6	72.3	77.3	71.7	75.3	74.1
Total	98.7	97.9	92.6	89.4	74.6	64.5	71.7	59.7	70.2	59.2	67.4	58.3	65.6	54.8

*The sequence was compared with those from sheep (Patri and Nau, 1992), pig (Bosch *et al.*, 1992), rabbit (Bernstein *et al.*, 1984), human (Harindranath *et al.*, 1993), horse (Schrenzel *et al.*, 1997) and mouse (Kawakami *et al.*, 1980).The gaps inserted to optimize the homologies were considered as mismatches.

Fig. 7.3 Comparison of the deduced amino acid sequences of cattle Cμ gene from Holstein (BLV7G1) and Angus x Hereford cross-bred (B5D8) cows with the predicted amino acid sequences of Cμ gene from Brown Swiss (BS) cow (Mousavi *et al.*, 1998), sheep (Patri and Nau, 1992), pig (Bosch *et al.*, 1992), rabbit (Bernstein *et al.*, 1984), human (Harindranath *et al.*, 1993), horse (Schrenzel *et al.*, 1997) and mouse (Kawakami *et al.*, 1980).The dash (-) indicates identical amino acid and dot (.) indicates lack of an amino acid. The gaps are inserted to optimize homology. *Unique amino acids substitutions in the IgM antibodies of cattle and sheep in contrast to conserved residues at these positions in other species.

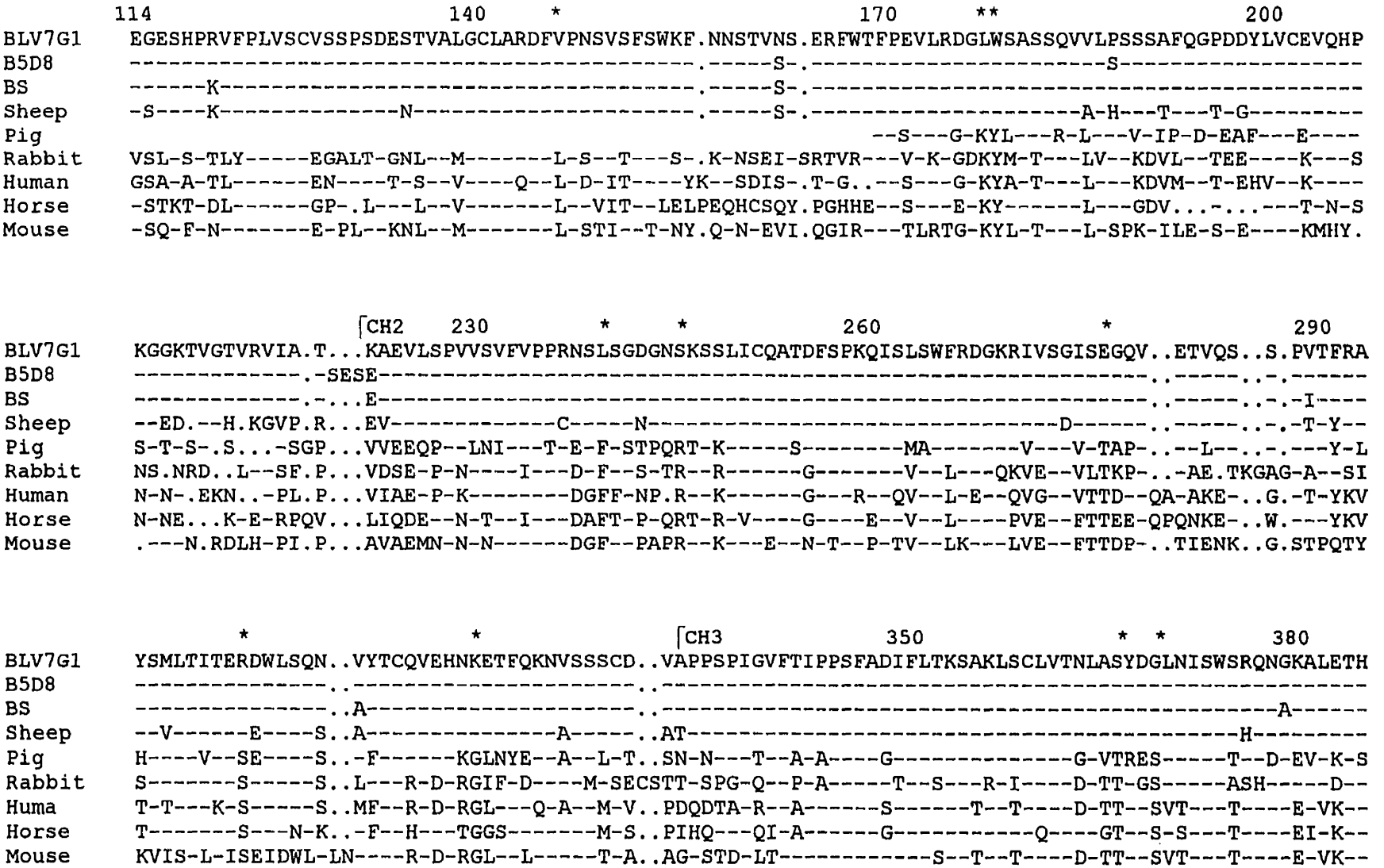
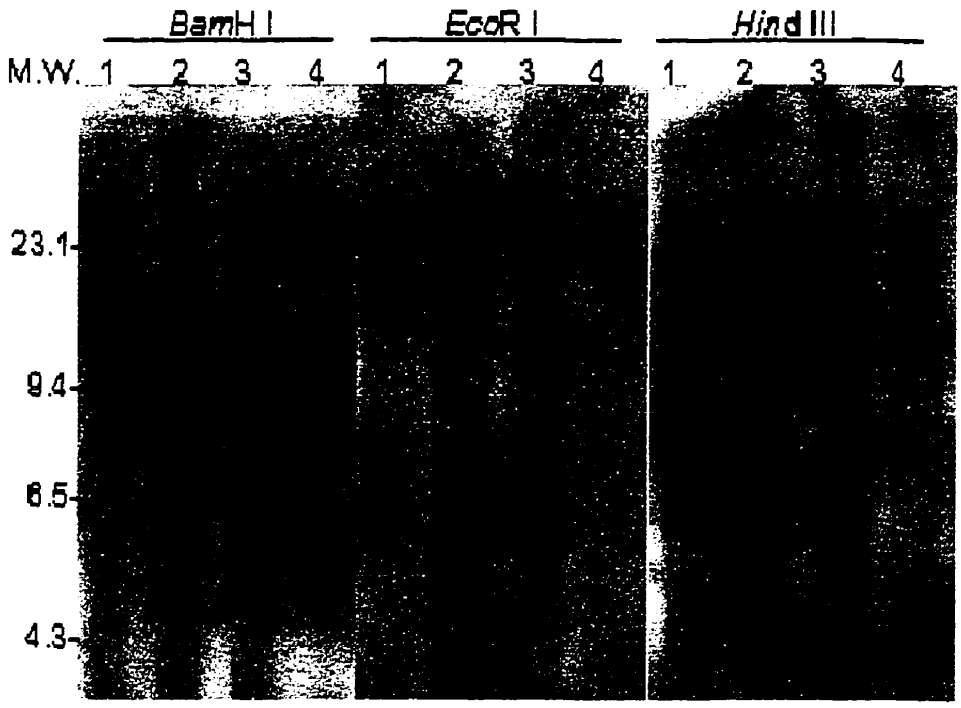


Fig. 7.3 (Continued)

410 * ⌈CH4 * 470
BLV7G1 T.YF.ERHLNDTFSARGEASVCSEDWESGEEFTCTVAHSDLPFPKKNSVSKPKDVAMKPPSVYLLPPTREQLSLRESASVTCLVKGF.APADVFVQ
B5D8 -.--.----------E--T----------.--------
BS -.--.----------E--A----------A-.--------
Sheep -.--.----------Y------L-----E-SAI----------V----------.--------
Pig IVFS.-IYA-RA-G.Q------V------DR-----T-T---S-L-Q-----RGM-RHM----V---AP-E---Q-W----------.S-------
Rabbit M.NIT-S-P-A----M------A-------Q-----T-A-----L-HTI--SRE--KH--A--V---A----V-----T--------.S-------
Human -NIS.-S-P-A----V----I-ED--N---R-----T-T---S-L-QTI-R--G--LHR-D------A----N-----TI----T--.S-------
Horse -NIS.-S-P-G----L---TI-V------DDYI---T-T-----L-QVI-R-DA-GKH-----V---H----------I-------LT-P--S--
Mouse I.KIM-S-P-G----K-V----V---NNRK--V---T-R---S-Q-KFI---NE-HKH---------A----N-----T--------.S---IS--
500 530 550
BLV7G1 WLQRGEPVTKSKYVTSSPAPEPQ..DPSV.YFVHSIPTVAEEDWSKGETYTCVVGHEALPHMVTERTVDKSTGK
B5D8 ----------..----.------L----------
BS ----------AR-----..----V------L----------.----------
Sheep ---K----A----------..---A.------L--T----------
Pig ---K----SAD-----A-V---EPKA-AS.---Q-VL--SAK---D----------T----------
Rabbit -Q---Q-LSSD-----A------..A-GL.--T--TL--T----NS---F----------
Human -M---Q-LSPE-----A-M----..A-GR.--A---L--S--E-NT--------A-----NR----------
Horse ---E-Q-LSPD-----A-MLD-S..PQAL.------L--S-----S----------V---------HC-
Mouse -----QLLPQE-----A-M---G..A-GF.--T---L--T--E-NS----------L----------

Fig. 7.3

Fig. 7.4 The RFLP analysis of the bovine genomic DNA from Holstein (1), Jersey (2), Hereford (3) and Charolais (4) cows digested with *Bam*H I, *Eco*R I and *Hin*d III restriction enzymes and hybridized with [α-^{32}P]dCTP radiolabeled cattle Cμ-specific DNA probe.


BamH I
EcoR I
Hind III
M.W.
1 2 3 4 1 2 3 4 1 2 3 4
23.1
9.4
6.5
4.3

Janzen and Womack, 1992). Further, RFLP analysis on sperm genomic DNA from 7 Holstein bulls showed, at least, two RFLP patterns, upon digestion with either *Bam*H I or *Eco*R I enzymes (not shown). Overall, the RFLP analysis suggests that, at least, four allelic forms of Cμ gene exist in cattle.

7.5 Discussion

The experiments outlined here provide structural evidence for extensive polymorphism at the bovine Cμ-locus based on nucleotide substitutions in all the Cμ exons as well as nucleotide addition at the Cμ1-Cμ2 junction. These observations are in agreement with the previous reports of bovine IgM allotypes based on recognition of different epitopes by anti-bovine Cμ-specific monoclonal antibodies (Naessens *et al.*, 1988; Williams *et al.*, 1990). The RFLP analysis of *Bam*H I, *Eco*R I and *Hin*d III restriction enzyme digested genomic DNA from Holstein, Jersey, Hereford and Charolais cattle revealed four allelic forms of bovine Cμ gene. The differences in the RFLP patterns were also noted in *Bam*H I and *Eco*R I digested sperm genomic DNA from seven Holstein bulls that showed polymorphism at the Cμ-locus within the Holstein breed. It could be argued, however, that such intra-breed polymorphism in Holstein breed might be associated with commercial breeding practices or outbred Holstein population. Further family studies of Holstein breed are required to confirm the intra-breed polymorphism observed in this study. Nevertheless, these observations indicate extensive polymorphism at the bovine Cμ-locus, similar to bovine Cγ1-, Cγ2- and Cγ3-loci (Rabbani *et al.*, 1997). Interestingly, addition of nine extra nucleotides at Cμ1-Cμ2 junction in Angus x Hereford cross-bred cow is unique and not observed in Holstein and Brown Swiss breeds. In fact, these nucleotides are retained from the intron sequence intervening Cμ1 and Cμ2

exons as a result of cleavage of pre-mRNA at an alternate cryptic 5' splice donor site (Mount, 1982). This suggests that cleavage of pre-mRNA at two alternate 5' splice donor sites results in two different structural variants of cattle IgM antibodies. A previous report demonstrating addition of codon GTG between positions 506 and 507 and deletion of codon GGC at position 531 (Mousavi *et al.*, 1998) in the conserved Cμ4 domain (Matsunaga *et al.*, 1994) is inconsistent with our observations in Brown Swiss and other cattle breeds.

The Cμ gene is known to exist as a single copy gene in the genome of all the vertebrates except elasmobranchs (horned shark, skates and rays), the earliest lineage in the radiation of the jawed vertebrates (Kokubu *et al.*, 1988a). Our observation with regard to the existence of a 6.2 kb faintly hybridizing band in *Hin*d III digested genomic DNA is consistent with the existence of IGHML (immunoglobulin M-like 1) gene in the bovine genome (Tobin-Jazen and Womack, 1992). A high amino acid homology (89.4%) between cattle and sheep IgM antibodies shows a close phylogenetic relationship between IgM molecules in the ruminant species. In contrast to other species, bovine IgM has the lowest number of proline residues (five) at the junction of the CH1-CH2 domain (position 200 to 260) which corresponds to the hinge region of IgG and IgA antibodies. Since the proline residues provide flexibility in the hinge region, it is likely that bovine IgM antibodies are relatively less flexible as compared to other species. Further, unique amino acid substitutions are noted at 12 positions in the cattle and sheep IgM antibodies as compared to other species. This offers the possibility of synthesizing peptides in the region of such specific substitutions to develop reagents that could be used to detect both sheep and cattle IgM antibodies. Bovine IgM, similar to sheep, pig, rabbit, human, horse and mouse, bears four N-linked glycosylation sites

in the first three Cμ domains. The carbohydrate residues provide functional configuration to IgM antibody by holding its domains in proper position and prevent its degradation (Jefferis and Lund, 1997).

In conclusion, four allelic forms of Cμ gene are noted in the bovine genome. Sporadic nucleotide substitutions resulting in amino acid replacements provide a structural basis of bovine IgM allotypes. Most remarkable, however, is the retention of additional nucleotides at the Cμ1-Cμ2 junction associated with the splice site selection mechanisms. The bovine IgM has the lowest number of proline residues at the CH1-CH2 junction which may result in relatively low flexibility in this region that corresponds to the hinge region of IgG and IgA antibodies.

Chapter 8

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

Unlike mice and humans, restricted germline genetic elements, both at V_H and V_λ loci, encode the primary antibody repertoire in cattle. A single predominantly expressed bovine V_H gene family, designated as Bov V_H1, is identified in cattle. This observation is consistent with other species like sheep, swine, rabbit and chicken in which a single or restricted set of V_H genes encode the entire antibody repertoire. The Bov V_H1 gene family shares maximum nucleotide homology with the murine V_H-Q52 (71.8-78%) and human V_H 4 (67.4-69.8%) gene families and, thus, is related to evolutionarily less conserved mammalian group I V_H genes. This is in contrast to swine, rabbit and chicken V_H genes which are related to evolutionarily conserved mammalian group III V_H genes. The Bov V_H 1 gene family is polymorphic and its genomic complexity ranges from 13 to 15 genes. The bovine V_H genes have a conserved CDR1 length of five amino acids and CDR2 length of eleven amino acids. Extensive nucleotide substitutions resulting in amino acid replacement in CDR1 and CDR2 of the rearranged V_H genes suggest that somatic hypermutations contribute significantly to the diversification of bovine IgM antibody repertoire. A majority of the VDJ rearrangements express CDR3H length ranging from 4 to 31 codons. The most striking observation, however, is the occurrence of an exceptionally long CDR3H (55 to 61 amino acids), with multiple cysteine residues, in approximately 9% of the adult VDJ rearrangements that encode functional IgM antibodies. Such an exceptionally long CDR3H length in a functional IgM

antibody is the first ever to be documented in a species. An even number of cysteine residues, with remarkable conservation of cysteine in the middle of CDR3H (position 100j11), are noted in the exceptionally long CDR3H which are likely to confer hitherto unknown configurational ability as well as rigidity to the antigen combining site via intra-CDR3H disulfide bridging. Information from X-ray crystallography and molecular modelling based on known co-ordinates of the bovine IgM antibody with an exceptionally long CDR3H will provide novel insight into the structural configuration of the combining site. The VDJ rearrangements with unusually long CDR3H are also noted in the splenic B lymphocytes of a 125-day-old bovine fetus. All VDJ rearrangements resulting in the exceptionally long CDR3H express a single germline V_H gene (gl.110.20) with few somatic hypermutations. Similar to other species, the bovine D_H genes are preferentially expressed in a hydrophilic reading frame. The D_H encoded CDR3H region of the fetal VDJ rearrangements shows repetitive GGT (glycine) and TAT (tyrosine) codons that probably favor recruitment of somatic hypermutations. Since all fetal VDJ rearrangements have structurally similar D_H encoded CDR3H regions and are remarkable for repetitive codons, it appears that few closely related D_H genes exist in the bovine genome. It remains unclear at present whether multiple D-D fusions, existence of long bovine D_H genes or processes intrinsic to VDJ recombination contribute to the generation of long CDR3H. Isolation and characterization of bovine germline D_H genes is likely to shed light on this question.The bovine antibodies with exceptionally long CDR3H offer an unique opportunity for the production of antigenized antibodies for vaccination against infectious diseases.Two bovine J_H genes, most homologous to human J_H4 (82.1-87.2%) and J_H 5 (84.6-89.7%) genes, are identified in the expressed

antibody repertoire.

Another remarkable observation relates to the preferential association of bovine $V_\lambda 1x$, hitherto reported to be not expressed in the bovine antibody repertoire, and novel $V_\lambda 1$ genes ($V_\lambda 1d$ and $V_\lambda 1e$) with VDJ rearrangements that encode exceptionally long CDR3H. In contrast to predominantly expressed $V_\lambda 1a$ and $V_\lambda 1b$ genes, the conservation of alanine-serine-alanine residues in the $V_\lambda 1x$, $V_\lambda 1d$ and $V_\lambda 1e$ gene encoded CDR3 region appears to provide structural requirements for the restricted $V_H + V_\lambda$ gene pairing. Such restricted $V_H + V_L$ pairings in polyclonally activated B cells from fetal and adult cattle are unique and deserve further investigations. Eighty available bovine V_λ gene sequences are classified into three V_λ gene families, $V_\lambda 1$, $V_\lambda 2$ and $V_\lambda 3$. The genomic complexity of $V_\lambda 1$ genes comprises 9 to 15 members and polymorphism exists at the bovine $V_\lambda 1$-locus.

The nucleotide structure of cattle Cμ gene has been determined and evidence for the occurrence of, at least, four allelic variants of Cμ gene is provided. Bovine IgM has fewer proline residues at the CH1-CH2 junction. Therefore, bovine IgM antibody is likely to have low flexibility in this region as compared to other species. The occurrence of sporadic nucleotide substitutions through out the Cμ gene provide the molecular basis of different bovine IgM allotypes. An insertion of additional nucleotides at the CH1-CH2 junction as a result of selection of an alternate splice donor site provides another source of bovine IgM variants. The availability of molecular markers for bovine IgM allotypes is likely to be useful for family studies of commercially bred cattle.

Overall, the experiments oulined in this thesis demonstrate that limited germline sequence diversity exists, both at V_H and V_λ loci, in cattle. The antibody diversification in this

species results from the generation of an exceptionally long CDR3H, junctional flexibility and inherent high predisposition to somatic hypermutations. These novel observations on molecular immunogenetics of bovine antibodies are likely to contribute towards future research and development in the area of genetic engineering of antibodies and development of transgenic cows producing antibodies of therapeutic and clinical significance via milk.

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APPENDIX A

MATERIAL GENERATED DURING THE COURSE OF RESEARCH

I. Bovine IgM-secreting mouse x cattle hetero-hybridomas:

Source	Number	Clone number(s)
Peripheral blood lymphocytes (PBL) of a bovine leukemia virus infected Holstein cow	45	BLV1C1, BLV1F3, BLV1G9, BLV1H12, BLV2A9, BLV2C7,BLV2G4, BLV2H6, BLV3D7, BLV3E9, BLV3F12, BLV4A8, BLV4B8, BLV4H9, BLV5B6, BLV5B8, BLV5B12, BLV5D3, BLV5H1, BLV5H4, BLV6D10, BLV7A2, BLV7A8, BLV7B7, BLV7F8, BLV7G1, BLV8A5, BLV8B3, BLV8B11, BLV8C11, BLV8E10, BLV8F3, BLV8G4, BLV8H1, BLV9A7, BLV9B8, BLV9C12, BLV9F2, BLV9H3,BLV10A11, BLV10C12, BLV10E12, BLV10H2, BLV10H8, BLV11F3
PBLs of an Angus x Hereford cross-bred cow	1	B5D8
Splenic B lymphocytes of a 125-day-old fetus	7	BF1H1*, BF2D12, BF2B5*, BF3A11*, BF3H11*, BF4E9*, BF5F10

*Hetero-hybridomas did not secrete bovine IgM, but were positive for VDJ rearrangement in a PCR assay.

II. Cloned DNA probes:

Clone number(s)	Gene cloned	Source/Hetero-hybridoma
p1H12. (1, 2, 3, 4)	VDJ	BLV1H12
p2C7. (5, 10)	VDJ	BLV2C7
p4H9. (1, 14)	VDJ	BLV4H9
p5B8. (1, 3)	VDJ	BLV5B8
p5D3. (1, 2, 3, 5)	VDJ	BLV5D3
p5H4. (1, 2, 3, 5)	VDJ	BLV5H4
p7B7. (2, 4, 5, 7)	VDJ	BLV7B7
p7G1. (5, 15)	VDJ	BLV7G1
p8C11. (1, 2, 3, 4)	VDJ	BLV8C11
p9907. (S1, S2, S3)	VDJ	HB-9907 (ATCC, Rockville, MD, USA)
p9908. (S1, S8, S9, S10)	VDJ	HB-9908 (ATCC, Rockville, MD, USA)
pA70. (1, 2)	VDJ	ILA70 (Dr. J. Naessens, ILRI, Nairobi, Kenya)
pBA14.1	VDJ-Cμ	Uni-ZAP™ XR cDNA library (Stratagene)
pBF1H1 LC. (1, 2)	VJ (λ)	BF1H1
pBLV8C11 LC. (4, 5, 9)	VJ (λ)	BLV8C11
p7G1. (9, 11)	Cμ	BLV7G1

Note: The bovine immunoglobulin genes were cloned in Bluescript KS+/- vector (Stratagene, La Jolla, CA, USA) and DH10B *E. Coli* host cells (GIBCO BRL, Gaithersburg, MD, USA).

APPENDIX B

NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA Graphic

Links: MEDLINE Protein Related Sequences

```
LOCUS       BTU36823      375 bp    mRNA            MAM       03-JUN-1998
DEFINITION  Bos taurus hybridoma S1-BL5C2.870005 anti-bovine herpes virus-1
            IgG1 mRNA, V region, partial cds.
ACCESSION   U36823
NID         g1493796
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 375)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     A single predominantly expressed polymorphic immunoglobulin VH gene
            family, related to mammalian group, I, clan, II, is identified in
            cattle
  JOURNAL   Mol. Immunol. 34 (8-9), 641-651 (1997)
  MEDLINE   98054116
REFERENCE   2  (bases 1 to 375)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (21-SEP-1995) Azad Kaushik, Vet. Micro.& Immuno.,
            University of Guelph, Guelph, Ont. N1G 2W1, Canada
COMMENT     On Aug 16, 1996 this sequence version replaced gi:1040979.
FEATURES             Location/Qualifiers
     source          1..375
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="hybridoma clone S1-BL5C2.870005 producing
                     anti-bovine herpes virus-1 IgG1"
                     /plasmid="pb7s2"
     CDS             <1..>375
                     /note="anti-bovine herpes virus-1 IgG1"
                     /codon_start=1
                     /product="IgG1 heavy chain"
                     /db_xref="PID:g1493797"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTVSGFSLSGNSVGWVRQTPGKA
                     LEWLGNMDGIGTTDYNPALKSRLSITKDNSKSQVSLSLSSVTTEDTATYYCAKCTGAY
                     CWRFDDAYGYDDWGQGLLVTVSS"
BASE COUNT       88 a    104 c    103 g     80 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctgtccctc
       61 acctgcacgg tctctggatt ctcattaagc ggtaatagtg taggctgggt ccgccagact
      121 ccaggaaagg cgctggagtg gctcggtaac atggatggta taggaaccac agactataac
      181 ccagccctga aatcccggct cagcatcacc aaggacaact ccaaaagcca agtctctcta
      241 tcactgagca gcgtaacaac tgaggacacg gccacatact attgtgcgaa gtgtactggt
      301 gcttattgct ggaggtttga tgacgcttat ggttatgatg actggggcca aggactcctg
      361 gtcaccgtct cctcg
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: MEDLINE | Protein | Related Sequences

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LOCUS       BTU36824      450 bp    mRNA            MAM       03-JUN-1998
DEFINITION  Bos taurus hybridoma S2/BL5C2.870009 anti-bovine herpes virus-1
            IgG1 mRNA, V region, partial cds.
ACCESSION   U36824
NID         g1493798
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 450)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     A single predominantly expressed polymorphic immunoglobulin VH gene
            family, related to mammalian group, I, clan, II, is identified in
            cattle
  JOURNAL   Mol. Immunol. 34 (8-9), 641-651 (1997)
  MEDLINE   98054116
REFERENCE   2  (bases 1 to 450)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (21-SEP-1995) Azad Kaushik, Vet. Micro.& Immuno.,
            University of Guelph, Guelph, Ont. N1G 2W1, Canada
COMMENT     On Aug 16, 1996 this sequence version replaced gi:1040981.
FEATURES             Location/Qualifiers
     source          1..450
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="hybridoma clone S2/BL5C2.870009 producing
                     anti-bovine herpes virus-1 IgG1"
                     /plasmid="pb8s1"
     CDS             <1..>450
                     /note="anti-bovine herpes virus-1 IgG1"
                     /codon_start=1
                     /product="IgG1 heavy chain"
                     /db_xref="PID:g1493799"
                     /translation="MNPLWTLLFVLSAPRGVLSQVQLRESGPSLVKPSQTLSLTCTVS
                     GFSLNSWGVGWVRQAPGKALECLGDISSNGYTGYKPALKSRLSITWDNSQSQVSLSLS
                     SVTSDDTATYYCVKSGSIGFSGGGCGVGGVAYAYVDAWGEGLRVTVSS"
BASE COUNT       88 a    125 c    134 g    103 t
ORIGIN
        1 atgaacccac tgtggaccct cctctttgta ctctcagccc ccagaggggt cctgtcccag
       61 gtgcagctgc gggagtcggg ccccagcctg gtgaagccct cacagaccct ctccctcacc
      121 tgcacggtgt ctggattctc attgaacagc tggggtgtag gctgggtccg ccaggctcca
      181 gggaaggcgc ttgagtgtct tggtgatata agtagtaatg gatacacagg gtataagcca
      241 gccctgaaat cccggctcag catcacctgg gacaactcac agagccaagt ctctctgtca
      301 ctgagcagcg tgacaagtga cgacacggcc acatactact gtgtgaaatc tggtagtatt
      361 ggcttcagtg gcggtggttg tggagtgggt ggtgttgctt atgcttacgt cgatgcctgg
      421 ggcgaaggac tccgggtcac cgtctcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: MEDLINE | Protein | Related Sequences

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LOCUS       BTAF000012    381 bp    mRNA            MAM       02-JUN-1998
DEFINITION  Bos taurus hybridoma BL2C7 immunoglobulin heavy chain variable
            region mRNA, partial cds.
ACCESSION   AF000012
NID         g2145026
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 381)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     A single predominantly expressed polymorphic immunoglobulin VH gene
            family, related to mammalian group, I, clan, II, is identified in
            cattle
  JOURNAL   Mol. Immunol. 34 (8-9), 641-651 (1997)
  MEDLINE   98054116
REFERENCE   2  (bases 1 to 381)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-APR-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..381
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /cell_line="BL2C7"
                     /cell_type="hybridoma"
     CDS             <1..>381
                     /codon_start=1
                     /product="immunoglobulin heavy chain variable region"
                     /db_xref="PID:g2145027"
                     /translation="QVQLRESGPSLVKPSQTLSLTRTISGFSLSNYAVGWVRQAPGKA
                     LEMPGDISSGGSTNYNPALKSRLSITKDNSKSQVSLSMSSVTTEDTATYYCAKYTYTS
                     SGFLCDGYNHYRDAWGQGLLVTVSS"
BASE COUNT       92 a    114 c     96 g     79 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctctccctc
       61 acccgcacga tcctctggatt ctcattgagc aactatgctg taggctgggt ccgccaggct
      121 ccagggaagg cgctggaaat gcccggtgac ataagtagtg gtggaagcac aaattataac
      181 ccagccctaa aatcccgact cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcaatgagca gcgtgacaac tgaggacacg gccacatact actgtgcgaa gtatacttat
      301 acgtctagtg gtttcctttg tgacggttac aatcactatc gcgatgcctg gggccaagga
      361 ctcctggtca ccgtctcctc a
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA Graphic
Links: Protein Related Sequences

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LOCUS       BTAF000013    390 bp    mRNA            MAM       02-JUN-1998
DEFINITION  Bos taurus hybridoma BL4H9 immunoglobulin heavy chain variable
            region mRNA, partial cds.
ACCESSION   AF000013
NID         g2145028
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 390)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     A single predominantly expressed immunoglobulin VH gene family,
            related to mammalian group I/clan II genes, is identified in cattle
  JOURNAL   Mol. Immunol. (1997) In press
REFERENCE   2  (bases 1 to 390)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-APR-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..390
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /cell_type="hybridoma"
                     /cell_line="BL4H9"
     CDS             <1..>390
                     /codon_start=1
                     /product="immunoglobulin heavy chain variable region"
                     /db_xref="PID:g2145029"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTISGFSLSSYSVYWVRQTPGKA
                     LECLGGISGSGTAGYHPALKSRLSVSKDNSKSQVSLSVSSVTTEDTATYYCAKSTSGA
                     AYSSSCSAGNFASYLDAWGQGLLVTVSS"
BASE COUNT       80 a    112 c    109 g     89 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctctccctc
       61 acctgcacga tctctggatt ctcattgagc agctatttctg tatactgggt ccgccagact
      121 ccagggaagg cgctggagtg tctgggtggt ataagtggta gtggaaccgc tggctatcac
      181 ccagccctga aatcccggct cagcgtcagc aaggacaact ccaagagcca ggtctctctg
      241 tcagtgagca gcgtgacaac tgaggacacg gccacatact actgtgcgaa gtcaactagt
      301 ggtgctgctt atagtagtag ttgtagtgct ggtaattttg cttcctacct cgatgcctgg
      361 ggccaaggac tcctggtcac cgtctcctca
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NCBI Entrez Nucleotide QUERY

Other Formats: FASTA | Graphic

Links: MEDLINE | Protein | Related Sequences

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LOCUS       BTAF000014     369 bp    mRNA            MAM       02-JUN-1998
DEFINITION  Bos taurus hybridoma BL5H4 immunoglobulin heavy chain variable
            region mRNA, partial cds.
ACCESSION   AF000014
NID         g2145030
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 369)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     A single predominantly expressed polymorphic immunoglobulin VH gene
            family, related to mammalian group, I, clan, II, is identified in
            cattle
  JOURNAL   Mol. Immunol. 34 (8-9), 641-651 (1997)
  MEDLINE   98054116
REFERENCE   2  (bases 1 to 369)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-APR-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..369
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /cell_type="hybridoma"
                     /cell_line="BL5H4"
     CDS             <1..>369
                     /codon_start=1
                     /product="immunoglobulin heavy chain variable region"
                     /db_xref="PID:g2145031"
                     /translation="QVQLRESGPSLVKPSQTLSLTCSVSGFSLSSYASGWVRQAPGKA
                     LEWVAAIDPGGGTYYNPALKSRLSITKDNSKSQVSLSVRSVTPEDTATYYCTTDYSGS
                     CYKLTWSVGVWGQGLLVTVSS"
BASE COUNT       80 a    109 c    103 g     77 t
ORIGIN
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       61 acctgctcgg tctctggatt ctcattgagc agctatgcgt caggctgggt ccgccaggct
      121 ccggggaagg cactggagtg ggttgctgca atagatcctg gtggaggcac atactataac
      181 ccagccctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtgagga gcgtgacacc tgaggacacg gccacatact actgtacgac agactacagt
      301 ggtagttgtt ataaacttac ttggagtgtc ggtgtctggg gccaaggact cctggtcacc
      361 gtctcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: MEDLINE | Protein | Related Sequences

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LOCUS       BTAF000015    372 bp    mRNA            MAM       02-JUN-1998
DEFINITION  Bos taurus hybridoma BL7B7 immunoglobulin heavy chain variable
            region mRNA, partial cds.
ACCESSION   AF000015
NID         g2145032
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 372)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     A single predominantly expressed polymorphic immunoglobulin VH gene
            family, related to mammalian group, I, clan, II, is identified in
            cattle
  JOURNAL   Mol. Immunol. 34 (8-9), 641-651 (1997)
  MEDLINE   98054116
REFERENCE   2  (bases 1 to 372)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-APR-1997) Pathobiology, University of Guelph,-Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..372
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                     /db_xref="taxon:9913"
                     /cell_type="hybridoma"
                     /cell_line="BL7B7"
     CDS             <1..>372
                     /codon_start=1
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                     /db_xref="PID:g2145033"
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                     LEYVGGVDADGGARYNPTLKSRLSITKDNSKSQVSLSVSSVTTEDTATYYCAKNSLFN
                     GDGDACGRLEVWGQGLLVTVSS"
BASE COUNT       85 a    109 c    102 g     76 t
ORIGIN
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       61 acctgcacgg tctctggatt ctcattgagc agctatgctg tgctctgggt ccgccaggct
      121 ccaggaaaga cgctggagta cgttggtggt gtagatgctg atggcggcgc aagatataac
      181 ccgaccctga aatcccggct cagcatcacc aaagacaact ccaagagcca agtctctctg
      241 tcagtgagca gcgtgacaac ggaagacacg gccacatact actgtgcaaa aaattcgtta
      301 tttaatggcg atggtgacgc ctgtggtcgg ctcgaagtct ggggccaagg actcctggtc
      361 accgtctcct ca
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA Graphic

Links: MEDLINE Protein Related Sequences

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LOCUS       BTAF000016    378 bp    mRNA            MAM       02-JUN-1998
DEFINITION  Bos taurus hybridoma BL7G1 immunoglobulin heavy chain variable
            region mRNA, partial cds.
ACCESSION   AF000016
NID         g2145034
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 378)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     A single predominantly expressed polymorphic immunoglobulin VH gene
            family, related to mammalian group, I, clan, II, is identified in
            cattle
  JOURNAL   Mol. Immunol. 34 (8-9), 641-651 (1997)
  MEDLINE   98054116
REFERENCE   2  (bases 1 to 378)
  AUTHORS   Saini,S.S., Hein,W.R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-APR-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /cell_type="hybridoma"
                     /cell_line="BL7G1"
     CDS             <1..>378
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                     /db_xref="PID:g2145035"
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                     DRGGCGYADAYDAWGQGLLVTVSS"
BASE COUNT       80 a    106 c    108 g     84 t
ORIGIN
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       61 acctgcacga tctctggatt ctcattgagc agctatgctg tagactgggt ccgccaggct
      121 ccagggaagg cactggagtg gcttggtggt ataaataata ctggacgcac aggctataac
      181 gcagccctga aatcccggct cagcatcgcc aaggacaact ccaggagcca agtctctctt
      241 tcagtgagca gcgtgacaac tgaggacacg gccacatact actgtgcgaa gtgtacttttt
      301 ggtggtgacc gtggtggttg tggttatgcg gatgcttatg atgcctgggg ccaaggactc
      361 ctggtcaccg tctcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015490      336 bp    mRNA            MAM       04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015490
NID         g2353725
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 336)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 336)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /clone="BLV1F3"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>336
                     /codon_start=1
                     /product="immunoglobulin variable region"
                     /db_xref="PID:g2353726"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTVSGFSLNDYGVKWVRQAPGKA
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                     GQGLLVTVSS"
BASE COUNT       73 a    101 c     95 g     67 t
ORIGIN
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       61 acctgcacgg tctctggatt ctcattgaac gactatggtg ttaaatgggt ccgccaggct
      121 ccggggaagg cactggagtg ggttggtgtt atgtggagtg gagggaactc cgattataac
      181 ccagccctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtgagca gcgtgacacc tgaggacacg gccacatatt actgtgcaac gtacgccagt
      301 gcctggggcc aaggactcct ggtcaccgtc tcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez Help

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015491      387 bp    mRNA            MAM      04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015491
NID         g2353727
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 387)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 387)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:9913"
                     /clone="BLV1G9"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>387
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                     /product="immunoglobulin variable region"
                     /db_xref="PID:g2353728"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTISGFSLSSYSVVWVRQAPGKA
                     LECLATIGSLGGTDYNPALKSRLSITKDNSKSQVSLSVSSVSTEDTATYYCAKSNSPY
                     CFRCVSSSYPDRYIDAWGQGLLVTVSS"
BASE COUNT       82 a    119 c     97 g     89 t
ORIGIN
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       61 acctgcacga tctctggatt ctcattgagc agctattctg tagtctgggt ccgccaggct
      121 ccagggaagg cgctggaatg tcttgctact attggtagtc tcggaggcac agactacaac
      181 ccagccctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtgagca gcgtgtcaac tgaggacacg gccacatact actgtgcgaa gtcaaatagt
      301 ccttactgct ttcgttgtgt tagtagttct taccctgatc gctacatcga tgcctggggc
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015492      336 bp    mRNA            MAM       04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015492
NID         g2353729
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 336)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 336)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..336
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                     /db_xref="taxon:9913"
                     /clone="BLV3D7"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>336
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                     /product="immunoglobulin variable region"
                     /db_xref="PID:g2353730"
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                     LEWVGVMWSGGNSDYNPALKSRLSITKDNSKSQVSLSVSSVTPEDTATYYCATYASAW
                     GQGLLVTVSS"
BASE COUNT       73 a    101 c     95 g     67 t
ORIGIN
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       61 acctgcacgg tctctggatt ctcattgaac gactatggtg ttaaatgggt ccgccaggct
      121 ccggggaagg cactggagtg ggttggtgtt atgtggagtg gagggaactc cgattataac
      181 ccagccctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtgagca gcgtgacacc tgaggacacg gccacatatt actgtgcaac gtacgccagt
      301 gcctggggcc aaggactcct ggtcaccgtc tcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015493      375 bp    mRNA            MAM       04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015493
NID         g2353731
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 375)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 375)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..375
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLV3D11"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>375
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                     /db_xref="PID:g2353732"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTVSGFSLSIYTVAWVRQAPGKA
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                     SLFNEEYSYLDAYGQGLLVTVSS"
BASE COUNT       85 a    110 c     98 g     82 t
ORIGIN
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       61 acctgcacgg tctctggatt ctcattgagc atctatactg tagcctgggt ccgccaggct
      121 ccggggaagg cactggagtg ggttggtaca attcgtgctg gtggaaacat agaccgtaac
      181 ccagccctag aatcccgtgt cagcatcagc aaggacaact ccaggagcca agtctctctg
      241 tcagtaagcg acgtgacacc tgacgacgcg gccacatact actgtgccaa aaatacggat
      301 ggccattctc tttttaatga ggagtatagt tacctcgatg cctacggcca aggactcctg
      361 gttactgtgt cctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015494      375 bp    mRNA            MAM       04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015494
NID         g2353733
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 375)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 375)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:9913"
                     /clone="BLV4B8"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>375
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                     /product="immunoglobulin variable region"
                     /db_xref="PID:g2353734"
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                     HPYDHLCGDGSAWGQGLLVTVSS"
BASE COUNT       84 a    105 c    103 g     83 t
ORIGIN
        1 caggtgcagc tgcgggagtc aggccccagc ctggtgaagc cctcacagac cctctccctc
       61 acctgcacgg tctctggatt ctcattgagc agctatgctg taggctgggt ccgccaggct
      121 ccggggaagg cactggagtg ggttggtggt ataacgagtg gtggaaggac agtctataat
      181 ccagccctga aatcccggct caccatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtaaata gcgtgacacc tgaggacacg gccacatact actgtgcaaa aagttgtgct
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      361 gtcaccgtct cctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA Graphic
Links: Protein Related Sequences

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LOCUS       AF015495      417 bp    mRNA            MAM      04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015495
NID         g2353735
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 417)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 417)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:9913"
                     /clone="BLV6D10"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>417
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                     /product="immunoglobulin variable region"
                     /db_xref="PID:g2353736"
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                     LEWVGGMLTDGLTYYNPALKSRLGITRDTSKSPVSLSVSSVATEDTATYYCAKCSVTH
                     GDIGSMGYINAGRYGYNSGETANVDAWGQGLLVTVSS"
BASE COUNT       91 a    113 c    119 g     94 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctctccctc
       61 acctgcacgg tctctggatt ctcactgagc agatatttctg tagtctgggt ccgccaggct
      121 ccagggaagg cgctggagtg ggttggtggt atgttaactg atggactcac atactataat
      181 ccagccctga aatcccggct cggcatcacc agggacacct ccaagagccc cgtctctctc
      241 tcagtgagca gcgtagcaac tgaggacacg gccacatatt actgtgcgaa gtgcagtgtt
      301 acacatggtg atattggtag tatgggatat ataaatgctg gacgttatgg gtataatagt
      361 ggtgagaccg cgaacgtcga tgcctggggc caaggactcc tggtcaccgt ctcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015496      363 bp    mRNA            MAM      , 04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015496
NID         g2353737
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 363)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 363)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:9913"
                     /clone="BLV7A8"
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     CDS             <1..>363
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                     /product="immunoglobulin variable region"
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                     DNNDWLYTWGQGLLVTVSS"
BASE COUNT       86 a    108 c     93 g     76 t
ORIGIN
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       61 acctgcacgg tctctggatt ctcattaagc ggcgctactg taggctgggt ccgccaggct
      121 tcaggaaagg cgctggagtg gctgagtgtc tacacttata acggaatagc agactataac
      181 ccagccctga aatcccgcct caccatcacc aaggacagtt ccaagagcca attcttcctg
      241 tcactgaaca gcctgacacc tgaggacacg gccacatatt actgcataag atggactgga
      301 ggtggcgata acaatgattg gctgtatact tggggccaag gactcctggt caccgtctcc
      361 tca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015497      372 bp    mRNA            MAM       04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015497
NID         g2353739
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 372)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 372)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada.
FEATURES             Location/Qualifiers
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                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLV7D7"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
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                     LEWVGGMSSGGSTNYNPALKSRLRMTKDKSKSQVSLSVSGVTPEDTATYYCSKVTGGT
                     YGYIESIYVDAWGQGLLVTVST"
BASE COUNT       86 a    102 c    107 g     77 t
ORIGIN
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       61 acctgcacgg tctctggatt ctcattgagc agttatgctg taggctgggt ccgccaggct
      121 ccggggaagg cactggagtg ggttggtggt atgagcagtg gtggaagcac aaactataac
      181 ccagccctga aatcccggct caggatgacc aaggacaagt ccaagagcca agtctctctc
      241 tcagtgagtg gagtgacacc tgaggacacg gccacatact actgttcaaa agtcactggt
      301 gggacttatg gttatataga aagtatctac gtcgatgcct ggggccaagg actcctggtc
      361 accgtctcca ca
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015498      375 bp    mRNA            MAM      . 04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015498
NID         g2353741
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 375)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 375)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada.
FEATURES             Location/Qualifiers
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                     /organism="Bos taurus"
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                     /clone="BLV7F8"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
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                     GTYLCYPSGVDAWGQGLLVTVSS"
BASE COUNT       79 a    107 c    107 g     82 t
ORIGIN
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       61 acctgcacgg tctctggatt ctcattgagc agctatactg taatctgggt ccgccaggct
      121 ccaggaaagg cgctggagtg gcttggtagt ggaaggtatg atggatacac atcgtataat
      181 tcagccctga aatcccggct cagcatcacg aaggacaact cccagagcca agtctctctg
      241 tcagtgagca gcgtgacaac tgaggacacg gccacatact actgcgcgaa gtgtctgtct
      301 ggtggtggca cgtatttatg ttatcccagt ggggtcgatg cctggggcca aggactcctg
      361 gtcaccgtct cctca
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NCBI Entrez **Nucleotide QUERY** BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015499      372 bp    mRNA            MAM       04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015499
NID         g2353743
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 372)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 372)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /clone="BLV9B8"
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                     antibody"
     CDS             <1..>372
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                     CTYSYVDDIDAWGQGLLVTVSS"
BASE COUNT       88 a    109 c    100 g     75 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctctccctc
       61 acctgcacga tctctggatt ctcattgagc agctatactg tagactgggt ccgccaggct
      121 ccggggaagg cgctggagtg ggttggtcag ataaatagtg gtggaagcac atgccttaac
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      241 tcactgagca gcgtgacaac tgaggacacg gccacatact actgtgcgaa aaatacacac
      301 actggttgta cgtatagtta tgttgacgac atcgatgcct ggggccaggg actactggtc
      361 accgtctcct ca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015500      375 bp    mRNA            MAM       04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015500
NID         g2353745
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 375)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 375)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /clone="BLV9C12"
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                     AAICSSGNYVDSWGQGLLVTVSS"
BASE COUNT       83 a    104 c    108 g     80 t
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       61 acctgcacga tctctggatt ttcaatgagc agctatggtg tagactgggt ccgccaggct
      121 ccggggaagg cgctggagtg ggttggtgga atacatgcta gtggaagtac ataccgtaac
      181 ctagccctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcactgagca gcgtgacaac tgaggacacg gccacatact actgtgcgaa gggtagtagt
      301 cgcaccgctg cgatttgtag tagtggtaat tatgtggatt cttggggcca aggactcctg
      361 gtcaccgtct cctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015501      375 bp    mRNA            MAM      04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015501
NID         g2353747
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 375)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 375)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..375
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                     /clone="BLV9H3"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>375
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                     /product="immunoglobulin variable region"
                     /db_xref="PID:g2353748"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTVSGFSLSNNGVFWVRQAPGKA
                     LEWLGGICGGGGTTFNPALKSRLSITKDTSKNQVSLSVSSVTPEDMATYYCAKNGGPR
                     CQFIYPYDYIDAWGQGLLVTVSS"
BASE COUNT       81 a    110 c    101 g     83 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctctccctc
       61 acctgcacag tctctggatt ctcattgagc aacaatggtg tattctgggt ccgccaggct
      121 ccagggaagg cgctggagtg gctcggtggt atatgcggtg gtggcggcac aacatttaac
      181 ccagccctga aatcccggct cagcatcacc aaggacacct ccaagaacca ggtctctctg
      241 tcagtgagca gcgtgacacc tgaggacatg gccacatact actgtgcaaa aaatggtggt
      301 ccgcgttgtc agtttattta tccttatgat tacatcgatg cctggggcca aggactcctg
      361 gtcacagtct cctca
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NCBI Entrez **Nucleotide QUERY** BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015502      399 bp    mRNA            MAM       04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015502
NID         g2353749
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 399)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 399)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..399
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLV10H2"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>399
                     /codon_start=1
                     /product="immunoglobulin variable region"
                     /db_xref="PID:g2353750"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTTSGFSLSSATVDWVRQAPGKA
                     LEWVGGIDNYGDICYNPALKSRLSITKDNSKSQVSLSVSSVTTEDTATYYCAKRKPGY
                     DDYDYGDYDCYSYGDHYFDAWGQGLIVTVSS"
BASE COUNT       97 a    105 c    106 g     91 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctctccctc
       61 acctgcacga cctctggatt ctcactgagc agcgctactg tagactgggt ccgccaggct
      121 ccggggaagg cgctggagtg ggttggtggt atagataatt atggagacat atgttataac
      181 ccagccctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctcta
      241 tcagtgagca gcgtgacaac tgaggacacg gccacatact actgtgcgaa aaggaaacct
      301 ggatatgatg attatgatta tggtgattat gactgttata gttatggtga tcactacttt
      361 gatgcctggg gccaaggact cattgtcacc gtctcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF097202      498 bp    mRNA            MAM       05-NOV-1998
DEFINITION  Bos taurus clone pBLV8C11 immunoglobulin heavy chain variable
            region mRNA, partial cds.
ACCESSION   AF097202
NID         g3834644
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 498)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Generation of exceptionally long CDR3H provides a novel mechanism
            of antibody diversity
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 498)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..498
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="pBLV8C11"
                     /cell_type="mouse x Bovine heterohybridoma"
     CDS             <1..>498
                     /note="VDJ region"
                     /codon_start=1
                     /product="immunoglobulin heavy chain variable region"
                     /db_xref="PID:g3834645"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTASGFSLSDKAVGWVRQAPGKA
                     LEWLGSIDAAGSTGYNPGLKSRLSITKDNSKSQVSLSVSSVAAEDSATYYCTTVHQKT
                     TRKTCCSDAYRYDSGCGSGCDCCGADCYVFGACTFGLDSSYSYIYIYQWLVDAWGQGL
                     LVTVSS"
BASE COUNT      111 a    122 c    137 g    128 t
ORIGIN
        1 caggtgcagc tgcgcgagtc gggccccagc ctggtgaagc cgtcacagac cctctcgctc
       61 acctgcacgg cctctggatt ctcattgagc gacaaggctg taggctgggt ccgccaggct
      121 ccagggaagg cgctggagtg gctcggtagt atagacgctg caggaagcac aggctataac
      181 ccaggcctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtgagca gcgtggcagc tgaggactcg gccacatact actgtactac tgtacaccaa
      301 aaaacaacaa gaaaaacttg ttgttctgat gcttatagat atgatagtgg ttgtggtagt
      361 ggttgtgatt gttgtggtgc cgattgttat gtttttggtg cttgtacttt tggtcttgat
      421 agtagttata gttatattta tatttaccaa tggctcgtcg atgcctgggg ccaaggactc
      481 ctggtcaccg tctcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015503      390 bp    mRNA            MAM      04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015503
NID         g2353751
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 390)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 390)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..390
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLV11F3"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>390
                     /codon_start=1
                     /product="immunoglobulin variable region"
                     /db_xref="PID:g2353752"
                     /translation="QVQLRESGPGLVKPSQTLSLTCTTSGFSLSGWDVGWVRQAPGKA
                     LEWIGGINSAGATCLNSALKSRLSITKDNSKSQVSLSLSSVMSEDTATYYCGRTGGGG
                     WAECTGTCACYYAYVDSWGQGLLVTVSS"
BASE COUNT       77 a    109 c    124 g     80 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccggc ctggtgaagc cctcacagac cctctccctc
       61 acctgcacga cctctggatt ctcattgagc ggctgggatg taggctgggt ccgccaggct
      121 ccggggaagg cgctggagtg gattggtggt attaattcgg cgggagcgac atgcctcaat
      181 tcagccctga aatcccgtct cagcatcacg aaggacaact ccaagagcca agtctctctg
      241 tcactgagca gcgtgatgag tgaggacacg gccacatact actgtggaag aacaggtgga
      301 ggtggttggg cggagtgcac gggtacatgt gcgtgttact atgcctacgt cgattcctgg
      361 ggccaaggac tcctggtcac cgtctcctca
//
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015504      492 bp    mRNA            MAM      04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015504
NID         g2353753
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 492)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 492)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada.
FEATURES             Location/Qualifiers
     source          1..492
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLVp5B8.1"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>492
                     /codon_start=1
                     /product="immunoglobulin variable region"
                     /db_xref="PID:g2353754"
                     /translation="QVQLRESGPSLVQPSQTLSLTCTASGFSLSDKAVGWVRQAPGKA
                     LEWLGSIDTGGSTGYNPGLKSRLSITKDNSKSQVSLSVSSVTTEDSATYYCTTVHQET
                     RKTCSDGYIAVDSCGRGQSDGCVNDCNSCYYGWRNCRRQPAIHSYEFHVDAWGRGLLV
                     TVSS"
BASE COUNT      110 a    133 c    140 g    109 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgcagc cctcacagac cctctccctc
       61 acctgcacgg cctctggatt ctcattgagc gacaaggctg taggctgggt ccgccaggct
      121 ccagggaagg cgctggagtg gctcggtagt atagacactg gtggaagcac aggctataac
      181 ccaggcctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtgagca gcgtgacgac ggaggactcg gccacatact actgtactac tgtgcaccaa
      301 gaaaccagaa aaacctgttc tgatggttat attgctgtag atagttgtgg tcgtggtcag
      361 agtgatggtt gtgtcaatga ttgcaattca tgttattatg gttggcggaa ctgtcgcagg
      421 cagcctgcaa ttcacagtta cgaatttcac gtcgatgcct ggggccgagg actcctggtc
      481 accatctcct ca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015505      495 bp    mRNA            MAM       04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015505
NID         g2353755
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 495)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 495)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLVp5d3.2"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>495
                     /codon_start=1
                     /product="immunoglobulin variable region"
                     /db_xref="PID:g2353756"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTASGFSLSDKAVGWVRRAPGKA
                     LEWLGTTDTGGSAAYNPGLKSRLSITKDNSKSQVSLSVSNVATEDSATYYCSSVTQRT
                     HVSRSCPDGCSDGDGCVDGCCCSAYRCYTPGVRDLSCTSYSITYTYEWNVDAWGQGLL
                     VTVSS"
BASE COUNT      106 a    128 c    140 g    121 t
ORIGIN
        1 caggtgcagc tgcgcgagtc gggccccagc ctggtgaagc cgtcacagac cctctcgctc
       61 acctgcacgg cctctggatt ctcattgagc gacaaggctg taggctgggt ccgccgggct
      121 ccagggaagg cgctggagtg gctcggtact acagacactg gtggaagcgc agcctataac
      181 ccaggcctga aatcccgact cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtgagca acgtggcaac ggaggactcg gccacatact actgtagtag tgtaactcag
      301 agaacccacg tatcacgtag ttgtcctgat ggttgtagtg atggtgatgg ttgtgttgat
      361 ggttgttgtt gtagtgcata tcgttgttat actcctggtg ttcgtgatct tagttgtacc
      421 agttatagta ttacttatac ttacgaatgg aacgtcgatg cctggggcca aggactcctg
      481 gtcaccgtct cctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?:

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015506      507 bp    mRNA            MAM      . 04-SEP-1997
DEFINITION  Bos taurus immunoglobulin variable region mRNA, partial cds.
ACCESSION   AF015506
NID         g2353757
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 507)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable region genes
            originating from a BLV-infected cow
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 507)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (23-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..507
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLVp1H12.2"
                     /cell_type="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>507
                     /codon_start=1
                     /product="immunoglobulin variable region"
                     /db_xref="PID:g2353758"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTASGFSLSDKAVGWVRQAPGKA
                     LEWLGSIDTGGNTGYNPGLKSRLSITKDNSKSQVSLSVSSVTTEDSATYYCTSVHQET
                     KKYQSCPDGYRERSDCSNRPACGTSDCCRVSVFGNCLTTLPVSYSYTYNYEWHVDVWG
                     QGLLVTVSS"
BASE COUNT      120 a    129 c    135 g    123 t
ORIGIN
        1 caggtgcagc tgcgcgagtc gggccccagc ctggtgaagc cgtcacagac cctctcgctc
       61 acctgcacgg cctctggatt ctcattgagc gacaaggctg taggctgggt ccgccaggct
      121 ccagggaagg cgctggagtg gctcggtagt atagacactg gtggaaacac aggctataac
      181 ccaggcctga aatcccggct cagcatcacc aaggacaact ccaagagtca agtctctctg
      241 tcagtgagca gcgtgacaac tgaggactcg gccacatact actgtacttc tgtgcaccag
      301 gaaacaaaaa aataccaaag ttgtcctgat gggtatagag aacgttcgga ttgtagtaac
      361 agacctgctt gtggtactag tgattgttgt cgtgttagtg tttttggtaa ttgtcttact
      421 actcttcctg tgagttatag ttatacttac aattacgaat ggcacgtcga tgtctggggc
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF097203      489 bp    mRNA            MAM       05-NOV-1998
DEFINITION  Bos taurus clone BF1H1 immunoglobulin heavy chain variable region
            mRNA, partial cds.
ACCESSION   AF097203
NID         g3834646
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 489)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Bovine fetal VDJ rearrangements characterized by repeat codons in
            the CDR3H that encode Glycine and Tyrosine
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 489)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /db_xref="taxon:9913"
                     /clone="BF1H1"
                     /cell_type="mouse x Bovine heterohybridoma"
                     /dev_stage="fetal"
                     /note="PCR product"
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                     /note="VDJ region"
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                     /product="immunoglobulin heavy chain variable region"
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                     LEWLGGIDTGGSTGYNPGLKSRLSITKDNSKSQVSLSVSSVTTEDSATYYCTTVHPSP
                     DGYSYGYGCGYGYGCSGYDCYGYGGYGYGGYGGYSSYSYSYSPRKYYVDAWGQGLLVT
                     VSS"
BASE COUNT      104 a    114 c    145 g    126 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cgtcacagac cctctcgctc
       61 acctgcacgg cctctggatt ctcattgagc gacaaggctg taggctgggt ccgccaggct
      121 ccagggaagg cgctggagtg gctcggtggt atagacactg gtggaagcac aggctataac
      181 ccaggcctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtgagca gcgtgacaac tgaggactcg gccacatact actgtactac tgtgcacccc
      301 agtcctgatg gttatagtta tggttatggt tgtggttatg gttatggttg tagtggttat
      361 gattgttatg gttatggtgg ttatggttat ggtggttatg gtggttatag tagttatagt
      421 tatagttata gtccaaggaa atactacgtc gatgcctggg gccaaggact cctggtcacc
      481 gtctcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF097204      492 bp    mRNA            MAM      05-NOV-1998
DEFINITION  Bos taurus clone BF4E9 immunoglobulin heavy chain variable region
            mRNA, partial cds.
ACCESSION   AF097204
NID         g3834648
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 492)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Bovine fetal VDJ rearrangements characterized by repeat codons
            encoding glycine and tyrosine in the CDR3H region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 492)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..492
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BF4E9"
                     /cell_type="mouse x Bovine heterohybridoma"
                     /dev_stage="fetal"
                     /note="PCR product"
     CDS             <1..>492
                     /note="VDJ region"
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                     /product="immunoglobulin heavy chain variable region"
                     /db_xref="PID:g3834649"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTASGFSLSDKAVGWVRQAPGKA
                     LEWLGGIDTGGSTGYNPGLKSRLSITKDNSKSQVSLSVSSVTTEDSATYYCTTVHQIF
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                     TVSS"
BASE COUNT      103 a    111 c    146 g    132 t
ORIGIN
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       61 acctgcacgg cctctggatt ctcattgagc gacaaggctg taggctgggt ccgccaggct
      121 ccagggaagg cgctggagtg gctcggtggt atagacactg gtggaagcac aggctataac
      181 ccaggcctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtgagca gcgtgacaac tgaggactcg gccacatact actgtactac tgtgcaccag
      301 atttttgtc ctgatggtta tagttatggt tatggttgtg gttatggtta tggttgtagt
      361 ggttatgatt gttatggtta tggtggttat ggttatggtg gttatggtgg ttatagtagt
      421 tatagttata gttatagtta cgaatactac ggcgatgcct ggggccaagg actcctggtc
      481 accgtctcct ca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF097205       390 bp    mRNA            MAM       05-NOV-1998
DEFINITION  Bos taurus clone BF2B5 immunoglobulin heavy chain variable region
            mRNA, partial cds.
ACCESSION   AF097205
NID         g3834650
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 390)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Bovine fetal VDJ rearrangements characterized by repeat codons
            encoding glycine and tyrosine in the CDR3H region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 390)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..390
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /cell_type="mouse x Bovine heterohybridoma"
                     /clone="BF2B5"
                     /dev_stage="fetal"
                     /note="PCR product"
     CDS             <1..>390
                     /note="VDJ region"
                     /codon_start=1
                     /product="immunoglobulin heavy chain variable region"
                     /db_xref="PID:g3834651"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTVFGFSLSDNSVGWVRQVPGKA
                     LEWLGVIYSGGSTGYNPALKSRLSITKDNSKSQVSLSLSSVTTEDTATYYCARDCYSD
                     YGNGYGCGYGYGSYVDAWGQGLLVTVSS"
BASE COUNT       88 a    100 c    110 g     92 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctgtccctc
       61 acctgcacgg tgtttggatt ctcattaagc gataatagtg taggctgggt ccgccaggtt
      121 ccaggaaagg cgctggagtg gctcggtgtc atatatagtg gtggaagcac aggctataac
      181 ccagccctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctcta
      241 tcattgagca gcgtgacaac tgaggacacg gccacatact actgtgcaag agattgttat
      301 agtgattatg gtaatggtta tggttgtggt tatggttatg gttcctacgt cgatgcctgg
      361 ggccaaggac tcctggtcac cgtctcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA Graphic

Links: Protein Related Sequences

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LOCUS       AF097206      381 bp    mRNA            MAM       05-NOV-1998
DEFINITION  Bos taurus clone BF2D12 immunoglobulin heavy chain variable region
            mRNA, partial cds.
ACCESSION   AF097206
NID         g3834652
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 381)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Bovine fetal VDJ rearrangements characterized by repeat codons
            encoding glycine and tyrosine in the CDR3H region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 381)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..381
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /cell_type="mouse x Bovine heterohybridoma"
                     /clone="BF2D12"
                     /dev_stage="fetal"
                     /note="PCR product"
     CDS             <1..>381
                     /note="VDJ region"
                     /codon_start=1
                     /product="immunoglobulin heavy chain variable region"
                     /db_xref="PID:g3834653"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTISGFSLSSYAVGWVRQAPGKA
                     LEWLGGISSGGSTCLNPALKSRLSITKDNSKSQVSLSVSSVTTEDTATYYCTCYSGYG
                     YGYGYAYGYDYVDAWGQGLLVTVSS"
BASE COUNT       79 a    105 c    109 g     88 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctctccctc
       61 acctgcacga tctctggatt ctcattgagc agctatgctg taggctgggt ccgccaggct
      121 ccggggaagg cgctggagtg gcttggtggt ataagtagtg gtggaagcac atgccttaac
      181 ccagccctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtgagca gcgtgacaac tgaggacacg gccacatact actgtacgtg ttatagtggt
      301 tatggttatg gttatggtta tgcttatggt tatgactacg tcgatgcctg gggccaagga
      361 ctcctggtca ccgtctcctc a
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NCBI Entrez Nucleotide QUERY BLAST Entrez 2

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF097207      387 bp    mRNA            MAM      05-NOV-1998
DEFINITION  Bos taurus clone BF3H11 immunoglobulin heavy chain variable region
            mRNA, partial cds.
ACCESSION   AF097207
NID         g3834654
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 387)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Bovine fetal VDJ rearrangements characterized by repeat codons
            encoding glycine and tyrosine in the CDR3H region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 387)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..387
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /cell_type="mouse x Bovine heterohybridoma"
                     /clone="BF3H11"
                     /dev_stage="fetal"
                     /note="PCR product"
     CDS             <1..>387
                     /note="VDJ region"
                     /codon_start=1
                     /product="immunoglobulin heavy chain variable region"
                     /db_xref="PID:g3834655"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTVSGFSLSSNGVGWVRQAPGKA
                     LEWVGGIDNDGDTYYNPALKSRLSITKDNSKSQVSLSVSSVTPEDTATYYCANSCYSG
                     YGYGCGYGYGYDYVDAWGQGLLVTVSS"
BASE COUNT       85 a    102 c    111 g     89 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctctccctc
       61 acctgcacgg tctctggatt ctcattgagc agcaatggtg taggctgggt ccgccaggct
      121 ccagggaagg cgctggagtg ggttggtggt atagataatg atggagacac atactataac
      181 ccagccctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctctg
      241 tcagtgagca gcgtgacacc tgaggacacg gccacatact actgtgcaaa tagttgttat
      301 agtggttatg gttatggttg tggttatggt tatggttatg actacgtcga tgcctggggc
      361 caaggactcc tggtcaccgt ctcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF097208      351 bp    mRNA            MAM       05-NOV-1998
DEFINITION  Bos taurus clone BF3A11 immunoglobulin heavy chain variable region
            mRNA, partial cds.
ACCESSION   AF097208
NID         g3834656
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 351)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Bovine fetal VDJ rearrangements characterized by repeat codons
            encoding glycine and tyrosine in the CDR3H region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 351)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..351
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /cell_type="mouse x Bovine heterohybridoma"
                     /clone="BF3A11"
                     /dev_stage="fetal"
                     /note="PCR product"
     CDS             <1..>351
                     /note="VDJ region"
                     /codon_start=1
                     /product="immunoglobulin heavy chain variable region"
                     /db_xref="PID:g3834657"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTVSGFSLSDNSVGWVRQAPGKA
                     LEWLGVIYSGGSTGYNPALKSRLSITKDNSKSQVSLSLSSVTTEDTATYYCARGLVMV
                     MVVTWGQGLLVTVSS"
BASE COUNT       80 a     99 c     99 g     73 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctgtccctc
       61 acctgcacgg tctctggatt ctcattaagc gataatagtg taggctgggt ccgccaggct
      121 ccaggaaagg cgctggagtg gctcggtgtc atatatagtg gtggaagcac aggctataac
      181 ccagccctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctcta
      241 tcactgagca gcgtgacaac tgaggacacg gccacatact actgtgcaag aggtttggtt
      301 atggttatgg ttgtgacatg gggccaagga ctcctggtca ccgtctcctc a
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

```
LOCUS       AF097209      390 bp    mRNA            MAM       05-NOV-1998
DEFINITION  Bos taurus clone BF5F10 immunoglobulin heavy chain variable region
            mRNA, partial cds.
ACCESSION   AF097209
NID         g3834658
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 390)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Bovine fetal VDJ rearrangements characterized by repeat codons
            encoding glycine and tyrosine in the CDR3H region
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 390)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..390
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                     /db_xref="taxon:9913"
                     /cell_type="mouse x Bovine heterohybridoma"
                     /clone="BF5F10"
                     /dev_stage="fetal"
                     /note="PCR product"
     CDS             <1..>390
                     /note="VDJ region"
                     /codon_start=1
                     /product="immunoglobulin heavy chain variable region"
                     /db_xref="PID:g3834659"
                     /translation="QVQLRESGPSLVKPSQTLSLTCTVSGFSLSDNSVGWVRQAPGKA
                     LEWLGVIYSGGSTGYNPALKSRLSITKDNSKSQVSLSLSSVTTEDTATYYCARDCYSG
                     YGYGYGCGYGYGSYVDAWGQGLLVTVSS"
BASE COUNT       86 a    105 c    109 g     90 t
ORIGIN
        1 caggtgcagc tgcgggagtc gggccccagc ctggtgaagc cctcacagac cctctccctc
       61 acctgcacgg tctctggatt ctcattaagc gataatagtg taggctgggt ccgccaggct
      121 ccaggaaagg cgctggagtg gctcggtgtc atatatagtg gtggaagcac aggctataac
      181 ccagccctga aatcccggct cagcatcacc aaggacaact ccaagagcca agtctctcta
      241 tcactgagca gcgtgacaac tgaggacacg gccacatact actgtgcaag agattgttat
      301 agtggttatg gttatggtta tggttgtggt tatggttatg gttcctacgt cgatgcctgg
      361 ggccaaggac tcctggtcac cgtctcctca
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015791      333 bp    mRNA            MAM      13-AUG-1997
DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
            cds.
ACCESSION   AF015791
NID         g2323371
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..333
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLV1F3"
                     /note="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>333
                     /codon_start=1
                     /product="immunoglobulin light chain variable region"
                     /db_xref="PID:g2323372"
                     /translation="QAVLTQPSSVSGSLGQRVSITCSGSSSNVGYGSYVNWFQQIPGS
                     APRTLIYGATSRASGVPDRFSGSRSGNTATLTINSLQAEDEADYFCTSYHSSSKNRVC
                     GSGTTLTVL"
BASE COUNT       70 a    104 c     91 g     68 t
ORIGIN
        1 caggctgtgc tgactcagcc gtcctccgtg tccgggtccc tgggccagag ggtctccatc
       61 acctgctctg gaagcagcag caacgttgga tatggcagtt atgtgaactg gttccaacag
      121 atcccaggat cggcccccag aaccctcatc tatggtgcga ccagtcgagc ctctggggtc
      181 cccgaccgat tctccggctc caggtctggg aacacagcca ccctgaccat caactcgctt
      241 caggccgagg acgaggcgga ttatttctgt acatcttatc acagtagtag caaaaataga
      301 gtgtgcggca gcgggaccac actgaccgtc ctg
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NCBI Entrez Nucleotide QUERY BLAST Entrez2 2.4

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015792      333 bp    mRNA            MAM      13-AUG-1997
DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
            cds.
ACCESSION   AF015792
NID         g2323373
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..333
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLV1G9"
                     /note="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>333
                     /codon_start=1
                     /product="immunoglobulin light chain variable region"
                     /db_xref="PID:g2323374"
                     /translation="QAVLTQPSSVSGSLGQRVSITCSGSSSNVGRGNYVNWFQQIPGS
                     APRTLIYGATSRASGVPDRFSGSRSGNTATLTITSLQAEDEADYFCATYDSSSNNGVF
                     GSGTTLTVL"
BASE COUNT       66 a    103 c     94 g     70 t
ORIGIN
        1 caggctgtgc tgactcagcc gtcctccgtg tccgggtccc tgggccagag ggtctccatc
       61 acctgctcgg gaagcagcag caacgttggg cgtggcaatt atgtgaactg gttccaacag
      121 atcccaggat cggcccccag aaccctcatt tatggtgcga ccagtcgagc ctctggggtc
      181 cccgaccgat ttccggctc caggtctggg aacacagcca cactgaccat cacctcgctc
      241 caggccgagg acgaggcgga ttatttctgt gcaacttatg acagcagtag taataatggt
      301 gttttcggca gcgggaccac actgaccgtc ctg
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015793      333 bp    mRNA            MAM      , 13-AUG-1997
DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
            cds.
ACCESSION   AF015793
NID         g2323375
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..333
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLV3D11"
                     /note="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>333
                     /codon_start=1
                     /product="immunoglobulin light chain variable region"
                     /db_xref="PID:g2323376"
                     /translation="QAVLTQPSSVSGSLGQRVSITCSGSSSNVGRGNYVNWFQQIPGS
                     APRTLIYGATSRASGVPDRFSGSRSGNTATLTISSLQAEDEAAYFCAVYDSSSNNAVF
                     GSGTTLTVL"
BASE COUNT       63 a    106 c     95 g     69 t
ORIGIN
        1 caggctgtgc tgactcagcc gtcctccgtg tccgggtccc tgggccagag ggtctccatc
       61 acctgctctg gaagcagcag caacgttggg cgtggcaatt atgtgaactg gttccaacag
      121 atcccaggat cggcccccag aaccctcatc tatggtgcga ccagtcgagc ctctggggtc
      181 cccgaccgat tctccggctc caggtctggg aacacagcca cgctgaccat cagctcgctc
      241 caggccgagg acgaggcggc ttatttctgt gcagtttatg acagcagtag caataatgct
      301 gttttcggca gcgggaccac actgaccgtc ctg
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NCBI Entrez Nucleotide QUERY

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015794      333 bp    mRNA            MAM      13-AUG-1997
DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
            cds.
ACCESSION   AF015794
NID         g2323377
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..333
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLV3D11"
                     /note="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>333
                     /codon_start=1
                     /product="immunoglobulin light chain variable region"
                     /db_xref="PID:g2323378"
                     /translation="QAVKTQPSSVSGSLGQRVSITCCGSSSNVGRGNYVNWFQQIPGS
                     APRTLIYGATSRASGVPDRFSGSRCGNTATMTISSLQAEDEADYFSAVYDSSSNNAAF
                     GSGTTLTVL"
BASE COUNT       68 a    103 c     97 g     65 t
ORIGIN
        1 caggctgtga agactcagcc gtcctccgtg tccgggtccc tgggccagag ggtctccatc
       61 acctgctgtg gaagcagcag caacgttggg cgtggcaatt atgtgaactg gttccaacag
      121 atcccaggat cggcccccag aaccctcatt tatggtgcga ccagtcgagc ctctggggtc
      181 cccgaccgat tctccggctc caggtgtggg aacacagcca cgatgaccat cagctcgctc
      241 caggccgagg acgaggcgga ttatttcagt gcagtctatg acagcagtag caataatgct
      301 gccttcggca gcgggaccac actgaccgtc ctg
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015795      333 bp    mRNA            MAM      13-AUG-1997
DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
            cds.
ACCESSION   AF015795
NID         g2323379
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..333
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /clone="BLV4B8"
                     /note="bovine x mouse heterohybridoma secreting IgM
                     antibody"
     CDS             <1..>333
                     /codon_start=1
                     /product="immunoglobulin light chain variable region"
                     /db_xref="PID:g2323380"
                     /translation="QAVLTQPSSVSGSLGQRVSITCSGSSSNVGRGNYVNWFQQIPGS
                     APRTLIYGATSRASGVPDRFSGSRSGNTATLTISSLQAEDEADYFCAAYDSSTNNAVF
                     GSGTTLTVL"
BASE COUNT       65 a    106 c     94 g     68 t
ORIGIN
        1 caggctgtgc tgactcagcc gtcctccgtg tccgggtccc tgggccagag ggtctccatc
       61 acctgctctg gaagcagcag caacgttggg cgtggcaatt atgtgaactg gttccaacag
      121 atcccaggat cggcccccag aaccctcatc tatggtgcga ccagtcgagc ctctggggtc
      181 cccgaccgat tctccggctc caggtctggg aacacagcca cgctgaccat cagctcgctc
      241 caggccgagg acgaggcgga ttatttctgt gcagcttatg acagcagtac aaataatgct
      301 gttttcggca gcgggaccac actgaccgtc ctg
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF015796      330 bp    mRNA            MAM       13-AUG-1997
DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
            cds.
ACCESSION   AF015796
NID         g2323381
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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ACCESSION   AF015797
NID         g2323383
KEYWORDS    .
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  ORGANISM  Bos taurus
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            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
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Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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KEYWORDS    .
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            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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ACCESSION   AF015799
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SOURCE      Bos taurus.
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            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
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Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
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ACCESSION   AF015800
NID         g2323389
KEYWORDS    .
SOURCE      Bos taurus.
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            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
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            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
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Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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ACCESSION   AF015801
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SOURCE      Bos taurus.
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            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 333)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
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Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
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            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
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Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
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ACCESSION   AF015803
NID         g2323395
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            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
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Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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ACCESSION   AF015804
NID         g2323397
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SOURCE      Bos taurus.
  ORGANISM  Bos taurus
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            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
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  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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NID         g2323399
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  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
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ACCESSION   AF015806
NID         g2323401
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
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            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     SGTTLTVL"
BASE COUNT       66 a    103 c     94 g     67 t
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       61 acctgctctg gaagcagcag caacatcggt agatatggtg tgggctggta ccaacaggtc
      121 ccaggatcgg gcctcagaac catcatctat aatagtagca gtcgacccctc gggggtcccc
      181 gaccgattct ccggctccaa gtctggcaac acagccaccc tgaccatcag ctcgctccag
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
            cds.
ACCESSION   AF015807
NID         g2323403
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
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                     antibody"
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BASE COUNT       65 a    105 c     92 g     68 t
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
            cds.
ACCESSION   AF015808
NID         g2323405
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
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BASE COUNT       61 a    105 c     97 g     67 t
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
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ACCESSION   AF015809
NID         g2323407
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   Saini,S.S., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (24-JUL-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
            cds.
ACCESSION   AF023841
NID         g2555148
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 336)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 336)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (10-SEP-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     FGSGTTLIVL"
BASE COUNT       70 a    100 c     89 g     77 t
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
            cds.
ACCESSION   AF023842
NID         g2555150
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 327)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 327)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (10-SEP-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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BASE COUNT       66 a    107 c     87 g     67 t
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Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus immunoglobulin light chain variable region mRNA, partial
            cds.
ACCESSION   AF023843
NID         g2555152
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Structural features of immunoglobulin variable-region lambda light
            chain of BLV-infected cattle
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (10-SEP-1997) Pathobiolcgy, University of Guelph, Guelph,
            ON N1G 2W1, Canada.
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                     SGTTLTVL"
BASE COUNT       68 a    100 c     93 g     69 t
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Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus clone BF1H1 immunoglobulin light chain variable region
            mRNA, partial cds.
ACCESSION   AF097214
NID         g3834668
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 324)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Bovine Ig rearranged lambda light chain gene (clone BF1H1)
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 324)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
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      121 ccaggatcgg cccccagaac cctgatctat aatagtaaca aacgaccctc gggggtcccc
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DEFINITION  Bos taurus VJ rearrangement mRNA, complete cdn.
ACCESSION   AF109697
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 330)
  AUTHORS   .
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 330)
  AUTHORS   S. S. Saini and A. Kaushik.
  TITLE     Bovine rearranged light chain gene; clone BLV5B8
  JOURNAL   Unpublished
REFERENCE   3  (bases 1 to 330)
  AUTHORS   S. S. Saini and A. Kaushik.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-NOV-1998) Pathobiology, University of Guelph, Guelph,
            Ontario N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /note="PCR product"
BASE COUNT       68 a    102 c     92 g     68 t
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA Graphic

Links: Protein Related Sequences

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LOCUS       AF005274      1311 bp    mRNA            MAM       02-JUL-1997
DEFINITION  Bos taurus IgM heavy chain constant region mRNA, partial cds.
ACCESSION   AF005274
NID         g2232298
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Mammalia; Eutheria; Artiodactyla; Ruminantia; Pecora;
            Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 1311)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Structural analysis of a bovine IgM
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1311)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (26-MAY-1997) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /strain="Holstein"
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                     /cell_line="BL7G1"
                     /cell_type="mouse-bovine heterohybridoma"
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                     VSGISEGQVETVQSSPVTFRAYSMLTITERDWLSQNVYTCQVEHNKETFQKNVSSSCD
                     VAPPSPIGVFTIPPSFADIFLTKSAKLSCLVTNLASYDGLNISWSRQNGKALETHTYF
                     ERHLNDTFSARGEASVCSEDWESGEEFTCTVAHSDLPFPKKNSVSKPKDVAMKPPSVY
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BASE COUNT      276 a    422 c    374 g    239 t
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       61 gagagcacgg tggccctggg ctgcctggcc cgggacttcg tgcccaattc agtcagcttc
      121 tcctggaagt tcaacaacag cacagtcaac agcgagagat tctggacctt ccccgaagtc
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      481 gtgtctggaa tttctgaagg ccaggtggag actgtgcagt cctcacccgt aactttcagg
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      601 caggtagaac acaacaagga aaccttccag aagaacgtgt cctcctcatg tgatgttgca
      661 ccaccatctc ccatcggggt cttcaccatc ccccatcct tcgccgacat cttcctcacg
      721 aagtcagcca agctgtcctg tctggtcaca aacctggcct cctatgatgg cctgaacatc
      781 agctggtccc gtcagaacgg caaggccctg gagacccaca cgtattttga gcgacacctc
      841 aacgacacct tcagcgcccg gggtgaggcc tcggtctgct cggaggactg ggagtccgga
      901 gaggagttca cgtgcacagt ggcccactcg gacctgccct tcccaaaaaa gaacagcgtc
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1141 accagcagcc cggcgcccga gcctcaggac cccagcgtgt actttgtgca cagcatcccg
1201 acggtggccg aggaggactg gagcaaaggg gagacctaca cctgcgtcgt gggccacgag
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NCBI Entrez **Nucleotide QUERY** BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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DEFINITION  Bos taurus clone B5D8 immunoglobulin heavy chain constant region
            mRNA, partial cds.
ACCESSION   AF097213
NID         g3834666
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 1320)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Bovine IgM constant region gene sequence
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1320)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
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                     /clone="B5D8"
                     /note="PCR product"
     CDS             <1..>1320
                     /note="IgM"
                     /codon_start=1
                     /product="immunoglobulin heavy chain constant region"
                     /db_xref="PID:g3834667"
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                     KRIVSGISEGQVETVQSSPVTFRAYSMLTITERDWLSQNVYTCQVEHNKETFQKNVSS
                     SCDVAPPSPIGVFTIPPSFADIFLTKSAKLSCLVTNLASYDGLNISWSRQNGKALETH
                     TYFERHLNDTFSARGEASVCSEDWESGEEFTCTVAHSDLPFPEKNTVSKPKDVAMKPP
                     SVYLLPPTREQLSLRESASVTCLVKGFAPADVFVQWLQRGEPVTKSKYVTSSPAPEPQ
                     DPSVYFVHSILTVAEEDWSKGETYTCVVGHEALPHMVTERTVDKSTGK"
BASE COUNT      275 a    424 c    379 g    242 t
ORIGIN
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       61 gagagcacgg tggccctggg ctgcctggcc cgggacttcg tgcccaattc agtcagcttc
      121 tcctggaagt tcaacaacag cacagtcagc agcgagagat tctggacctt ccccgaagtc
      181 ctgagggacg gcttgtggtc ggcctcctct caggtggtcc tgtcctcctc aagcgccttt
      241 caagggccgg atgactacct ggtgtgcgaa gtccagcacc ccaagggagg aaagaccgtc
      301 ggcaccgtga gggtgatcgc tacaagtgag tcagaggcgg aagtgctgtc cccagtcgtg
      361 agtgtctttg tcccgcctcg caacagcctc tctggtgacg gcaatagcaa gtccagcctc
      421 atctgccagg ccacggactt cagccccaaa cagatctcct tgtcctggtt tcgtgatgga
      481 aagcggatag tgtctggcat ttctgaaggc caggtggaga ctgtgcagtc ctcacccgta
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      661 gatgttgcac caccatctcc catcggggtc ttcaccatcc ccccatcctt cgccgacatc
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF097210      390 bp    mRNA            MAM       05-NOV-1998
DEFINITION  Bos taurus clone B5D8 immunoglobulin heavy chain variable region
            mRNA, partial cds.
ACCESSION   AF097210
NID         g3834660
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 390)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Bovine VDJ rearrangement from adult IgM antibody repertoire
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 390)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
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                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /cell_type="mouse x Bovine heterohybridoma"
                     /clone="B5D8"
                     /dev_stage="adult"
                     /note="PCR product"
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BASE COUNT       82 a    112 c    109 g     87 t
ORIGIN
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       61 acctgcacgg tctctggatt ctcattgagc agctatgctg taaactgggt ccgccaggct
      121 ccagggaagg cgctggagtg ggttggtggt ataactgttg gtggaagcac atgccttaac
      181 ccagccctga aatcccggct caccatcacc aaggagaact ccaagagcca agtctctctg
      241 tcagtaaaca acgtgacacc tgaggacacg gccacatact actgtgcgag gaattatgct
      301 cctgtggcta attcttggta tggttgccat gattacggtg ttggtgacct cgatgcctgg
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NCBI Entrez Nucleotide QUERY BLAST Entrez ?

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF097211      381 bp    mRNA            MAM       05-NOV-1998
DEFINITION  Bos taurus clone pBA14.1 immunoglobulin heavy chain variable region
            mRNA, partial cds.
ACCESSION   AF097211
NID         g3834662
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 381)
  AUTHORS   Saini,S.S. and Kaushik,A.
  TITLE     Bovine VDJ rearrangement from adult IgM antibody repertoire
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 381)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
FEATURES             Location/Qualifiers
     source          1..381
                     /organism="Bos taurus"
                     /db_xref="taxon:9913"
                     /tissue_type="spleen"
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                     /dev_stage="adult"
     CDS             <1..>381
                     /note="VDJ region"
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                     /product="immunoglobulin heavy chain variable region"
                     /db_xref="PID:g3834663"
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BASE COUNT       93 a    106 c    100 g     82 t
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      121 ccagggaagg cacgagagtg gatcggtgat gcagccgctg atggaaacac gctctataac
      181 ccagacctga aatcccggtt cagcatcacc aaggacaact cgaagaatca agtctctctg
      241 tcagtgagta gcgtaacacc tgaggacacg gccacatatt attgtacaaa aagtagtcgt
      301 gagcgacatt atgcttctag tagtagtctc gccggctaca tcgatctttg gggccaagga
      361 ctccgagtta cggtttcctc a
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NCBI Entrez Nucleotide QUERY BLAST Entrez

Other Formats: FASTA | Graphic

Links: Protein | Related Sequences

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LOCUS       AF097212      375 bp    mRNA            MAM       05-NOV-1998
DEFINITION  Bos taurus clone pA70.1 immunoglobulin heavy chain variable region
            mRNA, partial cds.
ACCESSION   AF097212
NID         g3834664
KEYWORDS    .
SOURCE      Bos taurus.
  ORGANISM  Bos taurus
            Eukaryota; Metazoa; Chordata; Vertebrata; Mammalia; Eutheria;
            Artiodactyla; Ruminantia; Pecora; Bovoidea; Bovidae; Bovinae; Bos.
REFERENCE   1  (bases 1 to 375)
  AUTHORS   Saini,S.S., Naessens,J. and Kaushik,A.
  TITLE     Bovine VDJ rearrangement from adult IgG antibody repertoire
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 375)
  AUTHORS   Saini,S.S., Allore,B., Jacobs,R. and Kaushik,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (07-OCT-1998) Pathobiology, University of Guelph, Guelph,
            ON N1G 2W1, Canada
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       61 acctgcacgg tctctggaat ctcattgagc agcadcactg tagtctgggt ccgccaggct
      121 ccagggaagg cgctggagtg ggttggtact atgactgcgg gtgaaagtgt atactataat
      181 tcagccctga gatcccggct cagcatcaca aaggacaact ccaagagcca actctctctg
      241 tcagtgagta gcgtgacacc tgaggacacg gccacatact attgtgcgaa gtgtggtgcc
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