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EXPRESSION PROFILE OF IL-1β IN *PESTE DES PETITS RUMINANTS* (PPR) VACCINATED GOATS

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Abstract – *Peste des petits ruminant virus* (PPRV) is associated with peracute and acute multi-systemic disease of sheep and goat commonly known as goat plague. It is characterized by profound immune suppression and leucopenia. In healthy animals, innate viral recognition pathways trigger release of molecules with direct anti-viral activities and pro-inflammatory mediators which recruit immune cells to support viral clearance. Interleukin-1β (IL-1β) has critical roles in the establishment of inflammation. In this study, the gene expression of IL-1β was investigated by quantitative real time PCR (qRT-PCR) in peripheral blood mononuclear cells (PBMCs) of PPRV vaccinated goats. The expression level of IL-1β remained elevated from 14 days post vaccination (dpv) and relative fold change in mRNA expression was increased up to 150 folds.

INTRODUCTION

Goat, the poor man's cow, is an important small ruminant and one of the chief contributors in the meat industry. For efficient economic gain from animals, health management sets a pivotal role. Study of all aspect of various diseases provides a path to mitigate the problem in easier way as well as helps in betterment of health management to achieve the quality production.

Among prevalent diseases of small ruminants, *Peste des-petits ruminants* (PPR) – commonly known as Goat plague, is an important disease with heavy mortality and morbidity (Albina *et al*., 2013). It is endemic in South Asia and Africa (Balamurugan *et al*., 2012). Due to high mortality and heavy economic losses to poor farmers the disease is of the top priority disease for OIE which needs to be eradicated after Rinderpest (Kumar *et al*., 2017). The disease is caused by a negative sense single stranded RNA virus of genus *Morbilivirus* which belongs to family paramyxoviridae and order mononegavirals (Muthuchelvan *et al*., 2014). The incubation period of PPR is 4-6 days, after that clinical illness start with pyrexia which may last for 3-5 days accompanied by dryness of muzzle, anorexia, watery nasal and lachrimal discharges which gradually become muco-purulent. Erosive

lesion in the oral cavity may become necrotic. In severe cases, the necrotic lesion progress with the appearance of a deposit of fibrin on the tongue and foul smelling. Later course of the disease is characterized by diarrhoea and coughing with laboured abdominal breathing. Finally, the animal may become dyspnoeic, suffering progressive weight loss and emaciation, ultimately leading to death (Parida *et al*., 2015). PPRV infection leads to high morbidity (up to 100%) and up to 90% mortality in sheep and goats (Baron *et al*., 2016). There is no therapeutic agent available for treatment of the disease. Prophylactic vaccination is only way to control the PPR.

The immunity against any virus depends on innate immune response as well as on adaptive immune response. Previously, it was considered that the immune response depends only on adaptive immune response whereas now the role of innate immune system is well recognized (Suresh and Mosser, 2013). Innate immune receptors play a major role in pathogen recognition and known as PRRs. These are mainly three type of receptors- Toll like receptors (TLRs), RIG-I-like receptors **(**RLRs) and NOD like receptors (NLRs) (Kawai and Akira, 2009). Out of these receptors, NLRs are the important PRRs for transmission of signal for endogenous pathogens and results in activation of inflammsome. Inflammasome is a multi-protein oligomer, consists mainly of centrally located NLRs such as NLRP1, NLRP3, NLRC4, NLRP6 and NLRP12, N terminal Pyrin and /or Caspase-1, apoptosis-associated speck-like protein containing CARD (ASC) and at C terminal Lucine rich region (LRRs) (Broz and Dixit, 2016). The activation of Inflammasome leads to activation of pro-caspase-1 into caspase-1 and the activation of caspase-1 further activate the production of IL-1β which triggers the inflammation and release of various pro-inflammatory cytokines (Motta *et al*., 2015). The present study has been conducted to find expression profile of IL-1β in goats by the relative fold change of expression of its RNA copy number after PPR vaccination using qRT-PCR.

MATERIALS AND METHODS

Animals and experimental design

Sirohi goats (n=21) of either sex and more than five months of age from Livestock Research Station (LRS), Bojunda, Chittorgarh, Rajsathan, India were selected for the study. All the animals were kept on balanced ration as per their nutrient requirement. Animals were immunized with Raksha PPR (live attenuated) vaccine (Indian immunological Ltd.) with 10^3 TCID virus per dose of vaccine via subcutaneous route.

Sample collection and peripheral blood mononuclear cells isolation

Blood samples were collected aseptically from jugular vein from each goat on 0, 1st, 3rd, 14th, 30th, 60th and 90th days. Peripheral blood mononuclear cells (PBMCs) were separated from blood by using Hisep LSM1077 (HIMEDIA) as per the manufacturer protocol.

Total RNA isolation

Total RNA was isolated using TriReagent (Sigma Aldrich) as per the manufacturer protocol using PBMC as starting material. Isolated RNA was treated with DNase-1 (Thermo Fisher Scientific India Pvt Ltd) to remove contaminating genomic DNA at 37 ^{0}C for 30 minutes followed by the heat inactivation at 95 ^{0}C for 15 minutes. Total RNA was quantified with help of bio-spectrophotometer (Eppendorf BioSpectrometer) and the average concentration of RNA isolated was 1120 µg/mL. The RNA concentration was normalized and total RNA used in studied was restricted to 500 ng per 20 µL of reaction volume used in qRT-PCR study.

cDNA synthesis

cDNA was synthesized from normalized RNA using reverse transcriptase (SUPER SCRIPT-200 Thermo Fisher Scientific Pvt Ltd.) as per manufacturer protocol. Reverse transcription was carried out briefly as follows: 5 µL of extracted RNA was mixed and incubated with 1 µL of Oligo-dT at 65 ^{0}C for 5 minutes and snap chilled on ice for 5 minutes. The reaction mixture was spinned briefly and 4 µL of 5X RT buffer, 1 µL of 40U/µL RNase inhibitor, 1 µL of 200U/µL Revert Aid H Minus Reverse transcriptase, 2µL of 10mM dNTPs and 6 µL nuclease free water (NFW) were added to make final volume 20 µL. It was mixed and incubated at 25 ^{0}C for 10 minutes followed by 42 ^{o}C for 60 minutes and then 70 ^{0}C for 10 minutes in thermal cycler (BIO-RAD). The received cDNA was analyzed for concentration in bio-spectrophotometer and stored at -80 ^{0}C until used.

Quantification of mRNA by qRT-PCR

The IL-1β quantification was carried by qRT-PCR in

the area max Real time PCR system (Agilent Pvt. Ltd) using SYBR green universal master mix (Agilent Pvt. Ltd). Primers used to determine dynamics of IL-1β as mentioned in Table 1. These primers were designed using Primer 3 software and analyzed by online software available at IDT DNA. The assay was run in duplicates for each sample to avoid possible technical variability. Relative quantification of IL-1β was normalized $\Delta\Delta C_T$ method using in-house gene β actin and standard curve and statistics were generated in Graph Pad in excel work book (Yuan *et al*., 2006).

RESULTS

In the present study it was found that IL-1β expression started only after 14 dpv. The relative fold change in RNA copy number was carried out using the $\Delta\Delta C_T$ methods (Table 2). It revealed that after the initial decrease in level of gene expression at 1 and 3 dpv, the expression level increased at 14 dpv and remained elevated at all other time points (≥ 90dpi).

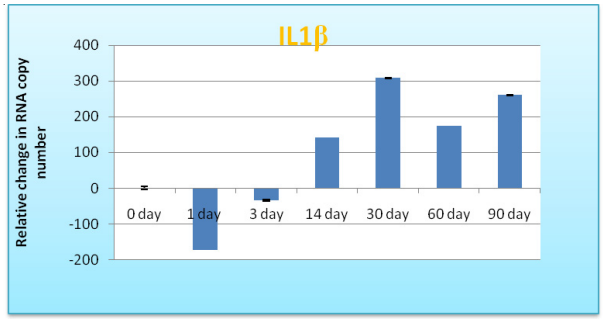

Fig. 1. Expression of IL1β starts to increase from 14 dpv and remained elevated up to 90 dpv.

Table 1. Detail of primer sequence used

S. No.	Gene	Name designated	Nucleotide sequences (5′-3′)	Product size (bp)	Melting temperature (^{0}C)	Annealing temperature (^{0}C)
1.	HUMB-ACTIN	NRG25 (Fp)	5′-CCCCAGCCATGTACGTTGCTATCC-3′	409	66	54
		NRG25 (Rp)	5′-GCCTCAGGGCAGCGGAACCGCTCA-3′		65.6	
2.	IL-1 β	NRG181 (Rp)	5′-CAAGATTCCTGTGGCCTTGG-3′	210	56.2	55
		NRG182 (Fp)	5′-GTGCTGATGTACCAGTTAGGG-3′		55.1	

Table 2. ΔC_T analysis to calculate the mean fold change in IL-1β gene expression level

Days post vaccination	Average C_T value IL1 β	Average $\Delta\Delta C_T$ value Actin	ΔC_T	$\Delta\Delta C_T$	Average $\Delta\Delta C_T$	$2^{\wedge-\Delta\Delta C_T}$
1 day	30.75	28.61	2.14	-7.32167	-7.435	
	31.52	28.87	2.65	-6.81167		173.0446
	30.67	29.38	1.29	-8.17167		
3 day	32.91	28.06	4.85	-4.61167	-5.078333333	33.78552
	26.75	19.965	6.785	-2.67667		
	29.47	27.955	1.515	-7.94667		
14 day	29.485	27.775	1.71	-7.75167	-7.153333333	142.3534
	29.935	27.455	2.48	-6.98167		
	27.86	25.125	2.735	-6.72667		
30 day	26.515	24.8	1.715	-7.74667	-8.266666667	307.9744
	26.695	26.02	0.675	-8.78667		
	26.985					
60 day	26.165	24.555	1.61	-7.85167	-7.444166667	174.1476
	25.895	23.47	2.425	-7.03667		
		25.13				
90 day	24.365	22.685	1.68	-7.78167	-8.021666667	259.8737
	26.8	24.16	2.64	-6.82167		
		23.855		-9.46167		
	23.33	18.06	5.27	-4.19167	5.92119E-16	
0 day	32.7	22.05	14.64	5.178333		1
	30.525	20.705	8.475	-0.98667		
			9.461667			

DISCUSSION

IL-1β is an important effector molecule having pleiotropic effects including the triggering inflammation and adaptive immune response. IL-1β is produced by hematopoietic cells such as blood monocytes, tissue macrophages, skin dendritic cells and brain microglia in response to TLRs, activated complement components, other cytokines (such as TNF-α) and IL-1β itself (Dinarello, 2011). The effect of IL-1β on the central nervous system includes fever as IL-1 is the classic endogenous pyrogen and activator of the hypothalamus-pituitary-adrenal axis. At elevated temperature, leukocyte migration is increased (Garlanda *et al*., 2013).

In this study, it was found that IL-1β up-regulated 14 dpv onwards and remained elevated throughout the study period (up to 90 dpv). Similar results, although using different virus, have also been found in other studies (Nain *et al*., 1990). In a study of cytokine kinetics in monocyte and macrophages after influenza virus exposure (Nain *et al*., 1990) found that only 14-day macrophages were able to secrete significant amounts of IL-1β in response to influenza A or Sendai virus infections. Sendai virus was a stronger inducer of IL-1β production compared with influenza A virus, whereas IL-18 secretion was low. Though not related but in this study of IL-1β following PPR virus vaccination increased at 14 day and being a RNA virus, the results might be correlated with this previously studied viruses. A study conducted by Patel and colleagues revealed the unique biphasic expression of IL-4 cytokine with up-regulation of INFkb in PPR infected and vaccinated goats but did not give any clue about IL-1β (Patel *et al*., 2012) .

The expression study of IL-1β was conducted in PBMC collected at various dpv from vaccinated goat in field conditions where other stress factors can influence the expression level of these molecules. Further, we studied relative fold change in gene expression hence, could not be assessed the pro-form of IL-1β or cleaved/active IL-1β, yet the data give a preliminary indication about the gene expression profile of this cytokine after exposure of PPR vaccine.

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