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Short communication

Association of microsatellite $(GT)_n$ polymorphism at 3′UTR of *NRAMP1* with the macrophage function following challenge with *Brucella* LPS in buffalo (*Bubalus bubalis*)

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Abstract

Brucella abortus is a facultative intracellular pathogen that survives and replicates in host macrophages. Hence, macrophage function plays an important role in influencing natural resistance/susceptibility to intracellular pathogen. The natural resistance associated macrophage protein 1 (*NRAMP1*; erstwhile referred as *Ity/Lsh/Bcg*), a transmembrane protein, regulates activity of macrophages against intracellular pathogens. In bovine, natural resistance to brucellosis is significantly associated with $(GT)_{13}$ allelic variant of microsatellite locus at 3′ untranslated region (3′UTR) of the *NRAMP1* gene. In the present study we screened 65 Murrah breed of buffalo (*Bubalus bubalis)* to identify polymorphism at 3′UTR of *NRAMP1* gene and evaluate the association of these polymorphisms with the macrophage function. Four allelic variants (viz., GT_{13}, GT_{14}, GT_{15} and GT_{16}) were identified. Majority of the buffaloes were of either homozygous $(GT)_{14}/(GT)_{14}$ or heterozygous $(GT)_{14}/(GT)_{15}$ with $(GT)_{14}$ allele occurring most frequently (62%). For association study, non-vaccinated and serologically negative animals were divided into three genotypic groups: group 1 ($n = 2$) comprising animals of homozygous $(GT)_{13}$ genotype, whereas, group 2 ($n = 4$) and group 3 ($n = 6$) consisted animals of heterozygous [$(GT)_{13}/(GT)_n$, where $n \neq 13$] and non-$(GT)_{13}$ [$(GT)_n/(GT)_n$, where $n \neq 13$] genotype, respectively. Macrophages, after maturation, were challenged with *Brucella* LPS to assay the macrophage function in terms of H_2O_2 and NO production. The $(GT)_{13}$ allele, either in homozygoous {$(GT)_{13}/(GT)_{13}$} or heterozygous {$(GT)_{13}/(GT)_n$, where $n = 14$, 15 or 16}, was significantly ($p < 0.01$) associated with increased production of H_2O_2 and NO. In this manuscript, for the first time, we have identified $(GT)_{13}$ allelic variant and demonstrated its significant association with the improved macrophage function in buffalo.

Keywords: *NRAMP1* gene; Microsatellite; 3′UTR; Brucellosis; Genetic resistance; Buffalo; *Buablus bubalis*; Macrophage function

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1. Introduction

Brucella abortus is a facultative intracellular pathogen that survives and replicates in host macrophages. Hence, macrophage function plays an important role in influencing natural resistance/susceptibility to infection by this intracellular pathogen. The natural resistance associated macrophage protein 1 (*NRAMP1*; erstwhile referred as *Ity/Lsh/Bcg*), a transmembrane protein of transporter family, regulates activity of macrophages against intracellular pathogens during the early stages of infection (Blackwell et al., 1994). After phagocytosis, *NRAMP1* is targeted to the membrane of the microbe-containing phagosome, where it modifies the intra-phagosomal milieu affecting microbial infection (Gruenheid et al., 1997). Though, physiological role of *NRAMP1* is not yet clear, it plays a significant role in inhibiting bacterial growth, production of reactive oxygen and nitrogen products, enhancing phagolysosomal fusion and regulation of cytokine production. Studies in mice have shown that a point mutation of G169D (Vidal et al., 1993) in *NRAMP*1 gene confers resistance towards a number of antigenically different intracellular microorganisms including *Mycobacterium bovis* (Bacille Calmette-Guérin, *Bcg*), *Salmonella typhimurium* (Ity) and *Leishmania donovani* (Bradley et al., 1979; Gros et al., 1981; Plant and Glynn, 1974; Skamene et al., 1982).

The G169D polymorphism has never been documented in other mammalian species including bovine. Nevertheless, macrophages from naturally resistant cattle, when challenged *in vitro* with virulent *B. abortus* S2308, exhibited superior ability to restrict the intracellular replication of *B. abortus* than those from susceptible cattle (Price et al., 1990; Qureshi et al., 1996; Templeton et al., 1990). Single-stranded conformational analysis (SSCA) revealed a highly significant ($P < 0.0089$) association of a polymorphic $(GT)_n$ microsatellite (where n is 13, 14, 15, or 16) located in the 3′ untranslated region (3′UTR) of bovine *NRAMP*1 with natural resistance to brucellosis (Adams and Templeton, 1998). A $(GT)_{13}$ microsatellite allele at 3′UTR of *NRAMP*1 gene has been reported to provide resistance. In an *in vitro* study, susceptible murine macrophage cell line (RAW264.7) was transfected with two different gene constructs containing either $(GT)_{13}$ or $(GT)_{16}$ allele under bovine *NRAMP*1 promoter. On challenge with infectious strain of *B. abortus*, cell lines transfected with $(GT)_{13}$ allele demonstrated more expression of *NRAMP*1 protein and restrictive replication of *Brucella* than those transfected with $(GT)_{16}$ allele (Barthel et al., 2001).

India has the distinction of possessing more than one half of the world buffalo population which contributes to more than 50% of total milk production of India and 2.03% of world meat production (FAO, 2005) earning annual foreign exchange revenue worth US$ 3122 million (APEDA, 2005). Brucellosis causes severe economic losses in buffalo and poses serious zoonotic threats (Guarino et al., 2000; Rathore et al., 2002). Although, association of natural resistance against brucellosis with polymorphisms at the bovine *NRAMP1* 3′UTR has already been established (Adams and Templeton, 1998), only a few reports are available describing polymorphic nature of *NRAMP1* gene in buffalo (Ables et al., 2002; Borriello et al., 2006). Accordingly, present study was aimed to explore $(GT)_n$ microsatellite polymorphism at 3′ UTR of *NRAMP1* gene in buffalo (*Bubalus bubalis*) and to evaluate the association of polymorphic variants with resistance/susceptibility to brucellosis using *in vitro* macrophage function assay.

2. Materials and methods

2.1. Animals

A total of 65 animals belonging to Murrah breed of buffalo (*B. bubalis*) maintained at the institute herd of Indian Veterinary Research Institute (IVRI), Izatnagar (UP), India, were included in the study. Records pertaining to their date of birth, parity, calving, abortion and vaccination were also collected.

2.2. Isolation of genomic DNA

Genomic DNA was isolated from the venous blood using standard phenol–chloroform extraction method (Sambrook et al., 1989).

2.3. Microsatellite polymorphism at 3′UTR of NRAMP1 gene

A region corresponding to 1804–1996 bp of 3′UTR with respect to cDNA sequence of buffalo *NRAMP*1