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Original Article

Polymorphism and disease resistance possessions of MHC Class II BoLA genes

R.K. Vandre[1*], A.K. Sharma[2], G.R. Gowane[3], Ravi Rajoriya[1], Shweta Rajoriya[1], Rakesh k. Sinha[4], Amod Kumar[5], Madhu Shivhare[1], D. D. Caser[1] and S. K. Meshram[1]

[1*]College of Veterinary science and Animal husbandry, Rewa (MP), India
[2]Temperate Animal Husbandry Division, Indian Veterinary Research Institute, Mukteshwar(UK), India
[3]Central Sheep and Wool Research Institute, Avikanagar, via-Jaipur (Rajasthan), India
[4]National Dairy Research Institute, Karnal (HR), India
[5]Indian Veterinary Research Institute, Bareilly (UP), India
Corresponding author: R.K. Vandre

Abstract

Polymorphism in the major histocompatibility antigen complex of the bovine, which is referred to as bovine leukocyte antigens (BoLAs), has been intensively investigated for identifying marker(s) for bovine diseases and immunological traits. This approach requires identification and documentation of the allelic diversity of BoLA among different animals across the globe. Major histocompatibility complex (MHC) molecules play a major role in immunological defence against pathogens. The MHC and their genes can be divided into three major classes: class I, class II and class III. PBC (Peptide binding cleft) is formed in between α1 and β1 domains with a beta pleated floor. The greatest polymorphic variability in the amino acids is in those facing the groove. This is a large region, 99% of the time these closely linked genes are transmitted to the next generation as a unit of MHC alleles. Genetic polymorphism of MHC genes in livestock have been generated, however, their association with disease and application to breeding programs will helpful for improve livestock.

Keywords: Major histocompatibility complex, Peptide binding cleft, BoLA, DQA, DRB

Introduction

The MHC and their genes can be divided into three major classes: class I, class II and class III. Class I MHC genes encode glycoprotein's expressed on the surface of nearly all nucleated cells; the major function of the class I gene products is presentation of peptide antigens to cytotoxic T cells. Class III MHC genes encode, in addition to other, various secreted proteins that have immune functions, including components of the complement system and molecules involved in inflammation. The Class II region of the MHC encodes both α and β chains that form the Class II heterodimers. There is a high degree of polymorphism within the Class II genes. The α and β chains are integral membrane glycoprotein's which are not ubiquitously expressed, unlike MHC Class I molecules, but are displayed

on the surface of antigen presenting cells (APCs), including macrophages, dendritic cells (DC) and B cells (Cresswell, 1994). In addition, MHC Class II genes are expressed on other cells in response to IFN-γ (Steimle *et al.,* 1994). The α1 and β1 subunit forms the polymorphic Peptide binding cleft (PBC). PBC is formed in between α1 and β1 domains with a beta pleated floor. The greatest polymorphic variability in the amino acids is in those facing the groove. This in turn determines the chemical structure of the groove and influences the specificity and affinity of peptide binding. Peptides associated with class II MHC are 13-25 amino acids long. The ends of peptide binding clefts are open so that peptides of 30 residues or more also can fit. Anchor sites for one or more amino acids also exist in the groove of the class II MHC molecule. α2 and β2 are largely non-polymorphic. During antigen presentation, CD4+ molecule of Th lymphocyte binds to β2 domain of the class II MHC molecules. The MHC class II genes are associated with resistance to the diseases and are extremely polymorphic in most vertebrates (Trowsdale, 1995). These genes have attracted much attention in farm animals due to the need of improved methods of disease control through the design of novel vaccines and selection of disease resistant animals.

Polymorphism of MHC genes

The MHC in cattle is known as the bovine leukocyte antigen (BoLA). A major rearrangement within the class II region has led to the division of the BoLA region into two distinct sub-regions, such as class IIa and class IIb, on chromosome 23. The class IIa sub-region contains the functionally expressed DR and DQ genes. These gene products, the DR and DQ molecules, represent the main class II restriction elements for CD4 T-helper cells (Aida, 1995; Glass *et al.,* 2000). Cattle have one DRA gene, three DRB genes (only one, DRB3, is thought to be functionally important) and several DQA and DQB genes depending on the haplotype (Takeshima and Aida, 2006). The BoLA-DRB3 gene is the most polymorphic class II locus in cattle and influences both the magnitude and epitope specificity of antigen-specific T cell responses to infectious diseases. The DQ region comprises five DQA loci and five DQB loci, with exon 2 of the DQA1, DQA2, DQA3, DQB1, and DQB2 genes being highly polymorphic (Takeshima and Aida, 2006). Till now, 51 *DQA* including 25 *DQA1* and 74 *DQB* alleles have been reported (http://www.ebi.ac.uk/ipd/mhc/bola/). DQ region has a layer of complexity unique to cattle, as in approximately half of the known haplotypes the *DQ* genes are duplicated (Andersson *et al.,* 1988; Ballingall, *et al.,* 1997). The main importance of DQ molecule is for priming CD4+T lymphocyte responses. Polymorphism of DQA and DQB is related with the duplication of the DQ genes. This has potential to markedly increase the variation at the cell surface because of inter and intra haplotype pairing of DQ α and β chains (Glass *et al.,* 2000). Duplicated DQ haplotypes increase the complexity of restriction element usage in cattle. Thus, cattle are likely to express

several class II gene products, all of which could make positive or negative contributions to the immune response to particular antigens. DQ molecules in cattle are relevant for peptide presentation to Th cells in immune responses against Peptides derived from FMDV (Glass *et al.*, 2000), *Babesia bovis* (Norimine *et al.*, 2004) and *Anaplasma marginale* (Brown *et al.*, 2002). DQA gene has been employed for studying the polymorphism in cattle (Davies *et al.* 1997; Ballingall *et al.* 1997; Ballingall *et al.* 1998; Donald *et al.* 2005; Takeshima *et al.*2007; Takeshima *et al.*2008; Miyasaka *et al.* 2011) and water buffaloes (*Bubalis bubalis)* (Niranjan *et al.,* 2009 and 2010).

The polymorphism of DQA gene can be determined by the several methods, such as Restriction fragment length polymorphism (RFLP), Polymerase chain reaction-Restricted fragment length polymorphism (PCR-RFLP), Polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP), Sequencing of genomic DNA, cDNAs, or cloned PCR products, Microarray-based typing method and Sequence based typing (SBT). There are multiple alleles, or forms, of each MHC gene. These alleles are expressed as proteins on the surface of various cells in a co-dominant manner. This diversity is important in maintaining an effective system of specific immunity. Altogether, the MHC genes span a region that is four million base pairs in length. Although this is a large region, 99% of the time these closely linked genes are transmitted to the next generation as a unit of MHC alleles. This unit is called a haplotype.

Structure of MHC molecule with immunogenic property:

In the MHC class II molecule following component are present:

Genes and Molecules

The Class II region of the MHC encodes both α and β chains that form the Class II heterodimers. In humans, the classical Class II gene pairs are DRA and DRB, DQA and DQB, DPA and DPB. There is a high degree of polymorphism within the Class II genes.

The α and β chains are integral membrane glycoprotein's which are not ubiquitously expressed, unlike MHC Class I molecules, but are displayed on the surface of antigen presenting cells (APCs), including macrophages, dendritic cells (DC) and B cells (Cresswell, 1994). In addition, MHC Class II genes are expressed on other cells in response to IFN-γ (Steimle *et al.,* 1994). The α1 and β1 subunits form the polymorphic PBC.

Antigen processing with the help of Peptide binding cleft of Class II molecules

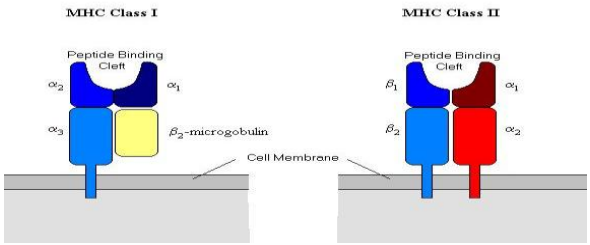
The function of the Class II molecules is to present bound peptide to CD4+ T cells. The peptides which are bound to the Class II heterodimer are primarily derived from internalized proteins. The proteins/pathogens are internalized through the process of phagocytises by macrophages, DCs and by Fc-mediated uptake by B cells. Once foreign proteins have been internalized they are degraded

through the action of enzymes in vesicles called endosomes (Pieters, 1997). Antigen presenting cells (APCs) not only present antigens to T lymphocytes, they are also the targets of T cell effectors functions. B cells and macrophage are principal cell types that present antigen to $CD4^+$ T cells. Macrophages that have engulfed microbes but unable to clear it presents microbial peptide antigen with MHC II proteins to antigen-specific $CD4^+$ T cells. The T cell in turn activates the macrophage and helps to eliminate the microbes. Similarly, B cells present endocytosed antigen with MHC II proteins to CD4+ T cells. The CD4+ T cells then stimulate the B cells finally resulting in production of antibodies against the foreign antigen.

The structure of the Class II molecule is similar to the Class I molecule, with 2 α helices and a β pleated sheet which form the functional PBC. These molecules form a complex with exogenous derived peptides of between 12-19 residues in length, although peptides of up to 30 amino acids have been reported (Engelhard, 1994). The PBC has a number of 'pockets' which bind the amino acid side chains of the peptide, these pockets have been numbered.

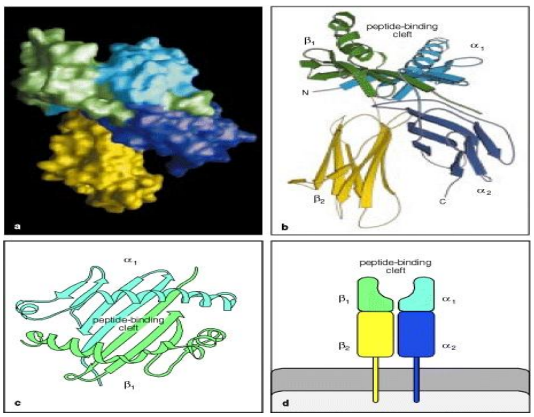
A peptide binding groove is formed in between α1 and β1 domains with a beta pleated floor (Fig 1 and Fig 2). As in the case for class I MHC, the greatest polymorphic variability in the amino acids is in those facing the groove. This in turn determines the chemical structure of the groove and influences the specificity and affinity of peptide binding. Peptides associated with class II MHC are 13-25 amino acids long. The ends of peptide binding clefts are open so that peptides of 30 residues or more also can fit. As with class I MHC, anchor sites for one or more amino acids also exist in the groove of the class II MHC molecule. α2 and β2 are largely non-polymorphic. During antigen presentation, CD4 molecule of Helper T lymphocyte binds to β2 domain of the class II MHC molecules.

Fig.1 Peptide binding cleft in MHC Class I and Class II molecules



Source:http://doctor jones.co.uk/Immunology/Tutorial/The%20Major%20Histocompatibility%20Complex.htm

Fig.2 Details of peptide binding cleft in MHC class II molecule



MHC class 2 molecule. (a) Crystallographic image, (b, c, d) molecule showing PB

Source: *Janeway et al. 2001, Immunobiology (Antigen recognition by T cells)*

MHC class II molecule

The MHC Class II molecules are found only on a few specialized cell types, including macrophages, dendritic cells and B cells, all of which are professional antigen-presenting cells (APCs). The peptides presented by class II molecules are derived from extracellular proteins. Hence, the MHC class II-dependent pathway of antigen presentation is called the endocytic or exogenous pathway. Loading of class II molecules must still occur inside the cell; extracellular proteins are endocytosed, digested in lysosomes, and bound by the class II MHC molecule prior to the molecule's migration to the plasma membrane. The *DQ* genes of MHC class II region are single-copy genes in the mouse, rat, pig, and rabbit. In humans and dogs, multiple *DQ* genes have been identified but only one DQ molecule appears to be expressed (Kappes and Strominger, 1988; Sarmiento *et al.* 1992, 1993). A feature unique to ruminants is certain variability in the number of *DQ* loci (Andersson and Rask 1988; Scott *et al.*, 1991). In cattle, most haplotypes carry duplicated *DQ* genes (Andersson and Rask 1988; Sigurdardóttir *et al.,* 1991b, 1992). Evidences has been obtained that in these cases both DQ molecules are expressed (Ballingall *et al.*, 1997; Bissumbhar *et al.*, 1994; Marello *et al.*, 1995; Russell *et al.*, 1997; Xu *et al.*, 1994).

The class IIa sub-region contains the functionally expressed DR and DQ genes. These gene products, the DR and DQ molecules, represent the main class II restriction elements for CD4+ T-helper cells (Aida, 1995; Glass *et al.*, 2000). Cattle have one DRA gene, three DRB genes (only one, DRB3, is thought to be functionally important), and several DQA and DQB genes depending on the haplotype (Takeshima and Aida, 2006). The BoLA-DRB3 gene is the most polymorphic class II locus in cattle and influences both the magnitude and epitope specificity of antigen-specific T cell responses to infectious diseases. In Buffalo, three BuLA DRB genes, including DRB1, DRB2 and DRB3 have been

reported, but among them only DRB3 gene appears to be functional (Lewin *et al*., 1999). Buffalo MHC DRA and MHC DRB gene show polymorphism. (Sena *et al*., 2003). Like DRB3 in cattle, DRB3 gene of buffalo also seems to be polymorphic. Total 28 DRB alleles were reported in buffalo (De *et al*., 2011). Apart from this many workers have reported polymorphism in MHC DRB3 gene either using Restriction fragment length polymorphism (RFLP) in Jaffarabadi & Mehsana (Acharya *et al*., 2002), Murrah (Singh *et al*., 2004) and Surti (Aravindakshan *et al*., 2000; Sumathi *et al.,* 2010), Nili Ravi (Kumar *et al*., 2008), Banni (*Behl et al.,* 2010) breeds of Indian buffalo and Iraninan buffalo (Rahimnahal *et al.,* 2010) or Single-Strand Conformation Polymorphism (SSCP) in Indian riverine buffalo (De *et al.*, 2002), Jaffarabadi and Mehsana (Pipalia *et al*., 2004), and Iranian water buffalo (Sheikhmohammadi *et al.,* 2010). MHC class II DRB gene of Riverine buffalo was shown to be homologous to cattle DRB3 gene (Iannazzi *et al.*, 1993)

Cattle MHC DQ region comprises five DQA loci and five DQB loci, with exon 2 of the DQA1, DQA2, DQA3, DQB1, and DQB2 genes being highly polymorphic (Takeshima and Aida, 2006). In water buffalo, 7 *DQA* alleles (Niranjan *et al.,* 2010) and 12 novel alleles at *DQB* loci (Sena *et al.,* 2011) are reported till today. High divergence among *DQ* allelic families and the isolation of two diverse *DQA* and *DQB* sequences from individual samples indicated that the duplication of *DQ* loci was similar in buffalo as in other ruminants (Niranjan et al., 2010). Among the DQB allele in buffalo MHC DQB gene showed homology to the BoLA- DQB gene. (Sena *et al.*, 2011; Niranjan *et al*., 2011). In cattle some individuals carry a single copy of *DQA* and DQB genes, whereas others have duplicated haplotypes (Andersson and Rask, 1988). By contrast with the DRA genes, DQA genes are highly polymorphic. This increases significantly the number of different DQ molecules that can be expressed on the cell surface in a given individual, thus expanding antigen presentation capability. In cattle, there are two or possibly three DQA genes (Ballingall *et al.,* 1997), whereas there are four different DQB genes (Sigurdardóttir *et al.,* 1992). The *DQB1* gene is the most frequent, whereas the *DQB2, DQB3* and *DQB4* genes can only be found in duplicated haplotypes. DQB1, DQB2 and DQB3 have been shown to be transcriptionally active in animals with duplicated DQ haplotypes (Marello *et al.,* 1995).

Nomenclature of MHC in animals

The collective name given to the protein encoded by MHC gene depends on the species. In humans these molecule are called human leukocyte antigen (HLA, chromosome no. 6), In Dog these are called Dog leukocyte antigen (DLA, chromosome no. 12), In sheep these are called ovine leukocyte antigen (OLA, chromosome no. 20), In goat these are called caprine leukocyte antigen (CLA, chromosome no. 23), In swine these are called swine leukocyte antigen (SLA, chromosome no. 7).

The MHC system in cattle is known as the bovine leukocyte antigen (BoLA). A major rearrangement within the class II region has led to the division of the BoLA region into two distinct sub-regions, such as class IIa and class IIb, on chromosome 23. In case of Buffalo, The MHC of buffalo is called buffalo lymphocyte antigen (BuLA) (Kumar *et al.,* 1993). MHC in river buffalo resides on the short arm of chromosome 2 (BBU2) and fall into two linkage groups, consistent with organization of the MHC in cattle (Rodrigues *et al.,* 2008).

Conclusion

After knowledge of thought related to the genetic variation to immune response in the population is a significant facade for designing disease prevention strategies for livestock. MHC has a determinant role in deciding the fate of antigen and initiating the immune response, therefore studying host pathogen relationship and intensifying research on variation at host genomic level combined with allelic diversity for immune response genes of MHC is required to support disease control strategy. In India, a lot of information regarding genetic polymorphism of MHC genes in cattle has been generated; however, their association with disease and application to breeding programs is not there. So need is to explore this missing link to develop best protection strategies for livestock health.

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