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Association between MHC gene and immune response to FMD vaccine in Malnad Gidda cattle

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ABSTRACT

Malnad Gidda cattle with specific qualities like disease resistance, heat tolerance, ability to survive and production under stress and low input conditions needs to be conserved for future. They are found to be less susceptible for foot and mouth disease (FMD) and are normally not vaccinated for FMD. The present study was conducted to determine the genetic polymorphism at MHC DRB3.2 loci and cellular immune responsiveness to FMD vaccination in Malnad Gidda compared to Hallikar × HF crossbred cattle. A total of 12 cattle, of which six Malnad Gidda and six Hallikar × Holstein Friesian crossbred animals aged between one to three years were selected. The genomic DNA was isolated and utilized to determine the genetic polymorphism at MHC DRB3.2 loci. The blood samples were collected from the same animals for estimation of CD4 and CD8 lymphocytes by flow cytometry to determine the cellular immune responsiveness to FMD vaccination. The data obtained were subjected to statistical analysis GraphPad Prism Version 5.01. The two allelic variants MHC DRB3.2*117 and *219 present between Malnad Gidda and Holstein Friesian crossbred cattle could be responsible for better cellular immune responsiveness with significantly higher CD4 lymphocytes population to FMD vaccination in Malnad Gidda cattle compared to Hallikar × HF crossbred cattle.

Keywords: Foot and mouth disease, Immune response, Malnad gidda, MHC gene

Malnad Gidda is dwarf Indian cattle with their home tract in Southern India and are found to be distinctly different from other breeds in the state of Karnataka (Anonymous 2009). They are mainly distributed in Shimoga, parts of Chickmagalur and Hassan, North and South Canara and Belgaum districts of Karnataka. The lots of variability are found among Malnad cattle and are named with their special character. The incidence of many tropical diseases in this breed is rare. The conservation of domestic cattle breeds with specific qualities like disease resistance, heat tolerance, ability to survive and production under stress and low input conditions need to be preserved for future (Ramesha *et al.* 2013). They are found to be less susceptible for foot and mouth disease (FMD) and are normally not vaccinated for FMD. The immune responsiveness and disease resistance are quantitative traits and are controlled by major histocompatability complex (MHC) genes (Maillard *et al.* 1996). The gene polymorphisms in bovine class II MHC influences the recognition of the individual epitopes, resulting in the animal-to-animal variation in both humoral and cellular immune responses (Garcia-Briones, 2000) and has reinforced the need for adequate T-cell activation for efficient peptide-based protection (De Leon *et al.* 2020). Accordingly, the present study was undertaken for better understanding of virus–host interaction (Rodriguez-Habibe *et al*. 2020) and association between cellular immune responsiveness to FMD vaccination and MHC genes in Malnad Gidda cattle.

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MATERIALS AND METHODS

A total of 12 cattle, of which six Malnad Gidda and six Hallikar × Holstein Friesian crossbred animals aged between one to three years were selected in an isolated herd maintained at Rayarakoppalu village of Alur taluk, Hassan district, Karnataka. Genomic DNA was isolated from venous blood by high salt method as described by Miller *et al*. (1988). The purity and concentration of DNA samples were estimated by spectrophotometer and agarose gel electrophoresis. The primers LA31 and LA32 which were of 30 bp and 27 bp length, respectively, are the flanking region of the 307 bp fragment of BoLA locus including 20 bp of the 5′ intron, and 269 bp of exon 2 including 18 bp of the 3′ intron (Gelhaus *et al*. 1995). The reaction volume was kept constant at 50 µl. An initial denaturation at 94°C for two minutes was done and subsequently denaturation and primer extension were carried out at 94°C for one

minute and 72°C for 1 min. respectively. The annealing temperature was 65°C for one minute and number of cycles was kept constant at 35. After the last cycle, a final primer, extension was carried out at 72°C for 7 min and the samples were then cooled down to 4°C until retrieved. Each reaction mix consisted of 48 µl of master mix and 2 µl (100 ng) of template DNA. All the 12 PCR products were subjected to sequencing (Automated Sequencer: Perkin-Elmer Applied Biosystems) by single pass method using both the forward and reverse primers used in the amplification of products. The resultant chromatogram and sequence were analyzed using CLC-Bio software. To analyze the significant difference between allelic frequencies between breeds, the chi-square test was used.

The same animals from which blood was collected for MHC analysis were subjected to FMD vaccination study. The animals were vaccinated subcutaneously at the neck region with 5 ml of polyvalent inactivated aluminium hydroxide adjuvant FMD virus vaccine (Indian Immunologicals, Hyderabad, India). The subsequent single booster vaccination was administered on day 21 post vaccination. All the animals were kept under the same management conditions. The blood samples were collected before vaccination and on 30 day post vaccination from both the groups. The blood samples from all the animals were subjected to flow cytometry for estimation of CD4 and CD8 lymphocytes. Flow cytometry was performed in a BD Biosciences FACS calibur flow cytometer and data were analyzed by using CellQuest Pro v5.2 software (BD Biosciences). Monoclonal anti-bovine CD4 mouse antibody tagged with FITC (Fluorochrome) and anti-bovine CD8 mouse antibody tagged with PE (Fluorochrome) were procured from M/s Serotech, USA.

The data obtained from the present study were subjected to statistical analysis. The data were analyzed with the aid of computerized statistical software, GraphPad Prism Version 5.01 (2007) by applying two-way ANOVA at 0.05% level of significance with the application of Tukey's post test. Mean values and standard error of mean were calculated and all the values are expressed as Mean±SE.

RESULTS AND DISCUSSION

In the present study, the genetic variation or molecular differences at MHC DBR3.2 region of MHC gene in Malnad Gidda and Hallikar × HF crossbred cattle was explored. The size of amplified product, i.e. 307 bp fragment of second exon of DRB3 gene was same in all the animals studied and thus suggested the conservation of this gene in the cattle. The alignment of consensus sequences of DRB3.2 gene from the animals revealed polymorphism at 117th and 219th positions of the nucleotide sequence. Thus two different allelic variants DRB3.2 *117 and DRB3.2 *219 were observed between Malnad Gidda and Hallikar × HF crossbred cattle and thereby suggested the high degree of polymorphism of this locus in the cattle taken up for the study (Table 1).

Similarly, the complexity of the bovine MHC with high levels of polymorphism and gene duplication was reported by Lewin *et al*. (1999), Ellis (2004) and Takeshima and Aida (2006) and De Leon *et al*. (2020). Gelhaus *et al*. (1995) identified 14 novel MHC DRB3.2 alleles in Holstein cattle. Sharif *et al*. (1998) reported MHC DRB3.2 *8, *11, *16, *22, *23 and *24 alleles in Holstein cows of Canada. Also, study by Takeshima *et al*. (2007) on exon 2 MHC DRB3 gene in Japanese Shorthorn cattle indicated high polymorphism. Behl *et al*. (2009) characterized the different allelic variants of the MHC DRB3 locus in the Kankrej, Sahiwal, Hariana, Rathi and Kankrej breeds.

Table 1. Allelic frequency at polymorphic sites of MHC DRB3.2 in Malnad Gidda and Hallikar × HF crossbred cattle

Poly-morphic sites	Alleles and their frequency				Chi-square exact test P values
	Malnad Gidda		Crossbred cattle		
44	G	A	G	A	0.90
	0.42 (5)	0.58 (7)	0.5 (6)	0.5 (6)	
85	G	C	G	C	0.90
	0.16 (2)	0.84 (10)	0.25 (3)	0.75 (9)	
95	T	C	T	C	1.00
	0.84 (10)	0.16 (2)	0.84 (10)	0.16 (2)	
101	T	C	T	C	0.99
	0.75 (9)	0.25 (3)	0.66 (8)	0.43 (4)	
117	A	T	A	T	0.012*
	0.16 (2)	0.84 (10)	0.75 (9)	0.25 (3)	
176	G	T	G	T	0.48
	0.16 (2)	0.84 (10)	0	1.00 (12)	
177	A	C	A	C	0.99
	0.42 (5)	0.58(7)	0.5 (6)	0.5 (6)	
185	T	C	T	C	0.99
	0.66 (8)	0.34 (4)	0.75 (9)	0.25 (3)	
216	G	A	G	A	1.00
	0.42 (5)	0.58 (7)	0.42 (5)	0.58 (7)	
219	A	G	A	G	0.039*
	0.25 (3)	0.75 (9)	0.75 (9)	0.25 (3)	
237	G	C	G	C	0.99
	0.84 (10)	0.16 (2)	0.75 (9)	0.25 (3)	
261	T	G	T	G	1.00
	0.84 (10)	0.16 (2)	0.84 (10)	0.16 (2)	
265	G	T	G	T	0.99
	0.84 (10)	0.16 (2)	0.75 (9)	0.25 (3)	

*Shows significant difference at 0.05% level of significance.

Further, in the present study the translation of consensus sequences into proteins resulted in change in the amino acid with Tyrosine and Lysine at 32nd and 66th position in Hallikar × HF crossbred and with phenylalanine and arginine amino acids at 32nd and 66th position in Malnad Gidda cattle (Fig.1). Similarly, De *et al*. (2011) in Indian cattle and buffalo breeds compared the predicted amino acid residues of DRB3 exon 2 alleles with similar alleles from other ruminants and observed considerable congruence in amino acid substitution pattern and high degree of nucleotide and amino acid polymorphism at peptide-binding regions. The MHC genes are the one which regulate the immune response (Xu *et al*. 1993) and thereby are the more

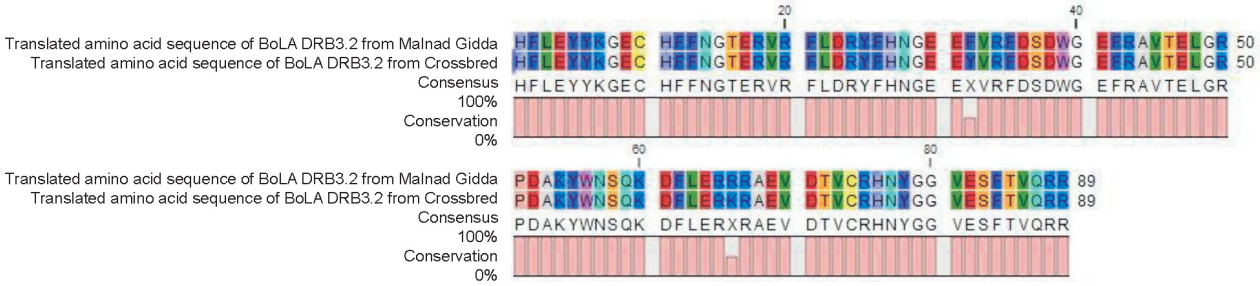

Fig. 1. Alignment of MHC DRB 3.2 translated amino acid sequence of Malnad Gidda and Hallikar × HF crossbred cattle.

suitable candidate genes to study the disease resistance and susceptibility exhibited by different breeds of animals (Vandre *et al*. 2014, De Leon *et al*. 2020). Collectively, the present findings advocate the use of alleles *117 and *129 of MHC DRB3.2 as reference points for more detailed mechanistic studies. But, this does not imply that the immune response for FMD vaccine should be based on MHC alleles. The information on a variety of other genes also needs to be taken into consideration.

The immune responsiveness to FMD vaccination in same Malnad Gidda and Hallikar × HF crossbred cattle aged between one to three years was evaluated. The per cent CD4 and CD8 lymphocyte population was studied by flow cytometry assay using the expression of specific antigens on the surface of lymphocytes. The mean per cent values of CD4 and CD8 lymphocyte on day 0 and day 30 post vaccination in Malnad Gidda and Hallikar × HF crossbred cattle were estimated. The per cent increase and the per cent of CD4 and CD8 lymphocyte on 0 day and 30 day post vaccination in Malnad Gidda and Hallikar × HF crossbred cattle are presented in Table 2.

Table 2. CD4 and CD8 lymphocyte per cent in Malnad Gidda and Hallikar × HF crossbred cattle

Group	CD4 lymphocyte (%)			CD8 lymphocyte (%)		
	0 day	30 day	Per cent increase	0 day	30 day	Per cent increase
Malnad Gidda	16.14	59.51*	3.68	12.76	57.29	4.49
Hallikar × HF crossbred cattle	26.84	50.22	1.87	13.38	55.99	4.20

*Shows significant difference at 0.05% level of significance between groups.

In the present study, there was significant (P<*0.05*) increase in the per cent of CD4 and CD8 lymphocyte on 30 day post vaccination compared to 0 day in both Malnad Gidda and Hallikar × HF crossbred cattle. But, the per cent of CD4 lymphocytes and per cent increase in CD4 lymphocyte on 30 day post vaccination was significantly (P<*0.05*) higher in Malnad Gidda than Hallikar × HF crossbred cattle. However, there was non-significant (P>*0.05*) difference in the per cent increase and per cent of CD8 lymphocyte on 30 day post vaccination between the groups. The increase in CD4 and CD8 lymphocytes per cent on 30 day post vaccination compared to 0 day levels observed in the present study clearly indicated the activation of humoral and CMI responses in vaccinated animals.

The present findings were in conformity with the observations made by Rigdena *et al*. (2003) who reported significant increases in titer of CD4 and CD8 lymphocytes in the animals vaccinated with adjuvant vaccine. Likewise, the specific T-cell antiviral responses, involving CD4 and CD8 cells, were observed in cattle and swine following infection and vaccination of FMD as reported by Grubman and Baxt (2004) and they attributed it to cell-mediated immunity for the clearance of virus from persistently infected animals. Similarly, Gerner *et al*. (2006) and Zhu and Paul (2008) demonstrated the role of T lymphocytes in the induction of antibody responses in ruminants based on the demonstration of FMD virus specific CD4 T lymphocytes proliferative responses following infection or vaccination with virus or peptide. Guzman *et al*. (2010) reported significantly higher levels of circulating virus-specific memory CD8 T lymphocytes at day 21 and beyond following infection with FMD virus or vaccination with inactivated virus.

The considerable animal to animal variation was reported in the context of FMDV challenge and immunization by Garcia-Briones *et al.* (2000) and Ledwidge *et al.* (2001). Likewise, Van Lierop *et al.* (1995) and Carr *et al.* (2013) showed FMD virus specific MHC class II restricted CD4 lymphocyte proliferative responses following infection or vaccination in cattle. Childerstone *et al.* (1999) showed CD8 lymphocytes mediate immune responses to FMD vaccine in cattle. In the present study, CD4 lymphocytes were present in greater per cent compared to the CD8 cells in blood following vaccination on day 30. This finding was in agreement with the previous studies on CD4:CD8 ratio in the bovine peripheral blood of mastitis-resistant and susceptible cows (Park *et al.* 2004). The activated CD4 T cells will go through a clonal expansion and assist in the production of humoral immunity through production of cytokines which lead to Thl and Th2 immune responses and interaction with B lymphocytes (Mosamann *et al*. 1986, O'Shea and Paul 2010) to undergo isotype switching to generate high affinity antibodies, increase microbicidal activity of macrophages and improve the efficiency of CD8

T-cell responses and CD8 T-cell memory (Stockinger *et al*. 2006).

The variations at both the genomic and transcriptomic level were found to correlate with immunological measures of vaccine responsiveness (O'Connor and Pollard 2013). The better immune responsiveness observed in Malnad Gidda cattle compared to Hallikar × HF crossbred cattle in the present study could be attributed to amino acid substitution which leads to unique conformational changes in protein products of DRB3.2 loci resulting in varied immune response (Gowane *et al*. 2013). Thus, the physiogenomic aspects responsible for better immunity in Malnad Gidda were evidenced. But, to better characterize the immune response to FMD vaccines there is need to analyze the kinetics and magnitude of the antibody and cell-mediated immune responses to FMD vaccines (Rizk *et al*. 2015).

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