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Research paper

Transcript profiling of pattern recognition receptors in a semi domesticated breed of buffalo, Toda, of India

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ABSTRACT

The primary objective of this study was to assess the expression profile and levels of toll-like receptor (TLR) mRNAs in the spleen, lung, mediastinal lymph node (MLN), jejunum, rectum, skin and peripheral blood mononuclear cells (PBMC) of Toda and Murrah buffalos. Spleen and PBMC had increased expression of TLR mRNAs 2, 4, 5, 6, 8, 9 and 10; lung had increased expression of TLR mRNAs 2, 4, 5, 6 and 8, MLN TLR mRNA 6, 9, 10 and decrease in TLR 3 and 7 mRNAs in skin. No significant differences were observed in the expression levels of any of the TLR mRNA in jejunum and rectum. Toda buffaloes showed significantly higher expression levels of TLR 9 mRNA in MLN, TLR mRNAs 1, 5, 6, 9 and 10 in skin and TLR mRNAs 2, 4, 7 and 9 in PBMC than Murrah buffaloes living in the vicinity. Toda and Murrah buffaloes were inoculated with TLR5 (flagellin) and TLR9 (CpG ODN) ligands *in vivo* and expression levels of the respective TLRs analyzed 12 h later. Following CpG inoculation, Toda buffaloes had significantly higher levels of TLR 9 mRNA expression but not in Murrah. However, flagellin induction did not increase TLR 5 mRNA expression in both these breeds. Histological sections of the skin were made and infiltrating cell clusters were graded and quantified. Following CpG inoculation, Toda buffaloes showed higher numbers of infiltrating grade 1 and grade 3 cell clusters while Murrah showed lower numbers of infiltrating grade 1 cells as compared to mock-inoculated skin sections. Flagellin treatment revealed no significant differences in infiltrating cell clusters in both the breeds. The results have shown differential expression of TLR mRNAs in various tissues between two divergent buffalo breeds with the highest difference in TLR expression profile seen in the skin, the largest portal of entry of pathogens, of Toda.

1. Introduction

Toda buffalo is an indigenous, genetically isolated, semi-wild breed with an estimated population of only 1800 which are maintained by the aboriginal Toda tribes in the Nilgiri hills of Tamil Nadu, Southern India. Nilgiri hills are a range of mountains with at least 24 peaks above 2000 m (6562 ft) from sea level in the westernmost part of Tamil Nadu. Its latitudinal and longitudinal dimensions are 130 km (Latitude: 11°08′ to 11°37′N) by 185 km (Longitude: 76°27′E to 77°4′E) with an area of 2479 sq km (957.1 sq m). Toda buffaloes are brown medium-sized animals with a fairly long body, long horns which are widely curved and directed downwards, broad and deep chest; short and sturdy legs and large, heavy head. The average

Abbreviations: TLR, toll-like receptor; PBMC, peripheral blood mononuclear cells; Poly I: C, polyinosinic–polycytidylic acid; qRT-PCR, quantitative real time polymerase chain reaction.

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daily milk yield of Toda buffaloes is 3.03 ± 0.06 kg with high milk fat content (8.23%). These buffaloes have phenotypic resemblance to the swamp buffalo but it is classified under riverine type as they have 50 chromosomes (Murali et al., 2009). Toda buffaloes might have separated from the other main breeds of India some 1800–2700 years ago as calculated from Nei's standard genetic distances based on genotyping data on seven breeds and 20 microsatellite loci (Kumar et al., 2006). The Todas are unique since they are the only tribe in India with buffalo-based dairying as their primary occupation (Nair et al., 1986). The Toda buffaloes are not fully domesticated and only natural mating is being practised. The level of husbandry is still primitive but is a better suited breed to the hilly tracts and is self-sustainable in the 'no-input' system of management. There is a dearth of information on the immunity of Toda buffaloes although anecdotal evidence suggests that they do not suffer from any clinical disease as much as other breeds of buffaloes in the vicinity (personal communication from local veterinarians). The contribution of their 'immunological superiority' or their less intensive methods of rearing to the above observation is not clearly known.

The more popular and extensively reared Murrah breed of buffalo is a major milch breed from Punjab and Haryana states of India with dark black coat colour, sometimes with white markings on face or legs.

Toll-like receptors (TLRs) are one of the important germ line encoded pattern recognition receptors which recognizes microbial pathogen associated molecular patterns and subsequently initiates innate and adaptive cellular immunity (Takeuchi et al., 2000; Akira et al., 2001; Beutler et al., 2003). TLRs are shown to be differentially expressed in various tissues and immune cells of mouse (Applequist et al., 2002), humans (Zarember and Godowski, 2002), bovines and ovines (Menzies and Ingham, 2006), buffalo (Vahanan et al., 2008), goats (Tirumurugaan et al., 2010) and chickens (Dhinakar Raj et al., 2009). The TLRs have broad specificity with particular PAMPs acting as agonists for each individual TLRs. The agonists includes lipopolysaccharide (LPS) from gram negative bacteria (TLR4), lipoprotein and peptidoglycan from gram positive bacteria (TLR1, 2 and 6), flagellin (TLR5), double stranded RNA (TLR3), unmethylated CpG dinucleotide motifs (TLR9), single stranded uridine rich RNA (TLR7) and the synthetic antiviral compound R-848 (TLR7 and TLR8). Activation of TLRs by its appropriate ligand leads to initiation of responses including cell proliferation or maturation and the production of various cytokines, chemokines or effector molecules, including nitric oxide and reactive oxygen intermediates (Thoma-Uszynski et al., 2000; Hemmi et al., 2002; Smith et al., 2003).

Differences in innate immune responses are likely to contribute significantly to different susceptibilities. Although various molecules contribute to the innate immune responses, TLR family could be important in determining the early outcome of infections. With respect to TLR mediated innate immunity, there could be several determinants of immune responses. Mutations or single nucleotide polymorphisms (SNPs) in TLR genes could affect downstream responses. Several SNPs in the TLR 2 gene of sheep and cattle have been incriminated with *Mycobacterium avium* subsp. *paratuberculosis* infections (Bhide et al., 2009).

Differential expression profiles and levels of TLR mRNAs could be another determinant of innate disease resistance of a species or breed of animal. Cows with mastitis were found to express 2.5 folds more TLR 2 than those with out mastitis (Fonseca et al., 2011). TLR 6 mRNA expression was higher in the Commercial White line of pigs than in the Piau breed after vaccination with *Mycoplasma hyopneumoniae* (Sousa et al., 2011). Genetic line effect was significant ($p < 0.05$) on TLR mRNA expression in the spleen of *Salmonella enteritidis*-infected birds. The Fayoumi line had higher TLR2 and TLR4 expression than Leghorn, higher TLR2 mRNA expression than broiler, and the broiler line had higher TLR5 expression than Leghorn and Fayoumi (Abasht et al., 2009).

Differences in the downstream cytokine profiles induced by different TLR ligands have also been shown to be different with respect to TLRs and among breeds/species/individuals (Trelis et al., 2011; Zaros et al., 2010; Nerren et al., 2009; Swaggerty et al., 2004). Further using sires within a broiler population with higher and lower pro-inflammatory cytokine mRNA expression levels in the heterophils progeny with increased or decreased levels of, pro-inflammatory cytokines have been produced (Swaggerty et al., 2009).

Hence this study was undertaken to determine the expression profiles of TLR 1–10 mRNAs in selected tissues and immune cells of Toda breed of buffaloes and comparing them with the Murrah breed. In addition, *in vivo* inoculation of two TLR ligands was performed followed by histological examination and quantification of the infiltrating cell clusters. The results have shown differential expression of TLR mRNAs in various tissues between two divergent buffalo breeds. Five TLR mRNAs were differentially expressed in the skin of Toda buffaloes, the largest portal entry of pathogens.

2. Materials and methods

2.1. Animals and tissue samples

Tissue samples were collected from apparently healthy 4–8 months old male Toda and Murrah calves ($n = 6$) within an area of 3–5 sq km in Nilgiri Hills of Tamil Nadu, India at the time of slaughter. The Toda tribes sacrifice their animals only during festivities in their family and slaughter for any other purposes is not normally done. They were maintained under the same nutritional and managemental conditions mostly by the same owners. No vaccinations had been done to these animals. This area is the home to the Toda buffaloes and they are not found anywhere else in the world.

The tissue samples such as lung, jejunum, mediastinal lymph node (MLN), spleen, rectum and skin were collected in sterile tubes and immediately transported to the laboratory in dry ice with in 2 h of collection for extraction of RNA. Sufficient care was ensured to avoid contamination with blood. The tissue samples were also collected in 10% formalin and processed using conventional histological techniques. No pathological changes were evident in these tissues.