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RESEARCH ARTICLE
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Evaluation and comparison of the constitutive expression levels of Toll-like receptors 2, 3 and 7 in the peripheral blood mononuclear cells of Tharparkar and crossbred cattle

Lakshya Veer Singh*, Shikha Saxena, Smita Gupta, Shishir Kumar Gupta*, G. Ravi Kumar, G. S. Desai, Aditya Prasad Sahoo, D. R. Harish and A. K.Tiwari

Molecular Biology Laboratory, Division of Veterinary Biotechnology, Indian Veterinary Research Institute, Izatnagar, Bareilly-243122, Uttar Pradesh, India
* Contributed equally
Corresponding author: A. K. Tiwari, email: aktiwari63@yahoo.com
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Abstract

Aim: This study was undertaken to assess the differential expression levels of toll-like receptors (TLRs) 2, 3 and 7 in peripheral blood mononuclear cells (PBMCs) isolated from Tharparkar and Crossbred cattle belonging to different regions of India.

Materials and Methods: PBMCs were isolated from blood samples of Tharparkar cattle from Indian Veterinary Research Institute (IVRI) farm (n=30); Suratgarh farm (n=61); Jaipur farm (n=8) and cross breed cattle from Jaipur (n=47). RNA was isolated from PBMCs and cDNA was synthesized using random hexamers. The expression profiles of TLR 2, 3 and 7 were estimated by real-time PCR and normalized to the expression of β-actin.

Results: PBMCs of Tharparkar cattle from Suratgarh, exhibited a significantly higher ($p<0.05$) constitutive expression levels of TLR2, TLR3 and TLR7 genes as compared to Tharparkar cattle from IVRI or Jaipur as well as the crossbred cattle from Jaipur. PBMCs of crossbred cattle from Jaipur showed higher expression profiles of all the TLRs than Tharparkar cattle from Jaipur and IVRI.

Conclusion: Our study indicates, expression levels of TLR2, TLR3 and TLR7 are significantly higher for Tharparkar cattle from Suratgarh than the cattle from Jaipur and IVRI and crossbred cattle from Jaipur. However, crossbred cattle from Jaipur showed higher basal expression levels of all the three TLRs than Tharparkar cattle from Jaipur and IVRI. Results also indicate that PBMCs of Tharparkar cattle show a regional variation in the expression pattern of TLRs.

Keywords: crossbred, immunity, resistance, Tharparkar cattle, Toll-like receptors.

Introduction

Toll-like receptors (TLRs) recognize the invading pathogens through the detection of 29 highly conserved pathogen associated molecular patterns (PAMPs) derived from a wide range of pathogens [1] and activate the innate immune response [2]. The term "Toll-like receptors" was proposed in 1997 for mammalian proteins structurally related to the "TOLL" cell surface receptor seen in the Drosophila larvae. TLRs trigger the activation of adaptive immune system by promoting the presentation of antigens and up-regulation of various cytokines and co-stimulatory molecules [3, 4]. Till date, at least 13 TLR members have been described in mammals [2, 5, 6]. Although, TLR family proteins are well conserved, the different members of the TLR family have very distinct functions in the host defence. The main characteristics that distinguish various TLRs are ligand specificity, signal transduction pathways and sub cellular localization [7]. Basal TLR expression profiles in different cells and tissues are suggestive of an individual's ability to respond to pathogens.

The expression pattern and distribution of the TLRs have been shown to be characteristic of each species [8-10]. Over the last two decades there has been great progress in identifying TLRs in different species of farm animals and exploring their roles in the disease susceptibility and resistance against the invading pathogens. Various breeds of animal show differences in their susceptibility to infectious diseases, while some breeds show a better resistance to a pathogen while others are susceptible to infectious diseases. Cattle also show differences in their susceptibility/ resistance to various invading organisms and this might be attributed to differential expression profile of TLR genes [11]. TLR2, TLR3 and TLR7 detect lipoproteins, dsRNA and ssRNA respectively, and are involved in defence against the bacterial and viral infections. The constitutive expression levels of these TLRs in different breeds of animal may explain the differential susceptibility status of various breeds.

Thus, this study was planned to assess the differential expression levels of TLR2, TLR3 and TLR7 in Tharparkar cattle from different regions and

Table-1: List of primers used in the study

Gene code	Gene	Primer sequence	Product length
MBL/NICRA/1	TLR 2F	TTCCGTCTCTTTGATGAG	114bp
MBL/NICRA/2	TLR 2R	CTTGGTGTTCATGATCTTC	
MBL/NICRA/3	TLR 3F	CTGGAGTATAATAACATAGAGC	108bp
MBL/NICRA/4	TLR 3R	CAGTGAAATGCTTTGTCTA	
MBL/NICRA/7	TLR 7F	CAAGCATTTGACAGAAATTC	77bp
MBL/NICRA/8	TLR 7R	CTGCTATGTGGTTAATGG	

crossbred cattle from Jaipur. This information might have functional implications to explain why despite of close similarity between Tharparkar and crossbred cattle, these animals respond very differently to many pathogens.

Materials and Methods

Ethical approval: Blood samples used in this study were collected from Indian Veterinary Research Institute (IVRI) farm, Suratgarh farm and Jaipur farm after the approval of Institutional Animal Ethics Committee.

Isolation of peripheral blood mononuclear cells (PBMCs) in Tharparkar cattle and Crossbred cattle: Blood was collected from the jugular vein using a sterile syringe containing heparin as an anticoagulant. The blood was slowly layered over on an equal volume of Ficoll Hypaque (Sigma, MO, USA) with specific gravity 1.077 g/ml. It was then centrifuged at 600g for 30 min and then the interface containing the PBMCs was collected and washed twice in sterile phosphate buffered saline (PBS). Their viability was determined by Trypan blue staining.

RNA extraction and cDNA synthesis: The PBMCs were centrifuged at 400g for 2 min and the resulting pellet was resuspended in 1 ml of TRIzol reagent (Invitrogen, CA, USA). The resulting mixture was extracted with chloroform and precipitated with isopropanol. The RNA pellet was briefly washed with 70% ethanol and resuspended in RNase free water (Invitrogen, CA, USA). Upon isolation of RNA, cDNA synthesis was carried out by using random hexamer primers (Fermentas, MD, USA).

Real-time PCR quantification (qRT-PCR): Expression levels of mRNA of TLR 2, TLR 3 and TLR 7 were analyzed by Real-time PCR using the QuantiTect SYBR Green qPCR system (Qiagen, CA, USA) on I-cycler (Bio-Rad, CA, USA) using gene specific primers. Beta actin gene was used as the reference gene (endogenous control). The primers used in the study were designed by Becon Designer software (Table-1). Real-time PCR was carried out in triplicate for each sample in a total volume of 20 µl consisting of 2 µl cDNA, 10 µl of Affimetrix SYBR Green Master Mix (USB, CA, USA), primers 0.5 µl each (20 pm/µl). Real-time PCR cyclic conditions were 95ºC for 2 min, 95ºC 30 sec, 51ºC for 30 sec, 72ºC for 15 sec, followed by 40 cycles. The final step was to obtain a melt curve for the PCR products to determine the specificity of the amplicons. Expression levels of the TLR 2, TLR3 and TLR7 genes were normalized relative to the expression of the β-actin gene and the relative expression of each sample was calculated using the $2^{-\Delta\Delta CT}$ method [12, 13, 14]. Results were analysed and shown as fold change ($2^{-\Delta\Delta CT}$).

Statistical analysis: All the experiments were run in triplicates. The results of three independent experiments were reported as mean ± SE. As the normality test failed, the Mann–Whitney nonparametric test was done in JMP 9 (SAS Institute Inc., Cary, NC, USA).

Results

PBMCs of Tharparkar cattle from Suratgarh, exhibited a significantly higher ($p<0.05$) constitutive expression levels of TLR2, TLR3 and TLR7 genes as compared to Tharparkar cattle from IVRI or Jaipur as well as the crossbred cattle from Jaipur. PBMCs of crossbred cattle from Jaipur showed higher expression profiles of all the TLRs than Tharparkar cattle from Jaipur and IVRI. The expression data of TLR2, TLR3 and TLR7 normalized to β-actin was found to be 0.519±0.17, 0.118±0.08, 0.121±0.07 (IVRI farm); 5.51±0.25, 6.19±0.18, 3.21±0.25 (Suratgarh farm); 0.56±0.18, 0.11±0.14, 0.20±0.34 (Jaipur farm) and for crossbred cattle 1.01±0.31, 0.063±0.18, 0.055±0.31, respectively.

Discussion

The innate immune system is essential for host defence and is responsible for early detection of invading pathogen. Effector mechanisms of innate immunity are activated immediately after infection, with the aim to control the replication of the infecting pathogen at the site of the entrance. The discovery of toll-like receptors (TLRs), particularly on the cells of innate arm of immune system [15, 16] and their critical role in providing immunity has aroused intense curiosity in the scientific arena in understanding the functions of the immune system [17, 18]. In Cattle, ten TLRs (1-10) have recently been reported [19, 20]. Overall, bovine TLRs seem to show a greater sequence homology to humans than to mice [11, 20, 21].

In the present study, we evaluated the expression profiles of TLR 2, TLR3 and TLR7 genes in the PBMCs of Tharparkar cattle from different regions and crossbred cattle from Jaipur. TLR2 is an extracellular receptor and implicated in the recognition of Gram-positive bacterial components, bacterial lipoproteins, and zymosan [22, 23]. TLR2-deficient mice displayed impaired cytokine production in response to *Staphylococcus aureus* peptidoglycon preparation and mycoplasmal lipopeptide [24]. TLR3 and TLR7 are

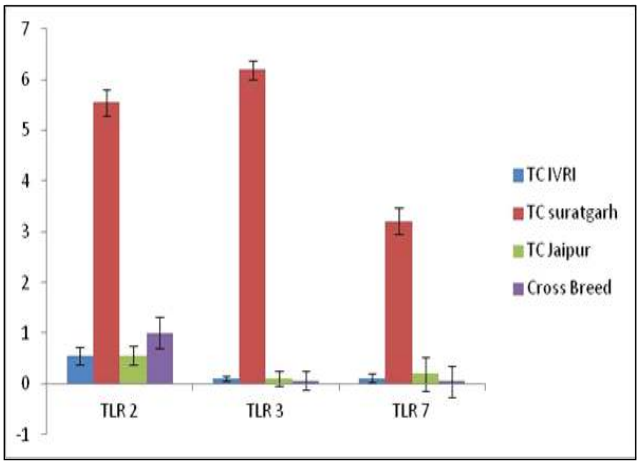

Figure-1: The expression profiles of TLR2, 3 and 7 mRNA by Real-time quantitative PCR in Tharparkar cattle from different regional farms and crossbred cattle PBMCs.

present on intracellular compartments and ligands for these TLRs, are double-stranded RNA (Poly I:C - Polyinosinic : polycytidylic acid) and single-stranded RNA, suggesting their role in the detection of RNA viruses [6].

We have found that all TLRs, under investigation, are expressed constitutively in the PBMCs of Tharparkar and crossbred cattle; however, PBMCs of Tharparkar cattle from Suratgarh, expressed higher mRNA transcripts of all three TLRs than that of PBMCs from Tharparkar cattle from Jaipur or IVRI and crossbred cattle (Figure-1). Further, though Tharparkar cattle from Suratgarh showed significantly higher expression levels of TLR2, TLR3 and TLR7 than crossbred from Jaipur, cattle from IVRI and Jaipur, did not show the same pattern and expressed very low levels of all the three TLRs than crossbred cattle from Jaipur. TLR3, particularly, showed a great variation among the breeds under study, and its high expression in Tharparkar cattle from Suratgarh shows this breed has a greater resistance for the infections by the RNA viruses.

Results suggest a regional variation in the expression pattern of TLR2, TLR3 and TLR7 in Tharparkar cattle that may be attributed to local environmental conditions. Moreover, differing expression profiles of TLRs in PBMCs of cattle indicate important functional consequences. The distinct relationship of cattle TLRs might be the possible reason for differences in the pathogen recognition receptor systems in these animals and consequently the differences in their susceptibility/resistance to various invading bacterial and viral pathogens. However, as we have not used any infectious model to ascertain the immune competence of these breeds by exposing them to the pathogen challenge, ingenious experimental designs combined with efficiency of latest tools and techniques are required to test this notion further.

Conclusion

Toll-like receptors play key role in the innate immune system by recognizing the invading pathogens. Higher expression of TLRs reflects a better innate immune response and disease resistance. Our study reveals the higher expression of TLR2, TLR3 and TLR7 in the PBMCs of Tharparkar cattle from Suratgarh in comparison to crossbred cattle. However, Tharparkar cattle from Jaipur and IVRI did not show the same pattern. Crossbred cattle from Jaipur exhibited a better immune competence than the Tharparkar cattle. Keeping in view the complexity of the innate immune system, further investigations are required to explain these differences in the expression levels of TLRs in Tharparkar and crossbred cattle from different regions. The knowledge generated will help us to recognize important alleles involved in disease resistance and in designing selective breeding programmes for efficient control and prevention of diseases.

Authors' contributions

LVS and SKG designed, conducted and drafted the manuscript. SS and SG enhanced the graphical presentation of the results. APS, RK, DRH, GSD and AKT analyzed the data and revised the manuscripts.

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Competing interests

The authors declare that they have no competing interests.

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