Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Review

'*Ovar-Mhc*' - ovine major histocompatibility complex: structure and gene polymorphisms

V.S.R. Dukkipati[1], H.T. Blair[1], D.J. Garrick[1,2] and A. Murray[1]

[1]Institute of Veterinary, Animal and Biomedical Sciences, Massey University, Palmerston North, New Zealand
[2]Department of Animal Sciences, Colorado State University, Fort Collins, CO, USA
Corresponding author: H.T. Blair
E-mail: h.blair@massey.ac.nz

Genet. Mol. Res. 5 (4): 581-608 (2006)
Received February 20, 2006
Accepted July 25, 2006
Published October 6, 2006

ABSTRACT. The major histocompatibility complex (MHC) in sheep, *Ovar-Mhc*, is poorly characterised, when compared to other domestic animals. However, its basic structure is similar to that of other mammals, comprising class I, II and III regions. Currently, there is evidence for the existence of four class I loci. The class II region is better characterised, with evidence of one DRA, four DRB (one coding and three non-coding), one DQA1, two DQA2, and one each of the DQB1, DQB2, DNA, DOB, DYA, DYB, DMA, and DMB genes in the region. The class III region is the least characterised, with the known presence of complement cascade (C4, C2 and Bf), TNFα and CYP21 genes. Products of the class I and II genes, MHC molecules, play a pivotal role in antigen presentation required for eliciting immune responses against invading pathogens. Several studies have focused on polymorphisms of *Ovar-Mhc* genes and their association with disease resistance. However, more research emphasis is needed on characterising the remaining *Ovar-Mhc* genes and developing simplified and cost-effective methods to score gene polymorphisms. Haplotype screening, employing multiple

markers rather than single genes, would be more meaningful in MHC-disease association studies, as it is well known that most of the MHC loci are tightly linked, exhibiting very little recombination. This review summarises the current knowledge of the structure of *Ovar-Mhc* and polymorphisms of genes located in the complex.

Key words: *Ovar-Mhc*, MHC, OLA, Sheep, Structure, Gene polymorphisms, Review

INTRODUCTION

The major histocompatibility complex (MHC) is an organised cluster of tightly linked genes with immunological and non-immunological functions, and is present in all vertebrates, except the jawless fish (Tizard, 2004). The MHC was discovered during tissue transplantation studies in mice (Gorer, 1937) and was first known for its role in histocompatibility. Subsequently, its role in immune regulation (Benacerraf and McDevitt, 1972) and several other functions (Bonner, 1986; Zavazava and Eggert, 1997; Penn and Potts, 1999) were discovered. The primary function of the MHC is to code for specialised antigen-presenting receptor glycoproteins, known as histocompatibility molecules or MHC molecules. These molecules bind processed peptide antigens and present them to T lymphocytes, thereby triggering immune responses.

The ovine MHC was first identified about 27 years ago by serological studies on sheep lymphocyte antigens (Millot, 1978). Since then, it has been generally referred to as ovine leukocyte antigen (OLA) or sheep lymphocyte antigen. In accordance with a nomenclature system for the MHC of vertebrates (Klein et al., 1990), it has now been designated as '*Ovar-Mhc*' ('*Ovar'* representing *Ovis aries*). However, this system of nomenclature has not been universally adopted amongst animal immunogeneticists (Rothschild et al., 2000). *Ovar* has been localised by *in situ* hybridisation to chromosome 20 between bands q15 and q23 (Mahdy et al., 1989; Hediger et al., 1991).

Owing to the immunological importance of MHC genes and their possible role in disease resistance, research on the ovine MHC received an impetus in the late 1980s and was comprehensively reviewed in 1996 (Schwaiger et al., 1996). Since that review, there have been a number of studies investigating the polymorphisms of genes within the *Ovar-Mhc* and their association with resistance to infectious diseases. This paper reviews the literature pertaining to the structure of the ovine MHC and polymorphisms of genes located in the region. A review of literature pertaining to the association of genes within the *Ovar-Mhc* with disease resistance has recently been completed (Dukkipati et al., 2006).

STRUCTURE OF THE OVINE MHC

Human and mouse MHCs have been investigated in much more detail than those of other mammals (Deverson et al., 1991), and among the domesticated species, the sheep MHC is poorly characterised (Kostia et al., 1998). The MHC of humans, designated as the human

leukocyte antigens (HLA), covers a region of about 3.6 megabasepairs. Its complete sequence and gene map locus have been reported (MHC Sequencing Consortium, 1999). It serves as a valuable reference for intra-species and inter-species comparative studies (Kulski et al., 2002). With over 224 gene loci (128 predicted to be expressed), it is the most gene-dense region of the human genome. The average gene density, including pseudogenes, over the entire region is one gene per 16 kilobasepairs. It is believed that about 40% of the expressed HLA genes are involved in immune system function.

The HLA complex is divided into three regions, the telomeric class I, the centromeric class II and the central class III (Klein, 1976). Analysis of the immediate-flanking regions has revealed that the classical class I and class II regions extend much further than originally thought and are referred to as extended class I and class II regions (Stephens et al., 1999). A set of more than 7 genes involved in inflammation, including the three members of the tumor necrosis factor (TNF) superfamily that is located at the telomeric end of the class II region, is sometimes specified as the class IV region (Gruen and Weissman, 1997).

The general structure of the MHC is conserved among mammalian species, including three main regions with different functional roles (Amills et al., 1998). However, when MHCs of different mammals are compared, some regions appear to be well conserved and others vary widely (Kelley et al., 2005). In general, the class II and class III regions are orthologous, i.e., they are clearly derived from a single ancestor without being subjected to major rearrangements (except in ruminants) and their gene order is conserved. In ruminants, the class II region is unique in that it is split into two distinct sub-regions, ‘a’ and ‘b’, separated by a distance of at least 15 cM (Andersson et al., 1988; van Eijk et al., 1995). The class I genes, in contrast, are paralogous, i.e., they are derived by duplication and have been reorganised several times (Kelley et al., 2005). The schematic structure of the ovine MHC is illustrated in Figure 1. Details of the genes harboured in the three regions and their known polymorphisms are summarised below.

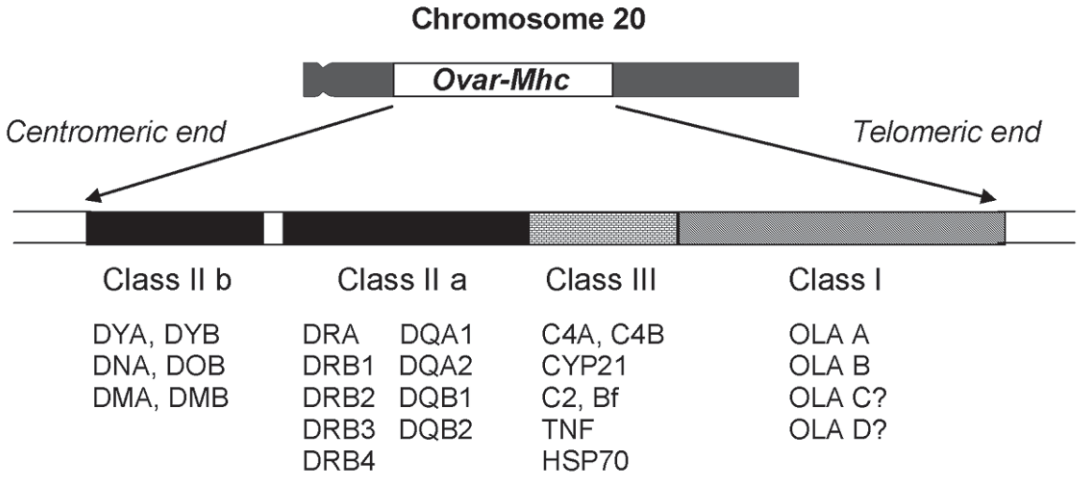

Figure 1. Schematic presentation of the structure of *Ovar-Mhc*.

CLASS I GENES

The class I loci include both classical and non-classical genes. The classical class I genes are members of the immunoglobulin gene family that are involved in the presentation of peptides, predominantly derived from intracellular proteins and parasites, to CD8+ cytotoxic T cells. They have also been found to interact with natural killer (NK) cells to prevent NK-mediated cell lysis (Reyburn et al., 1997). The non-classical class I genes are evolutionarily related and appear to have distinct functions related to immune response and NK cell recognition in specific settings (Lee et al., 1998). There are three classical (HLA-A, B and C) and three non-classical (HLA-E, F and G) class I genes in the HLA complex (Rhodes and Trowsdale, 1998).

In sheep, the class I region is poorly characterised and there is a significant controversy over the number of classical class I loci. Initial studies in this regard relied mainly on the use of alloantisera in micro-lymphocytotoxicity assays. Evidence for the presence of two closely linked class I loci, designated as OLA-A and B, was provided in 1978 (Millot, 1978). Several other studies confirmed the existence of two class I loci (Stear and Spooner, 1981; Cullen et al., 1982; Garrido et al., 1995; Stear et al., 1996; Jugo and Vicario, 2001; Jugo et al., 2002). Three different studies, one based on the micro-lymphocytotoxicity assay (Millot, 1984), one based on immunoprecipitation followed by 2-dimensional gel analysis (Puri et al., 1987a) and another based on restriction fragment length polymorphism (RFLP) (Grossberger et al., 1990), have indicated the existence of a third class I locus. In a recent study aimed at haplotype characterisation of transcribed ovine MHC class I genes, at least four distinct polymorphic loci were identified (Miltiadou et al., 2005).

Several molecular genetic investigations have been undertaken to study polymorphisms of class I genes. An RFLP study conducted employing a human class I probe revealed polymorphic bands co-segregating and correlating with serologically defined lymphocyte antigens (Chardon et al., 1985). This was the first evidence that the serologically detected class I sheep leukocyte antigens are coded by MHC genes. In a different study, a sheep thymus cDNA library was screened with a human cDNA probe derived from HLA-B27 (Grossberger et al., 1990). Thirteen clones were identified and partially sequenced. Based on the sequences, the clones could be categorised into 5 distinct groups, requiring the expression of at least 3 loci. These sequences were found to be more similar to bovine than to murine class I genes.

A purine-pyrimidine repeat of the form (CA)20 was identified in an ovine class I (*Ovar*-MHC I)-positive clone from a sheep genomic library (Groth and Wetherall, 1994). Polymerase chain reaction (PCR) amplification of this microsatellite region revealed the presence of 11 alleles at the locus, segregating in a Mendelian fashion. This microsatellite (SMHCC) was found to be highly polymorphic in different breeds of sheep (Buitkamp et al., 1996; Paterson, 1998; Paterson et al., 1998; Charon et al., 2001; Gruszczynska et al., 2002a). These studies revealed allele numbers ranging from 5 to 13, with high heterozygosity coefficients, indicating the usefulness of this locus as a genetic marker. This locus was found by recombination frequency to be 5.8 cM from the DRB1 locus (Buitkamp et al., 1996).

Recently, molecular genetic analyses in two heterozygous Scottish Blackface rams revealed 12 novel MHC class I transcripts (Miltiadou et al., 2005). Based on the class I sequence-specific genotypes of their progeny, these transcripts could be assigned to four individual haplotypes. Phylogenetic analyses of the more conserved exons (4 to 8) grouped the

transcripts into four clusters, while a combination of phylogenetic analyses, haplotype data and transcription levels suggested the transcripts to be products of at least four loci, three of which appeared together in a number of combinations in individual haplotypes.

CLASS I MOLECULES

Classical MHC molecules have four characteristics by which their function is defined: a high degree of polymorphism, high-level expression in particular cells, and the ability to bind small peptide molecules and present them to T cells (Kaufman et al., 1994). The class I MHC molecules, called class Ia molecules or class I classical molecules, are glycoproteins expressed on the surface of all nucleated somatic cells. They are found in highest concentration on lymphocytes and macrophages. The structure of the class I molecule was originally derived by X-ray crystallography (Bjorkman et al., 1987a,b). It is a heterodimer (Figure 2) consisting of an α- or heavy chain, non-covalently linked to a light β2-microglobulin chain. The chain is composed of three extracellular domains (α1, α2 and α3), a transmembrane domain and a cytoplasmic domain. The α1 and α2 domains form the peptide-binding region (PBR), lying above the α3 domain. The groove is formed by two α helices bordering a β-pleated sheet, and residues from both α1 and α2 domains contribute to the groove (Bjorkman et al., 1987a). The microglobulin chain has a single extracellular domain and probably serves to stabilise the structure. The known polymorphisms of the molecule, i.e., variations in the amino acid sequence, are concentrated in three or four discrete hypervariable regions within the PBR. The rest of the molecule is highly conserved and shows little sequence variation. The α-chains are encoded by polymorphic class I loci within the MHC, while β2-microglobulin is encoded by a non-polymorphic locus outside the MHC (Hughes and Yeager, 1998).

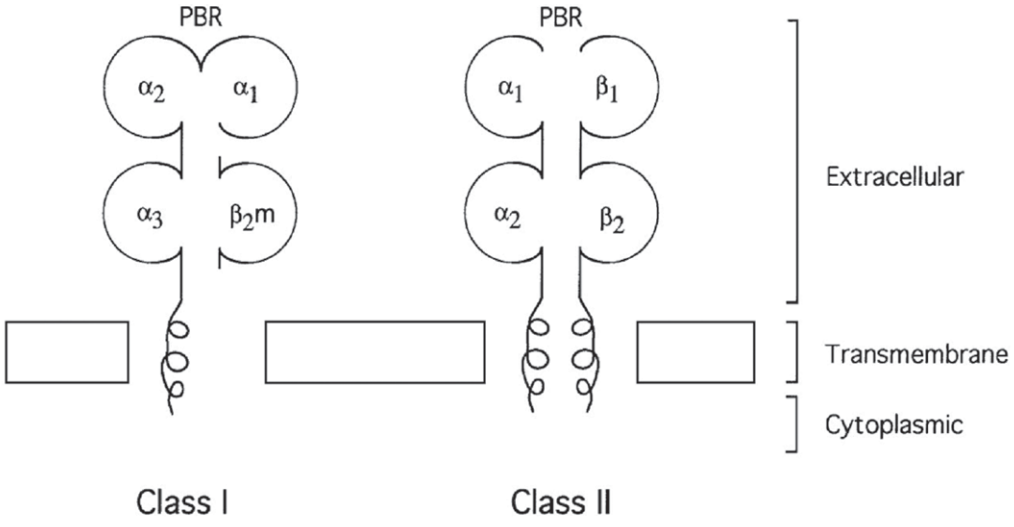

Figure 2. Schematic presentation of the structure of MHC class I and class II molecules. PBR = peptide-binding region. (Reprinted, with permission, from the Annual Review of Genetics, Vol. 32 ©1998 by Annual Reviews, www.annualreviews.org).

The class I molecules present antigenic peptides (8 or 9 amino acids long) to T cell receptors (TCRs) of CD8+ cytotoxic T lymphocytes (CTLs), the principal immune function of which is considered to be the killing of virus-infected cells and tumour cells (Rammensee et al., 1995). In all cells, there is constant turnover of cellular proteins that are broken down into small peptides by a multimeric proteolytic complex in the cytoplasm, known as a proteasome (Rivett, 1993). In mammals, there are two proteasome components encoded within the MHC class II region, called the low-molecular mass polypeptide 2 (LMP2) and LMP7. The expression of class I molecules and LMPs is enhanced by the cytokine interferon gamma. The peptides derived in the proteasome are transported across the membrane of endoplasmic reticulum (ER) by a dimeric transporter associated protein (TAP), encoded within the class II MHC region. In the ER, a complex involving the class I molecule, the peptide and β2-microglobulin is formed, and then transported to the cell surface.

The CTLs exercise a continual surveillance in the body by means of their TCRs. In the absence of any infection, the peptides bound by class I molecule are self-peptides. During infection by a virus or other intracellular parasite, some of the proteins broken down by the proteasome are of parasitic origin (non-self or foreign peptides). When CTLs encounter the complex of self-class I MHC and foreign peptide, a cytotoxic reaction is initiated that kills the infected cells. CTLs can only recognise foreign peptides in the context of self-class I MHC, a phenomenon referred to as class I MHC restriction of CTL (Zinkernagel and Doherty, 1974).

The molecular structure and tissue distribution of sheep classical class I molecules were studied using a panel of three monoclonal antibodies (Gogolin-Ewens et al., 1985). The class I heterodimer comprised a heavy chain of 44 kDa and a smaller β2-microglobin of 12 kDa. In similarity to the class I MHC molecules of other species, these molecules were found to be distributed on all sheep lymphocytes and many non-lymphoid tissues, with differential expression on mature and immature lymphocytes. They were found to be expressed equally on normal lymphocytes and antigen-activated lymphoblasts (Hopkins and Dutia, 1990).

The biosynthesis of sheep class I molecules was analysed by sodium dodecyl (lauryl) sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) separation of immunoprecipitates of splenocytes pulse-chase labelled with (35)S-methionine and (35)S-cysteine (Puri et al., 1987a). Two biosynthetic intermediates (39-40 and 41-42 kDa), finally resulting in a heavy chain (44 kDa) were noticed. Throughout the period of pulse-chase labelling, β-microglobulin could not be detected along with the heavy chain, indicating that sheep β-microglobulin either possesses very few methionine and cysteine residues or has a very low synthesis/turnover rate. A similar finding with β-microglobulin, that was corroborated in cattle (Joosten et al., 1988) but found to be contrary to that in humans, was reported in a study pertaining to immunoprecipitation and isoelectric focusing of sheep class I antigens (Jugo et al., 2002).

Another interesting aspect of sheep β-microglobulin is that it displays heterogeneity in 2-dimensional non-equilibrium pH gradient electrophoresis (NEPHGE)/SDS-PAGE analysis of class I molecules from (125)I-surface-labelled cells (Puri et al., 1987a). β-microglobulin can be resolved into two forms of varying charge and intensity, being consistent with either two primary gene products or allelic variation.

CLASS II GENES

Class II genes are members of the immunoglobulin superfamily of genes which are

functionally specialised for presentation of antigenic peptides mainly derived from extracellular proteins and parasites to the TCR on CD4+ helper T cells. In the HLA complex, these include five sets of the classical genes DP, DM, DO, DQ, and DR and non-classical genes such as LMP, TAP and TAPBP. Within each set of classical genes, genes for the α-chain are designated A, while genes for the β-chain are called B. The α- and β-chain genes in each set are located close together and resemble a two-gene duplication unit, with the exception of DOA and DOB genes, which are well separated from each other. Not all sets contain genes for both chains, although some contain many pseudogenes (Tizard, 2004).

Among the three regions of the ovine MHC, genes of the class II region are the best characterised. They are classified into different families, as in other mammalian species, using nomenclature adapted from humans and include DQ, DR, DP, etc. (Hein, 1997). Early studies of the class II region by genomic Southern analysis employing HLA gene probes resulted in a complex pattern of cross-hybridising bands, which suggested that sheep contained homologues of DQ and DR genes but probably not DP (Chardon et al., 1985; Puri et al., 1987d; Scott et al., 1987). In a subsequent study on two unrelated sheep, 7 distinct class II α and 24 distinct class II β or β-related sequences were identified (Deverson et al., 1991). Consistent with earlier predictions, DQ and DR homologues were detected but not DP. The *ovar*-DQ and *ovar*-DR loci, which constitute the class IIa sub-region, have been studied in detail. A number of other *ovar*-MHC II genes of the class IIb type have also been identified. These include DY (Wright et al., 1994), DM (Schwaiger et al., 1996) and DN/DO (Wright et al., 1995, 1996).

Ovar-DR genes

The DR genes are highly polymorphic and the classical class II molecules encoded by these genes are expressed in higher concentrations than the DQ molecules on the cell membranes of macrophages and B cells (Outteridge et al., 1996). Several studies have been undertaken to characterise DRA and DRB genes of sheep.

DRA genes

An early Southern hybridisation study (Scott et al., 1987) employing human HLA-D probes provided evidence for the existence of a single DRA gene in sheep that was later isolated and found to be expressed (Deverson et al., 1991; Ballingall et al., 1992). Although there was an indication for the presence of a second DRA gene in sheep (Deverson et al., 1991) that might have been the result of gene duplication (Ballingall et al., 1992), it has not been confirmed in any subsequent studies. Initial sequencing of exons 1 to 4 of the expressed DRA gene indicated that it was homologous to the human DRA gene. Complete sequencing of the gene (Fabb et al., 1993) has revealed that it could code for a polypeptide of 253 amino acids of which 24 constitute the signal peptide and the remaining 229 form the mature polypeptide. The DRA clones in the two studies differed at only two amino acid positions, one within exon2 (H^{50}/A^{50}) and the other in exon3 (T^{109}/I^{109}). This low level of *Ovar*-DRA sequence polymorphism was similarly reflected in RFLP studies (Fabb et al., 1993; Escayg et al., 1993, 1996). Three allelic fragments of 6.1, 4.9 and 2.4/2.8 kb with respective frequencies of 0.05, 0.875 and 0.075 were found to be associated with the enzyme *Bg*III in Merino and Romney sheep.

DRB genes

The most polymorphic among the MHC genes is the DRB locus (Andersson and Rask, 1988). *Ovar*-DRB genes have been reported to exist in multiple copies, some functional and others non-functional. Early serological and biochemical work on sheep MHC class II molecules detected seven β-polypeptides in association with DRA chains that provided evidence for the existence of more than one locus encoding them. Two distinct DRB-like genes were identified using RFLP studies on bacteriophage clones of a sheep genomic library (Scott et al., 1987), while a different study provided evidence for the expression of two distinct *Ovar*-DRB genes (Dutia et al., 1994). RFLP studies employing probes specific for *Ovar*-DRB exon 2 revealed 10 DRB alleles that required the presence of at least three DRB genes (Grain et al., 1993). Further evidence for the presence of two copies of the expressed DRB1 gene was provided in a study on single strand conformational polymorphism (SSCP) and sequence polymorphism of MHC-DRB exon 2 in Latxa and Karrantzar sheep (Jugo and Vicario, 2000). Apart from red deer (Swarbrick et al., 1995), sheep are the only ruminants in which the existence of two expressed DRB genes has been described, although a second DRB gene (DRB2) in cattle has been found to be expressed at very low levels (Groenen et al., 1990).

Four *Ovar*-DRB loci have been described by Scott et al. (1991b). The functional DRB1 gene is located at one of them and pseudogenes, DRB2, DRB3 and DRB4, are found at the remaining three loci. The pseudogenes lack defined exons 1 and 2, and also show numerous mutations in their sequences as well as stop codons in exons 3 and 4. There are indications that additional DRB pseudogenes exist (Schwaiger et al., 1996).

The whole *Ovar*-DRB region numbers several thousand base pairs and its basic structure is considered similar to other mammalian species (Schwaiger et al., 1996). However, almost all the studies on this region have concentrated on the polymorphisms found in exon 2 and adjoining intron 2 of the expressed gene DRB1. This is because DRB1 exon 2 encodes the β1 domain, which constitutes part of the PBR of the DR molecules. The highly variable residues concentrated in this region are in close contact with the peptides presented in the PBR or the TCR (Brown et al., 1993), and therefore, they are likely to be related to functionality such as disease resistance/susceptibility.

Another characteristic feature of *Ovar*-DRB1 is that a simple tandem repeat (STR) of the form [(GT)n(GA)m] exists in intron 2, 30 bp downstream from the 3' splice site of exon 2 (Schwaiger and Epplen, 1995). This STR with the same basic structure is present at virtually the identical positions in all the expressed DRB alleles of cattle, sheep, goat, red deer, and humans, indicating that it remained unchanged at a specific location across various species for nearly 100 million years of mammalian evolution. Two sheep DRB pseudogenes, DRB3 and DRB4, also harbour this STR either in the same or degenerated forms, while another pseudogene (DRB2) lacks it (Schwaiger and Epplen, 1995). In DRB3, the STR structure is highly disintegrated, and in DRB4 only three copies of each dinucleotide [(GT)3(GA)3] are detectable.

A different microsatellite of the form (AC)n is present in intron 5, adjacent to the 5'end of exon 6 of *Ovar*-DRB2 (Scott et al., 1991b; Blattman and Beh, 1992). Typing of this microsatellite together with that found in intron 2 of DRB1 in sheep belonging to the international mapping flock, AgResearch, New Zealand indicated a distance of 2.6 cM between the two loci (Schwaiger et al., 1996). This distance is almost the same as that between *Ovar*-DRB2 and

Ovar-MHC I. Also, haplotype analysis of unrelated animals has identified several haplotypes of the DRB region combining different DRB1 and DRB2 alleles, which underscores the genomic instability of the DRB sub-region. Similar distances between these loci have also been reported in a subsequent study (Paterson et al., 1998).

Typing of *Ovar*-DRB1 genes employing different methods in various sheep breeds has revealed extensive polymorphism at these loci (Table 1). Initial studies employed RFLP techniques utilizing DRB1 exon 2-specific probes (Blattman et al., 1993; Grain et al., 1993). However, this method has been considered unsuitable to study variation at the DRB1 owing to extensive cross-hybridisation between the DRB1 probe and the DQB locus (Escayg et al., 1996). Sequencing of the PCR-amplified DRB1 exon 2, either alone or together with the adjacent STR in intron 2, has revealed extensive polymorphism within the locus (Schwaiger et al., 1993b, 1994; Paterson, 1998; Konnai et al., 2003a; Sayers et al., 2005). SSCP and sequence analysis of DRB1 exon 2 is another method for DRB typing (Kostia et al., 1998; Tkacikova et al., 2005). However, in one of the studies employing this method (Jugo and Vicario, 2000), alleles from more than one DRB locus could be detected.

Another method for typing DRB1 alleles of farm animals, using PCR-RFLP analysis, has been suggested (Amills et al., 1996; Rasool et al., 2000; Konnai et al., 2003b; Dongxiao and Yuan, 2004; Gruszczynska et al., 2005). Using a pair of bovine specific primers, DRB1 exon 2 was amplified from cattle, buffalo, sheep, and guinea pig DNA samples. The amplified fragment was the same size in all the animals from the different species. Polymorphisms in exon 2 were detected by RFLP of the amplified product. Two recent studies looked at polymorphisms in exon 2 of the *Ovar*-DRB3 gene employing PCR-RFLP (Sun et al., 2003; Liu et al., 2004). An oligonucleotide method has also been described as a means for typing DRB genes (Schwaiger et al., 1993a). PCR fragments including exon 2 plus adjacent intron 2 are first separated on a polyacrylamide gel based on length variations of the microsatellite repeat and then hybridised with probes for both the intron repeat and exonic sequence. This polymorphism-specific oligonucleotide typing has been utilised for *Ovar*-DRB1 typing in various studies (Schwaiger et al., 1995; Stear et al., 1996; Buitkamp and Epplen, 1996; McCririe et al., 1997).

PCR amplification of exon 2 together with microsatellite in intron 2 and determination of the exact length of the amplified product using an automatic capillary sequencer is another method for typing *Ovar*-DRB1 alleles (Gruszczynska, 1999; Gruszczynska et al., 2000; Charon et al., 2002). Length polymorphism of the microsatellite in intron 2 of the expressed DRB gene in various artiodactyl species has been found to be strongly associated with sequence polymorphisms in exon 2 and thus could be utilised for DRB typing (Ellegren et al., 1993). This method was employed in several studies to detect *Ovar*-DRB1 alleles (Outteridge et al., 1996; Paterson, 1998; Paterson et al., 1998; Saberivand et al., 1998; Griesinger et al., 1999).

Length polymorphisms of the microsatellite in intron 5 of the pseudogene *Ovar*-DRB2 has also been studied in different breeds of sheep (Table 2). High heterozygosity (>78%) at this locus, reported in these studies, suggests the potential application of this locus as a genetic marker, especially for disease resistance. It has been shown in cattle that the resolution of microsatellite-based DRB3 typing was much better when the length polymorphism of another microsatellite located in DRB1 pseudogene was included (van Haeringen et al., 1999). However, no such typing studies in sheep involving the microsatellites located at DRB1 and DRB2 have been reported.

Table 1. Polymorphism of the expressed *Ovar*-DRB1 gene in various sheep breeds.

Typing method	Breed(s) analysed		Sheep screened	No. of alleles	References
A	Soay		15	5	Paterson (1998)
A	Perendale, Coopworth, Texel, Landrace, Merino, Romney		34	34	Schwaiger et al. (1994)
A	-		-	13	Schwaiger et al. (1993b)
A	Suffolk		71	28	Konnai et al. (2003a)
	Cheviot		20	14	
	Corriedale		6	9	
A	Texel		155	8	Sayers et al. (2005)
	Suffolk		179	7	
B	Polish Heath		675	20	Charon et al. (2002)
B	German Merino	*Parents*	43	36	Gruszczynska (1999)
		Progeny	37	28	
B	Polish Heath	*Parents*	52	36	Gruszczynska et al. (2000)
		Progeny	100	30	
C	Merino land		105	18	Griesinger et al. (1999)
	Changthangi		28	16	
	Red Maasai		35	15	
C	Soay		1209	8	Paterson (1998); Paterson et al. (1998)
C	-		363	12	Saberivand et al. (1998)
C	Merino		130	8	Outteridge et al. (1996)
C	Merino		234	16	Bot et al. (2004)
D	Scottish Blackface		21	8	McCririe et al. (1997)
D	Scottish Blackface		299	17	Buitkamp and Epplen (1996)
D	Scottish Blackface		200	19	Schwaiger et al. (1995); Stear et al. (1996)
D	-		-	16	Schwaiger et al. (1993a)
E	Merino		189	29 bands	Blattman et al. (1993)
E	Prealpe		89	10*	Grain et al. (1993)
F	Latxa, Karrantzar		83, 17	12*	Jugo and Vicario (2000)
F	Finsheep, Russian Ramanov		31	19	Kostia et al. (1998)
G	Suffolk		52	13 haplotypes	Konnai et al. (2003b)
G	Mongolian, Kazakh		53, 62	7	Sun et al. (2003)
G	Polish Heath		101	65 haplotypes	Gruszczynska et al. (2005)
	Polish Lowland		99	68 haplotypes	

A - PCR amplification and sequencing of exon 2 either alone or together with a part of adjacent intron 2
B - Length polymorphism of microsatellite in intron 2 together with exon 2
C - Length polymorphism of microsatellite in intron 2
D - Length polymorphism of STRs in intron 2 plus hybridization of oligonucleotides within exon 2
E - RFLP with exon 2-specific probe
F - SSCP and sequence analysis of exon 2
G - PCR-RFLP of exon 2
* - Existence of more than one loci has been indicated.

Ovar-DQ genes

The existence of DQ genes in sheep was first demonstrated by genomic Southern blot analysis employing probes homologous to the HLA DQ region (Chardon et al., 1985; Scott et al.,

Table 2. Polymorphism of pseudogenes *Ovar*-DRB2 and *Ovar*-DRB3 in different breeds.

Typing method	Breed(s) analysed	Sheep screened	No. of alleles	References
Pseudogene *Ovar*-DRB2				
A	German Rhonschaf	468	8	JanBen et al. (2002)
A	Heatherhead	190	11	Gruszczynska et al. (2002b)
	Polish Lowland	200	8	
A	Soay	887	6	Paterson (1998); Paterson et al. (1998)
A	Merino, Corriedale, Polworth, Southdown, Suffolk, Border Leicester, Romney, Dorset	58	13	Blattman and Beh (1992)
Pseudogene *Ovar*-DRB3				
B	Mongolian, Kazakh	-	7	Sun et al. (2003)
B	Dolang	-	24 haplotypes	Liu et al. (2004)

A - Length polymorphism of microsatellite in intron 5
B - PCR-RFLP of exon 2

1987). In the latter study, the presence of three DQA-like and four DQB-like genes was indicated. RFLP and sequence data derived from genomic clones (Scott et al., 1991a) and cDNA clones (Fabb et al., 1993) indicated the existence of two DQA genes per haplotype in sheep. This is consistent with a detailed genomic map of the ovine DQ sub-region (Wright and Ballingall, 1994), which revealed two DQ loci each containing one DQA and one DQB gene arranged in tail to tail orientation (Figure 3). The two loci are 22 kb apart and are linked on a linear tract of 130 kbp of DNA. The *Ovar*-DQ sub-region is more compact than the HLA-DQ sub-region, since a distance of 70 kb separates the two HLA-DQ loci (Campbell and Trowsdale, 1993). The *Ovar*-DQA1 and DQB1 genes at the first locus are separated by 11 kb, while the DQA2 and DQB2 genes at the second locus are 25 kb apart. The HLA-DQ1 genes and *Bota (Bos Taurus)*-DQ1 genes are also separated by a similar distance, while the HLA-DQ2 genes lie much closer together than the *Ovar*-DQ2 genes. The equivalents of *Bota*-DQA3 (Andersson, 1988) and HLA-DQB3 pseudogene (Ando et al., 1989) could not be detected in sheep.

Figure 3. Schematic presentation of the structure of the *Ovar*-DQ subregion**.** Arrows indicate the direction of transcription of the genes. Distances as per Wright and Ballingall (1994).

In a study on the linkage analysis between the *Ovar*-DQA1, DQA2, DQB1, DQB2, and DRA loci, no recombinants were observed between DQA1 and DQA2 loci or between DQA and DQB genes (Escayg et al., 1996). Also, there was no evidence of recombination

between the DRA locus and any of the DQ loci. This finding, despite the lack of any available information on the distance between the DQ and DR subregion, would suggest that these loci are physically close.

Ample evidence exists for both *in vitro* (Wright and Ballingall, 1994) and *in vivo* (Scott et al., 1991a; Fabb et al., 1993; Wright and Ballingall, 1994) transcription of *Ovar*-DQA genes. However, cell surface expression of DQ products has been detected only for the DQ1 locus (Wright and Ballingall, 1994). It is probable that despite expression of genes at the DQ2 locus, the lack of suitable monoclonal antibody (Wright and Ballingall, 1994) or the possibility of the DQ2 α- and β-chains mis-pairing (Snibson et al., 1998) may be the reason(s) for failure in detecting their products. This view is further supported by the fact that about 10 to 18% of sheep from different breeds (Scott et al., 1991a; Fabb et al., 1993; Escayg et al., 1996) lack the DQA1 gene in their haplotypes, indicating that any functional DQ molecule in these sheep would be the product of expressed genes at DQ2 locus (Snibson et al., 1998).

DQA genes

The nucleotide sequence of all exons and introns, excluding exon 1 of *Ovar*-DQA1 and DQA2 genes, has been determined and was found to be similar to respective analogues in humans (Scott et al., 1991a). The second exons in these two genes were less similar in terms of nucleotide (78%) and coding amino acid (71%) identities between them. Subsequently, full-length cDNA clones coding for these two *Ovar*-DQA genes, together with that for the *Ovar*-DRA gene, have been isolated and sequenced (Fabb et al., 1993). All of these encode polypeptides of 255 amino acids, with 23 of them accounting for signal peptide and the other 232 encoding the mature polypeptide. DQA1 and DQA2 could be discriminated mainly based on the nucleotide sequence of exon 2. The exon 2 nucleotide dissimilarity between DQA1 and DQA2 genes (19.5%) is far more than that between the alleles within either DQA1 (8.0%) or DQA2 (10.0%). Nucleotide variation was found to be minimal in exon 4 of both genes. Similar sequence polymorphisms in exon 2 were also observed in a different study (Snibson et al., 1998).

Several alleles of *Ovar*-DQA1 and DQA2 have been identified based on sequence variation of the PCR amplified exon 2. Twenty-three different DQA2 sequence alleles (1-Scott et al., 1991a; 1-Fabb et al., 1993; 1-Wright and Ballingall, 1994; 7-Snibson et al., 1998; 13-Hickford et al., 2004) and sixteen DQA1 sequence alleles (1-Scott et al., 1991a; 1-Fabb et al., 1993; 3-Wright and Ballingall, 1994; 2-Snibson et al., 1998; 3-Zhou and Hickford, 2001; 6-Zhou and Hickford, 2004) have been identified. PCR-SSCP is an ideal method for typing DQA sequence alleles (Snibson et al., 1998). A single set of PCR primers could amplify all known DQA2 alleles, while a separate set of primers amplified only the DQA1 gene. Two new DQA1 and nine DQA2 alleles were identified in the study using this method.

Employing PCR-SSCP, an extensive investigation on the DQA2 gene was carried out in 2000 sheep belonging to Merino, Corriedale, Borderdale, Romney, Awassi, and Finnish Landrace breeds (Hickford et al., 2004). As many as 23 exon 2 sequences could be identified, of which 5 were found to be more similar to bovine DQA3 or DQA4 sequences than to other sheep DQA2 and were designated as DQA2-like sequences. However, there was no evidence for the presence of the bovine DQA5-like sequences in sheep. Three or four unique DQA2 sequences could be recovered from individual sheep, suggesting the presence of two DQA2 loci.

A different study, but employing the same technique, on DQA1 in 300 sheep belonging to Merino, Corriedale, Borderdale, Romney, Awassi, and Finnish Landrace breeds revealed extensive polymorphism in the exon 2 sequence, with as many as 14 alleles (Zhou and Hickford, 2004). Comparison of the sheep DQA1 exon 2 sequences with those available from cattle revealed several clusters of ovine DQA1 sequences, with some of the sheep alleles being more similar to cattle alleles than to the other sheep alleles. It was suggested that this trans-species polymorphism might be the result of balancing selection at the DQA1 locus.

Polymorphisms of DQA genes have also been reflected in RFLP studies employing exon 2-specific probes (Scott et al., 1991a; Fabb et al., 1993; Escayg et al., 1996; Hickford et al., 2000). DQA2 was found to be more polymorphic than DQA1. Up to 8 and 16 alleles have been reported for DQA1 and DQA2, respectively. Another interesting feature of these studies is that in 11 to 36% of the sheep screened, no DQA1 allele could be detected and the allele in such animals was considered as null. Thus, sheep do have a variable number of DQA genes in their haplotypes. In some of the sheep that possessed null DQA1 allele, two DQA2-like sequences could be detected (Hickford et al., 2000), retaining the pattern of two DQA loci per haplotype. Duplication of DQA2 gene was suggested in these animals. Also, the similarity between the two DQA2 sequences would suggest that DQA1/DQA2 haplotype is ancestral to DQA1 null/DQA2 (duplicated) haplotype. Similarly, since the DQA1/DQA2 haplotype is more diverse than the DQA1 null/DQA2, it seems likely that DQA1 null/DQA2 haplotype preceded DQA1/DQA2. The presence of two DQA2-like sequences in animals with DQA1 null alleles has also been reported in cattle (Ballingall et al., 1997). However, the two DQA2 sequences were diverse and had been categorised as DQA2 and DQA3. In sheep, it was shown that some ovine DQA2 sequences exhibited much closer similarity to the cattle DQA3 gene than to other DQA2 sequences (Snibson et al., 1998). This suggests that the duplicated ovine DQA2 gene in animals with DQA1 null allele may be analogous to the cattle DQA3 gene (Hickford et al., 2000). However, there is no evidence with regard to the expression of this gene. The presence of two additional DQA loci in cattle, *Bota*-DQA4 (Ballingall et al., 1997) and DQA5 (Gelhaus et al., 1999) has been reported, but their homologues in sheep are yet to be identified.

DQB genes

The nucleotide sequence of *Ovar*-DQB gene, excluding exon 1 and parts of the introns, has been reported (Scott et al., 1991b). Comparison with human sequences revealed similarity with both HLA-DQB1 and DQB2, suggesting the presence of a common ancestor. Subsequently, exon 2 nucleotide sequences of two separate *Ovar*-DQB genes (DQB1 and DQB2), derived from cosmid clones, have been determined (Wright and Ballingall, 1994). The two genes could not be assigned to separate loci based on the nucleotide sequences, owing to >90% similarity. However, their proximity to an *Ovar*-DQA1 or DQA2 gene could be used to discriminate between these genes.

Several new DQB sequences have been determined in subsequent studies. Difficulty still exists in assigning these sequences to separate loci because of the high similarity between the two DQB genes. Ten distinct sequences were identified from an SSCP sequence analysis of PCR-amplified DQB exon 2 in 13 Merino sheep, demonstrating considerable variation in the ovine DQB region (van Oorschot et al., 1994). Twenty-nine percent of the total 267 nucleotide sites in exon 2 of these alleles, translating to 46% of amino acid sites, are polymorphic. The

presence of at least two separate OLA-DQB genes was demonstrated in that study. Phylogenetic analyses of the exon 2 nucleotide and amino acid sequences from sheep, cattle and humans showed that the ovine and bovine sequences are more closely related to each other than either are to the human sequences. The SSCP technique was shown to be capable of discriminating between all the *Ovar*-DQB sequences identified in the study.

Sixteen distinct PCR-amplified *Ovar*-DQB exon 2 sequences have been characterised from only 18 sheep in another study (Schwaiger et al., 1996). While three of these sequences could be assigned to DQB1 and two to DQB2, the rest could not be assigned to either locus. Reference-strand-mediated conformation analysis or double-strand conformational analysis, employing two reference alleles, has been shown to be a new method for high resolution typing of the *Ovar*-DQB genes (Feichtlbauer-Huber et al., 2000). The use of two different reference alleles would enable high resolution of many and probably all alleles and reduce the probability of missing new alleles. Using this method, 16 new sequences (from that of van Oorschot et al., 1994) were obtained from 10 unrelated Scottish black-faced sheep, increasing the number of known alleles to 28. However, the alleles could not be assigned to separate loci.

Ovar-DNA and DOB genes

The presence of the DNA (formerly DZA) gene in sheep had been inferred from Southern analysis of genomic DNA (Scott et al., 1987). Cosmid clones from the sheep MHC class II region were found to contain this gene (Deverson et al., 1991). Subsequently, the nucleotide sequence of the DNA gene, together with its predicted amino acid translations, were reported (Wright et al., 1995). It had all the salient features of a class IIA gene, including two exons coding for the two extracellular domains, and one coding for a proline rich connecting peptide, a hydrophobic transmembrane region and a cytoplasmic tail. Also, it has two conserved N-linked glycosylation sites NGT and NAT, and two conserved cysteine residues, forming a disulphide bond in the α2 domain. The ovine and human genes share 83% nucleotide identity (translating to 78% amino acid identity) at exons 2 and 3. Though transcription of the *Ovar*-DNA gene was detected by Northern hybridisation with an *Ovar*-DNA probe, there was no evidence of expression of the gene. Like that of the *Ovar*-DRA, the *Ovar*-DNA gene appears to be monomorphic (Schwaiger et al., 1996).

The B gene partner for HLA-DNA gene is the non-polymorphic HLA-DOB gene (Tonnelle et al., 1985), while the murine homologue of the *Ovar*-DNA gene expresses in combination with the H2-OB gene (Karlsson et al., 1991). There was an early indication in sheep for the existence of *Ovar*-DOB gene (Scott et al., 1987). The gene has been cloned and subsequently sequenced (Wright et al., 1996). Exons 1 and 2 have been found to exhibit amino acid identities of 62 and 80%, respectively, in comparison with the HLA-DOB gene. Neither transcription of the gene nor its expression in combination with *Ovar*-DNA gene could be detected in the study.

Ovar-DY genes

DYA and DYB (DIB) genes which are absent in HLA have been detected in cattle (Andersson et al., 1988). These were shown to segregate with the DOB gene in one region separated by a recombination distance of 17 cM from the region that contains DQ, DR and C4

loci. The *Bota*-DYA gene has been cloned and sequenced (van der Poel et al., 1990), while there has been no report of cloning of its B gene partner. A unique single copy class IIB gene, *Bota*-DIB has been cloned and sequenced from a phage library (Stone and Muggli-Cockett, 1990). The homologues of *Bota*-DYA and DIB genes in sheep, designated as *Ovar*-DYA and DYB, have been identified in sheep by screening a cosmid library with *Ovar*- and HLA-DQ probes at low stringency (Wright et al., 1994). The presence of DY genes, together with the absence of DP genes and variability in the number of DQ genes between haplotypes, has been considered as a distinguishing feature of the ruminant class II region.

The *Ovar*-DYA gene have shown high sequence similarity to the bovine and caprine DYA genes and much less so to the *Ovar*-DRA, DNA and DQA genes (Wright et al., 1994). Similarly, the *Ovar*-DYB gene exhibited a higher degree of sequence similarity to the *Bota*-DIB and was different from the *Ovar*-DQB and DRB genes. It was named DYB rather than DIB because of its close proximity to DYA gene. The DYA and DYB genes lie tail to tail with a distance of 11 kb between them. While transcription of the gene could be detected, there was no evidence for its expression. The authors suggested that evolution of the DY locus may be the result of duplication of a pair of DQ genes, with subsequent rapid divergence.

A polymorphic microsatellite (DYMS1) of the form (CA)n was found to be located in the region 5' of the DYA gene, 19 cM from the DRB1 locus (Buitkamp et al., 1996). Nineteen alleles were identified at the locus in this study. The polymorphism at this microsatellite locus was later confirmed in a different study in German Rhonschaf sheep that revealed 6 alleles (JanBen et al., 2002).

Studies on the second exon of DY genes employing SSCP have revealed 3 alleles for *Ovar*-DYA and 4 in DYB, with respective heterozygosities of 0.67 and 0.61 (Maddox, 1999). A recent study assessed the degree of conservation between ovine and bovine DYA gene sequences (Ballingall and McKeever, 2005). Nucleotide similarities of 97% in the immediate promoter, 94% in the coding and 91% in the intronic regions were observed between the species. The *Ovar*-DYA full length transcript revealed an open frame encoding a 288 amino acid protein compared with a 253 amino acid protein associated with the bovine DYA transcript.

Ovar-DM genes

The existence of DMA and DMB genes in sheep has been indicated based on PCR amplification of fragments from exons 2 and 3 of the *Ovar*-DMB gene and exon 2 of the *Ovar*-DMA gene, employing primers derived from murine and human gene sequences (Schwaiger et al., 1996). Only two exon 2 alleles could be detected in the case of the DMB gene by SSCP (Maddox, 1999).

CLASS II MOLECULES

Class II molecules have a much more restricted expression pattern than do class I molecules, in that they are expressed primarily on cells deemed to have antigen uptake, processing and presentation functions (macrophages, dendritic cells and B cells). Their expression varies among species and is enhanced in rapidly dividing cells and in cells treated with interferon (Tizard, 2004). Class II molecules are also heterodimers (Figure 2), but in contrast to class I molecules, are composed of an α- and a β-peptide chain. Each chain has two extracellular

domains, a connecting peptide, a transmembrane domain and a cytoplasmic domain. A third protein chain, called γ or invariant chain (Li or CD74), is associated with intracellular class II molecules (Tizard, 2004).

The class II PBR consists of two α helices bordering a β-pleated sheet (Hughes and Yeager, 1998), as with the class I molecule. The difference is that in class II, one of the α helices and about half of the β-pleated sheet are contributed by the α-chain, whereas the other α helix and other half of the β-pleated sheet come from the β-chain. Polymorphisms in the class II molecules result from variation in the amino acid sequences of the α helices at the sides of the groove. The α- and β-chains are encoded by genes in the class II region. In mammals, the class II subregions (designated as DR, DP and DQ in humans), each contain a functional α-chain gene and one or more functional β-chain genes.

The class II molecules present peptides derived from exogenous proteins to the TCR of CD4+ helper T cells (Germain and Margulies, 1993). In response to a foreign peptide, the helper T cells release cytokines that trigger the production of antibodies and cell-mediated immune responses. The class II molecules also possess the property of MHC restriction, in which the antigens bound to MHC molecules also need to be recognised by a TCR on a helper cell, in order to trigger an immune response. The peptides presented by class II molecules can vary substantially in length, between 11 and 17 residues (Rammensee et al., 1995).

The complex between the class II molecule and its peptide ligand is created by a mechanism quite different from that of class I. Before transport to the cell surface, the class II dimer forms a complex with the invariant chain (Li) in the ER, which acts as a chaperone to stabilise the heterodimer and prevents premature peptide loading. This complex then travels to an acidic endosome-like compartment (Peters et al., 1991), where the Li is degraded by a series of proteolytic cleavage events, leaving a residual peptide (class II-associated invariant chain peptide) occupying the PBR of the MHC molecule. The release of class II-associated invariant chain peptide and its replacement with antigenic peptides is catalysed by HLA-DM, which is independently targeted to endosomal compartments. The resultant MHC class II-peptide complex is then transported to the cell surface, where it awaits interaction with antigen-specific T cells. The expression of MHC class II, Li and HLA-DM genes is coordinately regulated at the level of transcription by a conserved set of factors and defined cis-acting elements (Boss and Jensen, 2003).

Immunoprecipitation and SDS-PAGE analysis of ovine class II molecules have revealed a non-covalently associated glycoprotein complex with a 30-32 kDa α-chain and a 24-26 kDa β-chain (Puri et al., 1985). A similar finding on the structure of class II molecules was reported by Hopkins et al. (1986). An interesting feature in both these studies, in contrast to that in humans, was that the sheep class II α- and β-chains could only be resolved under non-reducing conditions. Under reducing conditions, the β-polypeptide appeared to undergo a shift to a molecular mass of 30 kDa and thus co-migrated with the α-chain. Under non-reducing conditions, three bands, one corresponding to the α-chain, one to the β-chain and one to a probable invariant chain could be identified.

Another significant difference in the structure of the class II heterodimer of sheep, compared to mouse and human, is that it is unstable in the presence of 1% SDS at 20°C (Puri et al., 1987c). Under these conditions, 75% of the molecules were found to be dissociated into α- and β-chains and at a temperature of 100°C, almost all the molecules were found to be dissociated. The mouse and human class II molecules, on the other hand, are stable in SDS up to 38°C

(Shackelford et al., 1982). Also, the rate of biosynthesis of sheep class II molecules appeared to be similar to or slightly faster than that in humans (Puri et al., 1987a).

Studies have been undertaken to categorise the sheep MHC class II molecules (Puri et al., 1987a,b,d; Puri and Brandon, 1987). Sequential immunodepletion by a panel of monoclonal antibodies, followed by two-dimensional NEPHGE/SDS-PAGE analysis, revealed four structurally and serologically distinct subsets of class II molecules, similar to those found in humans. Also, these molecules exhibited structurally detectable allelic polymorphism. Three of the subsets displayed allelic polymorphism in β-polypeptides, while the fourth set showed allelic variation in both of their α- and β-polypeptides (Puri et al., 1987a). Approximately 10-12 different class II molecules were found to be expressed by a single sheep (Puri and Brandon, 1987). Subgroup-specific monoclonal antibodies against sheep MHC class II molecules, nine specific for the β-chain and four for the α-chain, have been developed (Dutia et al., 1990).

CLASS III GENES

Relative to other parts of the MHC, this region has the highest gene density, with the least number of pseudogenes (Kulski et al., 2002). However, some of the genes located in this region are not involved with the immune system. Class III genes with an obvious role in immunobiology include members of the complement cascade (C4A, C4B, C2, and Bf) and genes such as TNFα, LTA and LTB. C4, C2 and Bf are genes for complement proteins (Campbell et al., 1986). TNFα, LTA and LTB encode cachectin, lymphotoxin A and B molecules, respectively (Webb and Chaplin, 1990). Other genes of interest located in the region include HSP70, CYP21, G15, cytochrome p450, LST1, and 1C7. Of these, HSP70 is important as it encodes heat shock protein 70, which presents intracellular contents of cancer cells to the immune system and thus has a role in tumor rejection (Srivastava et al., 1998). The gene coding for HSP70 is duplicated and it has been shown recently that the loss of one of the duplicated genes in Holstein cattle is responsible for hereditary myopathy of diaphragmatic muscles (Sugimoto et al., 2003).

The class III region is poorly characterised in sheep. The existence of this region is based on circumstantial evidence derived from comparisons with related species, namely goats and cattle, and synteny between several loci (Schwaiger et al., 1996). The authors described a preliminary map of the *Ovar*-class III region. Cosmid clones containing C4 genes were isolated from a sheep genomic library by hybridisation with a bovine C4 cDNA probe. Additional cosmid clones containing the genes for 21-hydroxylase (CYP21), complement factor 2 (C2) and factor B (Bf) could also be obtained by a cosmid walking procedure employing respective human DNA probes. Relative positions of these loci were mapped within an approximate 150-kb DNA segment. Evidence could be obtained for duplication of C4 and CYP21 loci. Also, the order of CYP and C4 loci in sheep (CYP21B…C4…C4…CYP21) is quite different from that in humans, mouse and cattle (CYP21B…C4B…CYP21A…C4A). Furthermore, the two *Ovar*-C4 loci lie in tail-to-tail orientation. This evidence suggests the occurrence of a chromosomal inversion in this region of the sheep chromosome.

Complement cascade genes

The presence of the C4 gene in sheep was first indicated in RFLP studies employing

human C4 cDNA probe (Chardon et al., 1985). Neither any polymorphism nor linkage to MHC could be demonstrated. Subsequently, linkage between the C4 gene and OLA-SY1b antigen was established (Groth et al., 1987a). The presence of two polymorphic C4 loci has been indicated in a study on C3 and C4 concentrations in Merino and Suffolk sheep (Groth et al., 1987b). A rapid procedure for the isolation of complement factor, C4, from ovine plasma has been described, and two isotypes of C4 molecules, C4A and C4B, have been detected (Groth et al., 1988). The isotypes differed in the molecular mass of the α-chain (108 and 95 kDa, respectively). An RFLP of the C4 gene, employing Taq1 enzyme and the HLA-C4 probe, revealed linkage disequilibrium between C4 and DQB genes in unrelated sheep. Similar linkage of the C4 and DRB genes has also been reported (Wetherall et al., 1991). A C4*A2 phenotypic allele was found to be associated with a 19-kb DRB RFLP fragment in 18 of the 27 sheep studied.

In another study based on cloning and sequencing of DNA fragments obtained by PCR amplification of thioester and isotype determining sites of the sheep C4 genes, up to five distinct C4 gene loci were detected (Ren et al., 1993). The number of C4 genes per haplotype is thus similar to that in both humans and mice (Schwaiger et al., 1996). However, the sheep and cattle genes are believed to have evolved independent of those in primates and mice (Ren et al., 1993). Close to another complement factor gene, Bf, a polymorphic microsatellite locus, BfMS, has been detected (Groth and Wetherall, 1995). Eight alleles, differing in base-pair length, were detected at the locus in an Australian fine-wool Merino flock (Bot et al., 2004).

TNFα gene

TNFα is a cytokine with a wide range of effects on both lymphoid and non-lymphoid cell types. The existence of a single copy of the TNFα gene in sheep has been demonstrated (Nash et al., 1991). Ovine TNFα cDNAs were cloned and sequenced by three independent groups (Young et al., 1990; Green and Sargan, 1991; Nash et al., 1991). The sequences obtained in the first two studies were exactly the same, encoding for a 76-amino acid leader sequence and a 157-amino acid mature protein. The amino acid sequence was up to 88% homologous to the human TNFα protein. The cDNA sequence obtained in the third study was similar to that obtained in the first two studies, except that it lacked one amino acid in the leader sequence.

A recent study investigated allelic variation at the *Ovar*-TNFα locus (Alvarez-Busto et al., 2004). SSCP and sequence analysis of a 273-bp fragment, comprising part of the fourth exon and the 3' untranslated region of the gene, revealed three different alleles. These alleles differed in one deletion and one single nucleotide polymorphism. However, no difference was found in their frequencies in Latxa and Rasa breeds. An earlier attempt to detect polymorphism at this locus, employing RFLP with the use of human cDNA probes, was unsuccessful (Engwerda et al., 1996).

Other class III genes

There has been little research in the characterisation of genes other than the complement cascade and TNF genes of the *Ovar*-class III region. A dinucleotide microsatellite of the form (CA)n has been found to occur in at least one of the two cattle CYP21 genes (Moore et al., 1991). However, no such microsatellite could be detected in any of the *Ovar*-CYP21 genes

either by PCR using an oligonucleotide primer (Moore et al., 1991) or by Southern hybridisation (Schwaiger et al., 1996).

INHERITANCE AND POLYMORPHISM OF MHC GENES

A characteristic feature of the MHC antigens is their co-dominant expression, i.e., both the alleles at a given locus are expressed in a heterozygote individual. Also, the MHC is inherited *en bloc* as a haplotype with the exception of rare recombination (1-3% frequency). Hence, in the case of MHC genes, an association based on haplotypes is usually stronger and more meaningful than an allelic association (Dorak, 2005). Despite the enormous number of alleles at each expressed locus, the number of haplotypes observed in a population is much smaller than the theoretical expectations. This is because of certain alleles tending to occur together on the same haplotype rather than randomly segregating, a phenomenon referred to as linkage disequilibrium (Begovich et al., 1992).

Among the expressed loci in the human genome, the MHC shows the greatest degree of polymorphism (Dorak, 2005). The level of polymorphism is at such a degree that it is theoretically possible for each human to possess a different set of MHC alleles. Certain of the class I and class II loci that are involved in antigen presentation show extraordinarily high levels of polymorphism with several hundreds of allelic variants of the genes within the population (Klein, 1986). The genes at these loci are usually present as multiple copies, many of them being pseudogenes. The pseudogenes lack either one or more exons in them and even in the exons that exist, numerous mutations occur, rendering them non-functional. The presence of multiple copies is of evolutionary significance. Since it involves a birth and death process, new genes are created and some of them are maintained in the genome for a long time, while others are deleted or become non-functional through deleterious mutations (Klein et al., 1998). Class I loci undergo a faster rate of birth and death evolution than class II loci, and hence, it is difficult to establish the orthologous relationships of different class I genes among different orders of mammals (Hughes and Nei, 1989). On the other hand, the high longevity of class II genes enables such orthologous class II loci to be shared by different orders of mammals (Takahashi et al., 2000).

The mechanisms responsible for polymorphism in the MHC genes have been intensely debated and reviewed (Hughes and Yeager, 1998; Meyer and Thomson, 2001; Bernatchez and Landry, 2003). Parasite-mediated balancing selection and reproductive mechanisms constitute the two main types of mechanisms that operate to maintain the unusually high level of MHC polymorphism. Three different non-exclusive forms of balancing selection, symmetrical overdominance, negative frequency-dependent selection and fluctuation in selection pressure, are known to exist (Charbonnel and Pemberton, 2005). According to the hypothesis of heterozygote advantage or symmetrical overdominance (Doherty and Zinkernagel, 1975), an individual that is heterozygous, rather than homozygous, at the MHC loci has better immune surveillance against infectious organisms. The domains $\alpha1/\alpha2$ and $\alpha1/\beta1$ of class I and class II molecules, respectively, that form the peptide-binding groove in each case, constitute the driving force for heterozygote selection, in the presence of challenge from infectious agents. Several studies (Thursz et al., 1997; Carrington et al., 1999; Penn et al., 2002; Stear et al., 2005) have confirmed this selective advantage of MHC heterozygosity against infectious agents.

Under negative frequency-dependent selection or rare allele advantage (Clarke and Kirby, 1996), MHC genotypes with a rare allele are supposed to have a strong selective advan-

tage as few pathogens have been exposed and adapted to it. Conversely, the relative fitness of the common genotypes would be decreased. A study on the association between class II DRB alleles and resistance to gastro-intestinal parasitism in Soay sheep (Paterson et al., 1998) has provided evidence for rare allele advantage. The third form of balancing selection results from fluctuation in the selection pressure. Spatial and(or) temporal variation in the presence or density of pathogens could result in constant changes in the intensity of pathogen-mediated selection, thus maintaining polymorphism at the level of metapopulation (Hedrick, 2002). A recent study pertaining to a long-term genetic survey of Soay sheep supported this hypothesis (Charbonnel and Pemberton, 2005).

One early hypothesis explaining the high level of polymorphism within the MHC was the neutral theory of molecular evolution (Kimura, 1968). This theory suggested that the molecular mechanisms that result in polymorphism include point mutations, reciprocal recombination and gene conversion. However, the point mutation rate in MHC is by no means higher than elsewhere in the genome (Parham et al., 1995). However, accumulation of point mutations over millions of years as a result of the sharing of allelic lineages by related species, a fact referred to as trans-species polymorphism, brings about this extensive allelic polymorphism (Klein et al., 1993).

Other mechanisms that may bring about and maintain MHC gene diversity include MHC-based non-assortative mating preferences (Penn and Potts, 1999) and maternal-foetal incompatibility (Ober et al., 1998). However, these mechanisms together with the neutral theory have been discarded as the main cause of MHC polymorphism, as these processes should affect gene regions at random, rather than being concentrated in the PBRs (Jeffery and Bangham, 2000).

CONCLUSION

Several studies, over the past two and a half decades, have focused on the *Ovar-Mhc*. However, when compared to other domestic species, the ovine MHC is still poorly characterised. Several genes, across the three regions of the *Ovar-Mhc*, are yet to be characterised. Recent advances in large-scale cloning and large-scale sequencing have helped generate long genomic sequences, even complete MHC sequences, in several species (Kumanovics et al., 2003). The genomic sequences, in contrast to the cDNA sequences, provide the complete and ordered set of the MHC genes, including pseudogenes. The complete sequence of the HLA complex was available in late 1990s (MHC Sequencing Consortium, 1999) and it was evident that the class I and II regions extend well beyond the original boundaries (Stephens et al., 1999). Among the domesticated species, such large MHC genomic sequences have been reported for the B locus of the chicken (Kaufman et al., 1999), the class I region of the quail (Shiina et al., 1999), the class II region of the cat (Beck et al., 2001), and the class I region of the pig (Renard et al., 2001; Chardon et al., 2001). However, there are no reports of such sequences with regard to the *Ovar-Mhc*. Availability of the complete sequence of the *Ovar-Mhc* would enable the design of multiple markers that are more dense, equidistant and expansive throughout the region. This would facilitate the characterisation of individuals in terms of haplotypes rather than individual genes. MHC haplotypes are more meaningful, considering the existence of linkage disequilibrium among the MHC genes.

Most of the studies, aimed at characterizing the *Ovar-Mhc*, have focused on the class II region in general and on DR and DQ genes, in particular. The length polymorphisms of three

microsatellites (one each at the DRB1, DRB2 and DYA genes), and the exon 2 sequence variations at DRB1, DQA and DQB genes have been extensively studied in different breeds of sheep. In contrast, the class I region is poorly characterised. Controversy still exists with regard to the number of classical class I loci and there is no information on the non-classical class I genes. Studies pertaining to this region have focused mainly on the length polymorphism of a microsatellite located at one of the loci. Associations of the *Ovar-Mhc* genes with disease resistance have been reported in various studies (reviewed by Dukkipati et al., 2006). Several associations (especially of MHC antigens) with resistance/susceptibility to gastrointestinal nematodes have been revealed. However, those could not be utilised in screening sheep flocks for increased genetic resistance, owing to the complexity and labour intensiveness of MHC antigen serotyping methods. Hence, the development of accurate, simplified and cost-effective typing methods for various MHC loci enables more meaningful association studies to be carried out, followed by marker-assisted selection on a commercial basis.

ACKNOWLEDGMENTS

Financial support to the first author in the form of a Doctoral Scholarship from Massey University, New Zealand is gratefully acknowledged. The authors are also thankful to Annual Reviews, for permission to reprint the Figure 2 depicted in the review.

REFERENCES

Alvarez-Busto J, Ruiz-Nunez A, Mazon LI and Jugo BM (2004). Detecting of polymorphisms in tumour necrosis factor alpha candidate gene in sheep. *Eur. J. Immunogenet.* 31: 155-158.

Amills M, Francino O and Sanchez A (1996). A PCR-RFLP typing method for the caprine MHC class II DRB gene. *Vet. Immunol. Immunopathol.* 55: 255-260.

Amills M, Ramiya V, Norimine J and Lewin HA (1998). The major histocompatibility complex of ruminants. *Rev. Sci. Tech. - O.I.E.* (Off. Int. Epizoot.) 17: 108-120.

Andersson L (1988). Organisation of the bovine MHC class II region as revealed by genomic hybridizations. *Anim. Genet.* 19: 32-34.

Andersson L and Rask L (1988). Characterization of the MHC class II region in cattle: The number of DQ genes varies between haplotypes. *Immunogenetics* 27: 110-120.

Andersson L, Lunden A, Sigurdardottir S, Davies CJ, et al. (1988). Linkage relationships in the bovine MHC region. High recombination frequency between class II subregions. *Immunogenetics* 27: 273-280.

Ando A, Kawai J, Maeda M, Tsuji K, et al. (1989). Mapping and nucleotide sequence of a new HLA class II light chain gene, DQB3. *Immunogenetics* 30: 243-249.

Ballingall KT and McKeever DJ (2005). Conservation of promoter, coding and intronic regions of the non-classical MHC class II DYA gene suggests evolution under functional constraints. *Anim. Genet.* 36: 237-239.

Ballingall KT, Wright H, Redmond J, Dutia BM, et al. (1992). Expression and characterization of ovine major histocompatibility complex class II (OLA-DR) genes. *Anim. Genet.* 23: 347-359.

Ballingall KT, Luyai A and McKeever DJ (1997). Analysis of genetic diversity at the DQA loci in African cattle: evidence for a *Bota*-DQA3 locus. *Immunogenetics* 46: 237-244.

Beck TW, Menninger J, Voigt G, Newmann K, et al. (2001). Comparative feline genomics: a BAC/PAC contig map of the major histocompatibility complex class II region. *Genomics* 71: 282-295.

Begovich AB, McClure GR, Suraj VC, Helmuth RC, et al. (1992). Polymorphism, recombination, and linkage disequilibrium within the HLA class II region. *J. Immunol.* 148: 249-258.

Benacerraf B and McDevitt HO (1972). Histocompatibility-linked immune response genes. *Science* 175: 273-279.

Bernatchez L and Landry C (2003). MHC studies in nonmodel vertebrates: what have we learned about natural selection in 15 years? *J. Evol. Biol.* 16: 363-377.

Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, et al. (1987a). Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 329: 506-512.
Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, et al. (1987b). The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* 329: 512-518.
Blattman AN and Beh KJ (1992). Dinucleotide repeat polymorphism within the ovine major histocompatibility complex. *Anim. Genet*. 23: 392.
Blattman AN, Hulme DJ, Kinghorn BP, Woolaston RR, et al. (1993). A search for associations between major histocompatibility complex restriction fragment length polymorphism bands and resistance to *Haemonchus contortus* infection in sheep. *Anim. Genet*. 24: 277-282.
Bonner JJ (1986). Major histocompatibility complex influences reproductive efficiency: evolutionary implications. *J. Craniofac. Genet. Dev. Biol. Suppl*. 2: 5-11.
Boss JM and Jensen PE (2003). Transcriptional regulation of the MHC class II antigen presentation pathway. *Curr. Opin. Immunol*. 15: 105-111.
Bot J, Karlsson LJE, Greef J and Witt C (2004). Association of the MHC with production traits in Merino ewes. *Livest. Prod. Sci*. 86: 85-92.
Brown JH, Jardetzky TS, Gorga JC, Stern LJ, et al. (1993). Three-dimensional structure of the human class II histocompatibility antigen HLA-DR1. *Nature* 364: 33-39.
Buitkamp J and Epplen JT (1996). Major histocompatibility and T-cell receptor genes in artiodactyls: characterization, polymorphism and genetic resistance to a helminthic infection. *J. Anim. Breed. Genet*. 113: 287-291.
Buitkamp J, Filmether P, Stear MJ and Epplen JT (1996). Class I and class II major histocompatibility complex alleles are associated with faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. *Parasitol. Res*. 82: 693-696.
Campbell RD and Trowsdale J (1993). Map of the human MHC. *Immunol. Today* 14: 349-352.
Campbell RD, Carroll MC and Porter RR (1986). The molecular genetics of components of complement. *Adv. Immunol*. 38: 203-244.
Carrington M, Nelson GW, Martin MP, Kissner T, et al. (1999). HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. *Science* 283: 1748-1752.
Charbonnel N and Pemberton J (2005). A long-term genetic survey of an ungulate population reveals balancing selection acting on MHC through spatial and temporal fluctuations in selection. *Heredity* 95: 377-388.
Chardon P, Kirszenbaum M, Geffrotin C, Auffray C, et al. (1985). Analysis of the sheep MHC using HLA class I, II and C4 cDNA probes. *Immunogenetics* 22: 349-358.
Chardon P, Rogel-Gaillard C, Cattolico L, Duprat S, et al. (2001). Sequence of the swine major histocompatibility complex region containing all non-classical class I genes. *Tissue Antigens* 57: 55-65.
Charon KM, Moskwa B, Kuryl J, Gruszczynska J, et al. (2001). Relationship between polymorphism in locus OMHC1 (MHC class I) and resistance to nematodes in Polish Heatherhead sheep. *Anim. Sci. Pap. Rep*. 19: 285-292.
Charon KM, Moskwa B, Rutkowski R, Gruszczynska J, et al. (2002). Microsatellite polymorphism in DRB1 gene (MHC class II) and its relation to nematode faecal egg count in Polish Heath sheep. *J. Anim. Feed. Sci*. 11: 47-58.
Clarke B and Kirby DR (1996). Maintenance of histocompatibility polymorphisms. *Nature* 211: 999-1000.
Cullen PR, Bunch C, Brownlie J and Morris PJ (1982). Sheep lymphocyte antigens: a preliminary study. *Anim. Blood Groups Biochem. Genet*. 13: 149-159.
Deverson EV, Wright H, Watson S, Ballingall K, et al. (1991). Class II major histocompatibility complex genes of the sheep. *Anim. Genet.* 22: 211-225.
Doherty PC and Zinkernagel RM (1975). Enhanced immunological surveillance in mice heterozygous at the H-2 gene complex. *Nature* 256: 50-52.
Dongxiao S and Yuan Z (2004). Polymorphisms of the second exon of MHC-DRB gene in Chinese local sheep and goat. *Biochem. Genet*. 42: 385-390.
Dorak MT (2005). Major histocompatibility complex. http://dorakmt.tripod.com/mhc/mhc.html. Accessed November 1, 2005.
Dukkipati VSR, Blair HT, Garrick DJ and Murray A (2006). '*Ovar-Mhc*' - Ovine major histocompatibility complex: role in genetic resistance to diseases. *N. Z. Vet. J*. 54: 153-160.
Dutia BM, Hopkins J, Allington MP, Bujdoso R, et al. (1990). Characterization of monoclonal antibodies specific for alpha- and beta-chains of sheep MHC class II. *Immunology* 70: 27-32.
Dutia BM, McConnell I, Ballingall KT, Keating P, et al. (1994). Evidence for the expression of two distinct MHC class II DR beta like molecules in the sheep. *Anim. Genet*. 25: 235-241.

Ellegren H, Davies CJ and Andersson L (1993). Strong association between polymorphisms in an intronic microsatellite and in the coding sequence of the BoLA-DRB3 gene: implications for microsatellite stability and PCR-based DRB3 typing. *Anim. Genet.* 24: 269-275.

Engwerda CR, Dale CJ and Sandeman RM (1996). IgE, TNF alpha, IL1 beta, IL4 and IFN gamma gene polymorphisms in sheep selected for resistance to fleece rot and flystrike. *Int. J. Parasitol*. 26: 787-791.

Escayg AP, Montgomery GW, Hickford JG and Bullock DW (1993). A BgIII-RFLP at the ovine MHC class II DRA locus. *Anim. Genet.* 24: 217.

Escayg AP, Hickford JGH, Montgomery GW, Dodds KG, et al. (1996). Polymorphism at the ovine major histocompatibility complex class II loci. *Anim. Genet.* 27: 305-312.

Fabb SA, Maddox JF, Gogolin-Ewens KJ, Baker L, et al. (1993). Isolation, characterization and evolution of ovine major histocompatibility complex class II DRA and DQA genes. *Anim. Genet*. 24: 249-255.

Feichtlbauer-Huber P, Stear MJ, Fries R and Buitcamp J (2000). Reference-strand-mediated conformation analysis of MHC alleles: a new method for high-resolution typing of the *Ovar*-DQB genes. *Immunogenetics* 51: 65-68.

Garrido JJ, de Andres DF, Pintado CO, Llanes D, et al. (1995). Serologically defined lymphocyte alloantigens in Spanish sheep. *Exp. Clin. Immunogenet.* 12: 268-271.

Gelhaus A, Forster B, Wippern C and Horstmann RD (1999). Evidence for an additional cattle DQA locus, *Bota*-DQA5. *Immunogenetics* 49: 321-327.

Germain RN and Margulies DH (1993). The biochemistry and cell biology of antigen processing and presentation. *Annu. Rev. Immunol*. 11: 403-450.

Gogolin-Ewens KJ, Mackay CR, Mercer WR and Brandon MR (1985). Sheep lymphocyte antigens (OLA). I. Major histocompatibility complex class I molecules. *Immunology* 56: 717-723.

Gorer PA (1937). The genetic and antigenic basis of tumour transplantation. *J. Pathol. Bacteriol.* 44: 691-697.

Grain F, Nain MC, Labonne MP, Lantier F, et al. (1993). Restriction fragment length polymorphism of DQB and DRB class II genes of the ovine major histocompatibility complex. *Anim. Genet.* 24: 377-384.

Green IR and Sargan DR (1991). Sequence of the cDNA encoding ovine tumour necrosis factor-alpha: problems with cloning by inverse PCR. *Gene* 109: 203-210.

Griesinger I, Pruser F, Siemienski K and Geldermann H (1999). Extreme fragment lengths differences of the microsatellite in the expressed MHC-DRB gene of Merinoland sheep. *Anim. Genet.* 30: 77-78.

Groenen MA, van der Poel JJ, Dijkhof RJ and Giphart MJ (1990). The nucleotide sequence of bovine MHC class II DQB and DRB genes. *Immunogenetics* 31: 37-44.

Grossberger D, Hein W and Marcuz A (1990). Class I major histocompatibility complex cDNA clones from sheep thymus: alternative splicing could make a long cytoplasmic tail. *Immunogenetics* 32: 77-87.

Groth DM and Wetherall JD (1994). Dinucleotide repeat polymorphism within the ovine major histocompatibility complex class I region. *Anim. Genet*. 25: 61.

Groth DM and Wetherall JD (1995). Dinucleotide repeat polymorphism adjacent to sheep complement factor B. *Anim. Genet*. 26: 282-283.

Groth DM, Wetherall JD, Outteridge P and Stear MJ (1987a). Class I major histocompatibility antigens and C4 concentrations in sheep plasma. *Anim. Genet*. 18: 187-191.

Groth DM, Wetherall JD, Outteridge PM, Windon RG, et al. (1987b). Analysis of C3 and C4 in ovine plasma. *Complement* 4: 12-20.

Groth DM, Wetherall JD, Taylor L, Sparrow PR, et al. (1988). Purification and characterisation of ovine C4: evidence for two molecular forms in ovine plasma. *Mol. Immunol*. 25: 577-584.

Gruen JR and Weissman SM (1997). Evolving views of the major histocompatibility complex. *Blood* 90: 4252-4265.

Gruszczynska J (1999). Polymorphism of the OLA-DRB1 (MHC class II) gene in German Merino sheep. *Ann. Warsaw Agricult. Univ. - SGGW, Anim. Sci*. 35: 125-132.

Gruszczynska J, Charon K and Szdlowski M (2000). Effect of foeto-maternal immunization in sheep on birth weight and live weight gain of lambs. *Anim. Sci. Pap. Rep.* 18: 65-76.

Gruszczynska J, Charon KM, Swiderek W and Sawera M (2002a). Microsatellite polymorphism in locus OMHC1 (MHC class I) in Polish Heath Sheep and Polish Lowland Sheep (Zelazna variety). *J. Appl. Genet.* 43: 217-222.

Gruszczynska J, Swiderek W, Charon KM, Kuryl Jolanta Cieslak D, et al. (2002b). Microsatellite polymorphism of pseudo-gene OLA-DRB2 in intron 5 in two Polish sheep breeds. *Anim. Sci. Pap. Rep.* 20: 67-72.

Gruszczynska J, Brokowska K, Charon KM and Swiderek WP (2005). Restriction fragment length polymor-

phism of exon 2 Ovar-DRB1 gene in Polish Heath Sheep and Polish Lowland Sheep. *J. Appl. Genet.* 46: 311-314.
Hediger R, Ansari HA and Stranzinger GF (1991). Chromosome banding and gene localizations support extensive conservation of chromosome structure between cattle and sheep. *Cytogenet. Cell Genet.* 57: 127-134.
Hedrick PW (2002). Pathogen resistance and genetic variation at MHC loci. *Evol. Int. J. Org. Evol.* 56: 1902-1908.
Hein WR (1997). Molecular genetics of immune molecules. In: The Genetics of sheep (Piper L and Ruvinsky A, eds.). CAB International, New York, NY, USA, 183-197.
Hickford JG, Ridgway HJ and Escayg AP (2000). Evolution of the ovine MHC DQA region. *Anim. Genet.* 31: 200-205.
Hickford JG, Zhou H, Slow S and Fang Q (2004). Diversity of the ovine DQA2 gene. *J. Anim. Sci.* 82: 1553-1563.
Hopkins J and Dutia BM (1990). Monoclonal antibodies to the sheep analogues of human CD45 (leucocyte common antigen), MHC class I and CD5. Differential expression after lymphocyte activation *in vivo*. *Vet. Immunol. Immunopathol.* 24: 331-346.
Hopkins J, Dutia BM and McConnell I (1986). Monoclonal antibodies to sheep lymphocytes: I. Identification of MHC class II molecules on lymphoid tissue and changes in the level of class II expression on lymph-borne cells following antigen stimulation *in vivo*. *Immunology* 59: 433-438.
Hughes AL and Nei M (1989). Evolution of the major histocompatibility complex: independent origin of nonclassical class I genes in different groups of mammals. *Mol. Biol. Evol.* 6: 559-579.
Hughes A and Yeager M (1998). Natural selection at major histocompatibility complex loci of vertebrates. *Annu. Rev. Genet.* 32: 415-435.
JanBen M, Weisman C, Gauly M and Erhardt (2002). Associations between infections with *Haemonchus contortus* and genetic markers on ovine chromosome 20. Paper presented at the 7th World Congress on Genetics Applied to Livestock Production, August 19-23, 2002, Montpellier, France.
Jeffery KJ and Bangham CR (2000). Do infectious diseases drive MHC diversity? *Microbes Infect.* 2: 1335-1341.
Joosten I, Oliver RA, Spooner RL, Williams JL, et al. (1988). Characterization of class I bovine lymphocyte antigens (BoLA) by one-dimensional isoelectricfocusing. *Anim. Genet.* 19: 103-113.
Jugo BM and Vicario A (2000). Single-strand conformational polymorphism and sequence polymorphism of Mhc-DRB in Laxta and Karrantzar sheep: implications for caprinae phylogeny. *Immunogenetics* 51: 887-897.
Jugo BM and Vicario A (2001). Lymphocyte antigens in sheep: linkage to the MHC class II DRB1 gene. *Eur. J. Immunogenet.* 28: 451-458.
Jugo BM, Joosten I, Grosfeld-Stulemeyer M, Amorena B, et al. (2002). Immunoprecipitation and isoelectric focusing of sheep MHC class I antigens reveal higher complexity than serology. *Eur. J. Immunogenet.* 29: 391-399.
Karlsson L, Surh CD, Sprent J and Peterson PA (1991). A novel class II MHC molecule with unusual tissue distribution. *Nature* 351: 485-488.
Kaufman J, Salomonsen J and Flajnik M (1994). Evolutionary conservation of MHC class I and class II molecules - different yet the same. *Semin. Immunol.* 6: 411-424.
Kaufman J, Milne S, Gobel TW, Walker BA, et al. (1999). The chicken B locus is a minimal essential major histocompatibility complex. *Nature* 401: 923-925.
Kelley J, Walter L and Trowsdale J (2005). Comparative genomics of major histocompatibility complexes. *Immunogenetics* 56: 683-695.
Kimura M (1968). Genetic variability maintained in a finite population due to mutational production of neutral and nearly neutral isoalleles. *Genet. Res.* 11: 247-269.
Klein J (1976). Evolution and function of the major histocompatibility complex: facts and speculations. In: The major histocompatibility system in man and animals (Gotze D, ed.). Springer-Verlag New York Inc., New York, NY, USA, 339-378.
Klein J (1986). Natural history of the major histocompatibility complex. John Wiley and Sons Inc., New York, NY, USA.
Klein J, Bontrop RE, Dawkins RL, Erlich HA, et al. (1990). Nomenclature for the major histocompatibility complexes of different species: a proposal. *Immunogenetics* 31: 217-219.
Klein J, Satta Y, O'hUigin C and Takahata N (1993). The molecular descent of the major histocompatibility complex. *Annu. Rev. Immunol.* 11: 269-295.
Klein J, Sato A and O'hUigin C (1998). Evolution by gene duplication in the major histocompatibility

complex. *Cytogenet. Cell Genet*. 80: 123-127.
Konnai S, Nagaoka Y, Takesima S, Onuma M, et al. (2003a). Sequences and diversity of 17 new Ovar-DRB1 alleles from three breeds of sheep. *Eur. J. Immunogenet.* 30: 275-282.
Konnai S, Nagaoka Y, Takesima S, Onuma M, et al. (2003b). Technical note: DNA typing for ovine MHC DRB1 using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *J. Dairy Sci*. 86: 3362-3365.
Kostia S, Kantanen J, Kolkkala M and Varvio SL (1998). Applicability of SSCP analysis for MHC genotyping: fingerprinting of *Ovar*-DRB1 exon 2 alleles from Finnish and Russian breeds. *Anim. Genet.* 29: 453-455.
Kulski JK, Shiina T, Anzai T, Kohara S, et al. (2002). Comparative genome analysis of the MHC: the evolution of class I duplication blocks, diversity and complexity from shark to man. *Immunol. Rev*. 190: 95-122.
Kumanovics A, Takada T and Lindahl KF (2003). Genomic organization of the mammalian MHC. *Annu. Rev. Immunol*. 21: 629-657.
Lee N, Llano M, Carretero M, Ishitani A, et al. (1998). HLA-E is a major ligand for the natural killer inhibitory receptor CD94/NKG2A. *Proc. Natl. Acad. Sci. USA* 95: 5199-5204.
Liu YF, Yan GQ and Wang XF (2004). Polymorphism analysis of MHC-DRB3 gene in Dolang sheep with PCR-RFLP. *Yi Chuan* 26: 59-62.
Maddox JF (1999). Single strand conformational polymorphisms in the second exons of the ovine DMB, DYA and DYB genes. *Anim. Genet*. 30: 382-383.
Mahdy EA, Makinen A, Chowdhary BP, Andersson L, et al. (1989). Chromosomal localization of the ovine major histocompatibility complex (OLA) by *in situ* hybridization. *Hereditas* 111: 87-90.
McCririe L, Bairden K, Britton C, Buitkamp J, et al. (1997). Heterogeneity in the recognition of *Ostertagia circumcincta* antigens by serum antibody from mature, infected sheep. *Parasite Immunol*. 19: 235-242.
Meyer D and Thomson G (2001). How selection shapes variation of the human major histocompatibility complex: a review. *Ann. Hum. Genet*. 65: 1-26.
MHC Sequencing Consortium (1999). Complete sequence and gene map of a human major histocompatibility complex. *Nature* 401: 921-923.
Millot P (1978). The major histocompatibility complex of sheep (OLA) and two minor loci. *Anim. Blood Groups Biochem. Genet*. 9: 115-121.
Millot P (1984). The OLA major histocompatibility complex of sheep. Study of six new factors and evidence of a third locus of the complex: OLA-C. *Exp. Clin. Immunogenet.* 1: 31-42.
Miltiadou D, Ballingall KT, Ellis SA, Russel GC, et al. (2005). Haplotype characterization of transcribed ovine major histocompatibility complex (MHC) class I genes. *Immunogenetics* 57: 499-509.
Moore SS, Sargeant LL, King TJ, Mattick JS, et al. (1991). The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. *Genomics* 10: 654-660.
Nash AD, Barcham GJ, Brandon MR and Andrews AE (1991). Molecular cloning, expression and characterization of ovine TNF alpha. *Immunol. Cell Biol.* 69: 273-283.
Ober C, Hyslop T, Elias S, Weitkamp LR, et al. (1998). Human leukocyte antigen matching and fetal loss: results of a 10 year prospective study. *Hum. Reprod.* 13: 33-38.
Outteridge PM, Andersson L, Douch PGC, Green RS, et al. (1996). The PCR typing of MHC-DRB genes in the sheep using primers for an intronic microsatellite: application to nematode parasite resistance. *Immunol. Cell Biol*. 74: 330-336.
Parham P, Adams EJ and Arnett KL (1995). The origins of HLA - A, B, C polymorphism. *Immunol. Rev*. 143: 141-180.
Paterson S (1998). Evidence for balancing selection at the major histocompatibility complex in a free-living ruminant. *J. Hered*. 89: 289-294.
Paterson S, Wilson K and Pemberton JM (1998). Major histocompatibility complex variation associated with juvenile survival and parasite resistance in a large unmanaged ungulate population. *Proc. Natl. Acad. Sci. USA* 95: 3714-3719.
Penn DJ and Potts WK (1999). The evolution of mating preferences and major histocompatibility complex genes. *Am. Nat*. 153: 145-164.
Penn DJ, Damjanovich K and Potts WK (2002). MHC heterozygosity confers a selective advantage against multiple-strain infections. *Proc. Natl. Acad. Sci. USA* 99: 11260-11264.
Peters PJ, Neefjes JJ, Oorschot V, Ploegh HL, et al. (1991). Segregation of MHC class II molecules from MHC class I molecules in the Golgi complex for transport to lysosomal compartments. *Nature* 349:

669-676.
Puri NK and Brandon MR (1987). Sheep MHC class II molecules: II. Identification and characterization of four distinct subsets of sheep MHC class II molecules. *Immunology* 62: 575-580.
Puri NK, Mackay CR and Brandon MR (1985). Sheep lymphocyte antigens (OLA). II. Major histocompatibility complex class II molecules. *Immunology* 56: 725-733.
Puri NK, Gogolin-Ewens KJ and Brandon MR (1987a). Monoclonal antibodies to sheep MHC class I and II molecules: biochemical characterization of three class I gene products and four distinct subpopulations of class II molecules. *Vet. Immunol. Immunopathol.* 15: 59-86.
Puri NK, de Kretser T and Brandon MR (1987b). Monoclonal antibodies to sheep MHC class II molecules recognize all HLA-D or subsets of HLA-D region products. *Hum. Immunol.* 20: 195-207.
Puri NK, Gorrell MD and Brandon MR (1987c). Sheep MHC class II molecules: I. Immunochemical characterization. *Immunology* 62: 567-573.
Puri NK, Scott SC, Choi CL and Brandon MR (1987d). Biochemical and molecular analysis of sheep MHC class II molecules. *Vet. Immunol. Immunopathol.* 17: 231-241.
Rammensee HG, Friede T and Stevanoviic S (1995). MHC ligands and peptide motifs: first listing. *Immunogenetics* 41: 178-228.
Rasool TJ, Dechamma HJ and Sreekumar E (2000). A common method for MHC typing of farm animals using PCR-RFLP. *Indian J. Anim. Sci.* 70: 716-717.
Ren XD, Dodds AW and Law SK (1993). The thioester and isotypic sites of complement component C4 in sheep and cattle. *Immunogenetics* 37: 120-128.
Renard C, Vaiman M, Chiannilkulchai N, Cattolico L, et al. (2001). Sequence of the pig major histocompatibility region containing the classical class I genes. *Immunogenetics* 53: 490-500.
Reyburn H, Mandelboim O, Vales-Gomez M, Sheu EG, et al. (1997). Human NK cells: their ligands, receptors and functions. *Immunol. Rev.* 155: 119-125.
Rhodes D and Trowsdale J (1998). Genetics and molecular genetics of the MHC. http://www-immuno.path.cam.ac.uk/~immuno/mhc/mhc.html. Accessed October 14, 2005.
Rivett AJ (1993). Proteasomes: multicatalytic proteinase complexes. *Biochem. J.* 291: 1-10.
Rothschild MF, Skow L and Lamont SJ (2000). The major histocompatibility complex and its role in disease resistance and immune responsiveness. In: Breeding for disease resistance in farm animals (Axford RFE, Bishop SC, Nicholas FW and Owen JB, eds.). CABI Publishing, New York, NY, USA, 73-105.
Saberivand A, Sackey D, Hohenlraus MA and Outteridge PM (1998). Microsatellite genotyping of MHC-DRB genes in sheep and goats. Paper presented at the 6th World Congress on Genetics Applied to Livestock Production, January 11-16, Armidale, NSW, Australia.
Sayers G, Good B, Hanrahan JP, Ryan M, et al. (2005). Major histocompatibility complex DRB1 gene: its role in nematode resistance in Suffolk and Texel sheep breeds. *Parasitology* 131: 403-409.
Schwaiger FW and Epplen JT (1995). Exonic MHC-DRB polymorphisms and intronic simple repeat sequences: Janus' faces of DNA sequence evolution. *Immunol. Rev.* 143: 199-224.
Schwaiger FW, Buitkamp J, Weyers E and Epplen JT (1993a). Typing of artiodactyl MHC-DRB genes with the help of intronic simple repeated DNA sequences. *Mol. Ecol.* 2: 55-59.
Schwaiger FW, Weyers E, Epplen C, Brun J, et al. (1993b). The paradox of MHC-DRB exon/intron evolution: alpha-helix and beta-sheet encoding regions diverge while hypervariable intronic simple repeats coevolve with beta-sheet codons. *J. Mol. Evol.* 37: 260-272.
Schwaiger FW, Weyers E, Buitkamp J, Ede AJ, et al. (1994). Interdependent MHC-DRB exon-plus-intron evolution in artiodactyls. *Mol. Biol. Evol.* 11: 239-249.
Schwaiger FW, Gostomski D, Stear MJ, Duncan JL, et al. (1995). An ovine major histocompatibility complex DRB1 allele is associated with low faecal egg counts following natural, predominantly *Ostertagia circumcincta* infection. *Int. J. Parasitol.* 25: 815-822.
Schwaiger FW, Maddox J, Ballingall K, Buitkamp J, et al. (1996). The ovine major histocompatibilty complex. In: The major histocompatibility complex region of domestic animal species (Schook LB and Lamont SJ, eds.). CRC Press, Inc., Boca Raton, FL, USA, 121-176.
Scott PC, Choi CL and Brandon MR (1987). Genetic organization of the ovine MHC class II region. *Immunogenetics* 25: 116-122.
Scott PC, Gogolin-Ewens KJ, Adams TE and Brandon MR (1991a). Nucleotide sequence, polymorphism, and evolution of ovine MHC class II DQA genes. *Immunogenetics* 34: 69-79.
Scott PC, Maddox JF, Gogolin-Ewens KJ and Brandon MR (1991b). The nucleotide sequence and evolution of ovine MHC class II B genes: DQB and DRB. *Immunogenetics* 34: 80-87.
Shackelford DA, Kaufman JF, Korman AJ and Strominger JL (1982). HLA-DR antigens: structure, separation of subpopulations, gene cloning and function. *Immunol. Rev.* 66: 133-187.

Shiina T, Shimizu C, Oka A, Teraoka Y, et al. (1999). Gene organization of the quail major histocompatibility complex (*MhcCoja*) class I gene region. *Immunogenetics* 49: 384-394.
Snibson KJ, Maddox JF, Fabb SA and Brandon MR (1998). Allelic variation of ovine MHC class II DQA1 and DQA2 genes. *Anim. Genet.* 29: 356-362.
Srivastava PK, Menoret A, Basu S, Binder RJ, et al. (1998). Heat-shock proteins come of age: primitive functions acquire new roles in an adaptive world. *Immunity* 8: 657-665.
Stear MJ and Spooner RL (1981). Lymphocyte antigens in sheep. *Anim. Blood Groups Biochem. Genet.* 12: 272-276.
Stear MJ, Bairden K, Bishop SC, Buitkamp J, et al. (1996). An ovine lymphocyte antigen is associated with reduced faecal egg counts in four-month-old lambs following natural, predominantly *Ostertagia circumcincta* infection. *Int. J. Parasitol.* 26: 423-428.
Stear MJ, Innocent GT and Buitkamp J (2005). The evolution and maintenance of polymorphism in the major histocompatibility complex. *Vet. Immunol. Immunopathol.* 108: 53-57.
Stephens R, Horton R, Humphray S, Rowen L, et al. (1999). Gene organisation, sequence variation and isochore structure at the centromeric boundary of the human MHC. *J. Mol. Biol.* 291: 789-799.
Stone RT and Muggli-Cockett NE (1990). Partial nucleotide sequence of a novel bovine major histocompatibility complex class II beta-chain gene, BoLA-DIB. *Anim. Genet.* 21: 353-360.
Sugimoto M, Furuoka H and Sugimoto Y (2003). Deletion of one of the duplicated *Hsp70* genes causes hereditary myopathy of diaphragmatic muscles in Holstein-Friesian cattle. *Anim. Genet.* 34: 191-197.
Sun DX, Zhang Y and Li N (2003). Polymorphisms of exon 2 of MHC-DRB3 gene in Mongolian and Kazakh sheep. *Yi Chuan Xue Bao* (*Acta Genet. Sin.*) 30: 761-765.
Swarbrick PA, Schwaiger FW, Epplen JT, Buchan GS, et al. (1995). Cloning and sequencing of expressed DRB genes of the red deer (*Cervus elaphus*) Mhc. *Immunogenetics* 42: 1-9.
Takahashi K, Rooney AP and Nei M (2000). Origins and divergence times of mammalian class II MHC gene clusters. *J. Hered.* 91: 198-204.
Thursz MR, Thomas HC, Greenwood BM and Hill AV (1997). Heterozygote advantage for HLA class-II type in hepatitis B virus infection. *Nat. Genet.* 17: 11-12.
Tizard IR (2004). Acquired immunity: antigen-presenting receptors. In: Veterinary immunology: an introduction. Elsevier, Philadelphia, PA, USA, 67-77.
Tkacikova L, Bhide MR and Mikula I (2005). Asymmetric PCR-SSCP: a useful tool for detection OLA-DRB1 (MHC class II) gene polymorphism in Slovak improved Valachian sheep. *Acta Vet. Brno* 74: 275-278.
Tonnelle C, Demars R and Long EO (1985). DOB: a new B chain gene with a distinct regulation of expression. *Embo J.* 4: 2839-2847.
van der Poel JJ, Groenen MA, Dijkhof RJ, Ruyter D, et al. (1990). The nucleotide sequence of the bovine MHC class II alpha genes: DRA, DQA and DYA. *Immunogenetics* 31: 29-36.
van Eijk MJ, Beever JE, Da Y, Stewart JA, et al. (1995). Genetic mapping of BoLA-A, CYP21, DRB3, DYA and PRL on BTA23. *Mamm. Genome* 6: 151-152.
van Haeringen WA, Gwakisa PS, Mikko S, Eythorsdottir E, et al. (1999). Heterozygosity excess at the cattle DRB locus revealed by large scale genotyping of two closely linked microsatellites. *Anim. Genet.* 30: 169-176.
van Oorschot RA, Maddox JF, Adams LJ and Fabb SA (1994). Characterization and evolution of ovine MHC class II DQB sequence polymorphism. *Anim. Genet.* 25: 417-424.
Webb GC and Chaplin DD (1990). Genetic variability at the human tumor necrosis factor loci. *J. Immunol.* 145: 1278-1285.
Wetherall JD, Groth DM and Karlsson LJ (1991). DNA markers and parasite resistance in sheep: complement and major histocompatibility complex associations. In: Breeding for disease resistance in sheep (Gray GD and Woolaston RR, eds.). Wool Research and Development Corporation, Melbourne, Victoria, Australia, 109-114.
Wright H and Ballingall KT (1994). Mapping and characterization of the DQ subregion of the ovine MHC. *Anim. Genet.* 25: 243-249.
Wright H, Ballingall KT and Redmond J (1994). The DY subregion of the sheep MHC contains an A/B gene pair. *Immunogenetics* 40: 230-234.
Wright H, Redmond J, Wright F and Ballingall KT (1995). The nucleotide sequence of the sheep MHC class II DNA gene. *Immunogenetics* 41: 131-133.
Wright H, Redmond J and Ballingall KT (1996). The sheep orthologue of the HLA-DOB gene. *Immunogenetics* 43: 76-79.
Young AJ, Hay JB and Chan JY (1990). Primary structure of ovine tumor necrosis factor alpha cDNA.

Nucleic Acids Res. 18: 6723.
Zavazava N and Eggert F (1997). MHC and behavior. *Immunol*. *Today* 18: 8-10.
Zhou H and Hickford JG (2001) Rapid communication: three new allelic sequences at the ovine MHC class II DQA1 locus. *J*. *Anim. Sci*. 79: 779-780.
Zhou H and Hickford JG (2004). Allelic polymorphism in the ovine DQA1 gene. *J*. *Anim*. *Sci*. 82: 8-16.
Zinkernagel RM and Doherty PC (1974). Immunological surveillance against altered self components by sensitised T lymphocytes in lymphocytic choriomeningitis. *Nature* 251: 547-548.