IDENTIFICATION OF IL-1α GENE FROM BOVINE PERIPHERAL BLOOD MONONUCLEAR CELLS BY POLYMERASE CHAIN REACTION

S.D. Audarya[1,*], A. Sanyal[2], J.K. Mohapatra[2] and B. Pattnaik[2]

ABSTRACT

Interleukin-1 (IL-1) play its role in inflammation, fever and release of acute phase proteins by acting on major organs of the body. IL-1α gene along with other cytokines compels Th1 and Th17 inflammatory responses. The present study is aimed at standardization of polymerase chain reaction for amplification of IL-1α gene from bovine peripheral blood mononuclear cells. Blood samples were collected in containers with 0.1% Ethylenediaminetetraacetic acid (EDTA) anticoagulant (Sigma, USA). Peripheral blood mononuclear cells (PBMCs) were isolated using commercially available Histopaque®-1077. Extracted total ribose nucleic acid (RNA) was utilized in reverse transcription to prepare complementary deoxyribose nucleic acid (cDNA). IL-1α gene specific primers were used to amlify partial nucleotide sequence of IL-1α. The cDNA was employed in polymerase chain reaction (PCR). The resulting IL-1α DNA product (124 bp) was identified on agar gel electrophoresis and documented.

Keywords: buffaloes, *Bubalus bubalis*, bovine peripheral, blood mononuclear cells, Interleukin-1

INTRODUCTION

Cytokines are proteins released from cells to act on adjacent or distantly located cells and even on the cell itself and modulate the immune responses. Cytokines secreted by some leukocytes and act upon other leukocytes are referred as Interleukins. Cytokines exhibit attributes of pleiotropy, redundancy, antagonism, and synergy and cascade induction and hence regulate cellular activity in a co-ordinated, interactive way as per Kindt *et al*. (2007). Cytokines play a pivotal role in regulating immune responses of infected or vaccinated animals. Fate of the infectious process is also governed by antiviral cytokines induced after infection. Three primary classes of cytokines were divided into pro-inflammatory, anti-inflammatory and chemokines by Flint *et al*. (2004). Cytokine interleukin-1 (IL-1), tumour necrosis factor (TNF), IL-6 and IL-12 are examples of pro-inflammatory which promote leukocyte activation, while others IL-10, IL-4 and tumour growth factor (TGF) β are anti-inflammatory and suppress activity of pro-inflammatory cytokines and return system to basal "circulate and wait state". Adhesion molecules (intercellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1) are induced at site of inflammation by TNF-α, IL-1

[1]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Mhow Indore, Madhya Pradesh, India, *E-mail: asd_vet@yahoo.com
[2]Indian Veterinary Research Institute Campus, Mukteshwar-Kumaon, Nainital, Uttarakhand, India

and IFN-γ. Hence the present study is aimed to standardize and identify IL-1α gene from peripheral blood mononuclear cells from healthy bovines.

MATERIALS AND METHODS

Blood samples were collected asceptically in glass containers with anticoagulant (0.1% Ethylenediaminetetraacetic acid (EDTA) from the Jugular vein of bovine animals. The animals were kept at Dairy section of Indian Veterinary Research Institute (IVRI), Mukteshwar-Kumaon, Uttarakhand (Himalayan Holstein Friesian crossbred cattle residing at high altitude of more than 2000 m above sea level). IL-1α and 18S RNA custom oligonucleotide synthesized primers (Standard, 0.02 μmol) were obtained from Metabion GmBH (Germany) as per Lahmers *et al*. (2006). Primers were diluted as per instructions of the manufacturer and kept at -80°C as a stock. The details of which are given in the Table 1. Peripheral blood mononuclear cells were isolated by using Histopaque®-1077 by adopting the method of Boyum (1968). RNA was extracted as per Chomczynski and Sacchi (1987). Reverse Transcription (RT) method was used to produce cDNA by employing only Oligo $dT_{(15)}$ primers.

Table 1. List of primers used to amplify IL-1α and 18S RNA.

Item	Primer sequence	Product size, bp	Primer length
IL-1α	F-5'- CCA CTT CGT GAG GAC CAG ATG AAT-3'	124	24
	R-5'- TCT TCA GAA TCT TCC CAC TGG CTG-3'		24
18S RNA	F- 5'-CTG AGA AGA CGG TCG AAC TTG ACT-3'	90	24
	R-5'-TCC GTT AAT GAT CCT TCC GCA GGT-3'		24

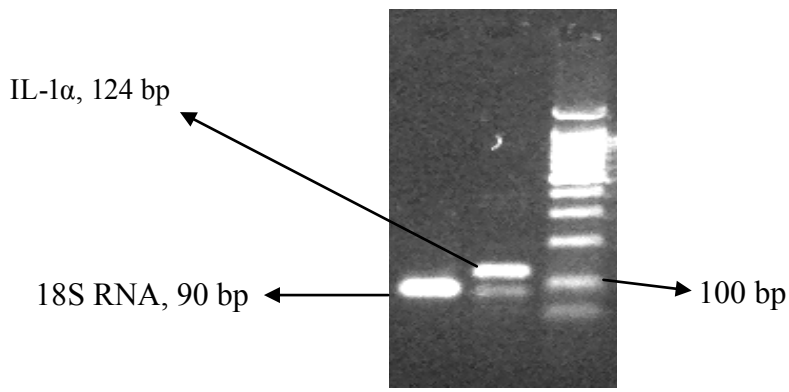

Figure 1. Agarose gel electrophoresis of amplified products. Marker: DNA Ladder

The procedures of isolation of PBMCs and RT were given by Audarya *et al*. (2014). Polymerase chain reaction (PCR) was conducted as per the following in a reaction volume of 13 µl for both 18S RNA and IL-1α; GoTq 5x buffer -2.5 µl, $MgCl_2$ (25 mM/µl) -0.75 µl, dNTP mix (10 mM/µl) -0.5 µl, cDNA -1 µl, GoTq Tq DNA polymerase (5 U/µl) -1 µl and Nuclease Free Water -6.25 µl and Primers (forward and reverse) -1 µl (0.5 µl each). The final concentration of the primers was 100 nM. Cycling conditions were as follows; i) Initial activation at 95°C for 2 minutes, ii) denaturation at 95°C for 30 seconds, annealing 60°C for 30 seconds and extension 72°C for 1 minute for 35 subsequent cycles and iii) final extension at 72°C for 10 minutes thereafter iv) hold at 4°C. Tris Borate EDTA buffer (TBE, Promega, USA) was used to prepare 1% agarose gel. 0.5 µg/ml Ethidium bromide (Electran®, BDH, UK) was added in the gel. Then, 5 µl of amplified DNA product of IL-1α and 18S RNA genes were loaded in the wells using 6x loading dye and the gel electrophoresis was run for 1 h duration at 100 V and thereafter documented using gel the results were documentation system.

RESULTS AND DISCUSSION

In the present investigation, peripheral blood mononuclear cells were isolated, total RNA extracted, cDNA prepared and used in PCR to document successful amplification of 18S RNA (90 bp) and IL-1α (124 bp) DNA products (Figure 1). 18S RNA is normally expressed by the cells and its detection makes sure about the vitality of cells. In Indian cattle and buffalo there was transient but marked decrease in the circulating leukocyte and monocyte levels on 1 day post Foot-and-Mouth disease virus (FMDV) infection (Mohan *et al.*, 2008). Mingala *et al*. (2009) studied expression of cytokines in water buffalo after inactivated FMDV vaccination and reported that IFN-γ, IL-10 and TNF-α was highest at three week post-vaccination than IL-2, IL-4 and IL-6. Increase in T lymphocyte activity after vaccination was thought to be linked with failure in detecting IL-1 after emergency FMDV vaccination by Barnett *et al*. (2002) while as per Zhang *et al*. (2006) in acute stage of infection, there was increased mRNA expression of IL-1α. There were changes in cytokine expression during the day and night in case of IL-6 as reported by Vgontzas *et al*. (2005). Hence successful standardization, amplification and identification of IL-1α will subsequently help in formulating strategies to study differential expression of cytokines in FMDV infection and vaccination.

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