

Cytokine profiling in the acute phase of viral vaccination in Poultry

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ABSTRACT

In this study, the status of T cell-dependent cytokine gene expressions in the acute phase (3-day post-infection and 5-day post-infection) of infection with vaccine virus strains of New castle disease virus (NDV) (R2B and Lasota), Avian infectious bronchitis virus (AIBV) (Massachusetts H120), and Infectious Bursal Disease virus (IBDV) in SPF chicken of seven day age was evaluated. The birds were divided into four groups, each having six birds. Each group of birds was inoculated with the prescribed dose of different vaccines at 7 days of age. Blood was collected before inoculation (uninfected), at the 3rd and 5th day post-inoculation. Presence of virus in peripheral blood confirmed by real-time reverse-transcription PCR assay and quantitation of cytokine was performed in peripheral blood by real time PCR assay. It was observed that the infection with different vaccine strains of viruses in poultry modulates cytokine expression in order to elicit antiviral immune responses. AIBV and NDV viruses markedly up-regulate *IL-2*, *IL-12*, *p40*, *IL-4*, *IL-5*, and *IL-13* whereas, IBDV induces prolonged up-regulation of *IFN-γ*, *IL-10* genes.

Keywords: Cytokine, *IFN-γ*, *IL-2*, *IL-4*, *IL-5*, *IL-12*, *IL-13*, Immunity, Poultry viruses and *p40*

The widespread distribution of Avian infectious bronchitis (IB), Newcastle disease (ND), and the epidemics of avian influenza (AI) that have occurred over the last ten years have resulted in heavy economic losses.

Cytokines are low molecular weight proteins that are secreted by many different types of cells (Chen *et al.* 2017, Ginting *et al.* 2017, Liu *et al.* 2018). Their main function is to orchestrate the functional activities of the cells of the immune system. The CD4⁺ helper T (Th) cells play crucial roles in immune responses. The CD4⁺ T cells have been classified as either Th1 or Th2 based on their cytokine profiles. Th1 cells have been involved to enhance the clearance of intracellular pathogens and are defined on the basis of their production of *IFN-γ*. Th2 cells are critical for the control of certain parasitic infections through the production of the clustered group of cytokines *IL-4*, *IL-5*, and *IL-13* (Bao and Reinhardt 2015).

During viral infection, Chickens are able to elicit immune response either by stimulating Th1-cell-mediated immunity or Th2-dependent humoral immunity (Bhuiyan *et al.* 2021). Though, cytokine secretion from these cells can correlate with adaptive immune responses against different viruses. Therefore, the present study was planned to investigate the expression level of Th1 dependent (Cell-mediated immunity) cytokines (*IFN-γ*, *IL-2* and *IL-12p40*

genes) and Th2 dependent (Humoral immunity) cytokines (*IL-4*, *IL-5*, *IL-10* and *IL-13* genes.) during the acute phase of infection against vaccine strains of NDV, AIBV and IBV.

MATERIALS AND METHODS

Institutional animal ethical committee permission: IAEC of CVAS, Bikaner granted permission vide letter no –CVAS/IAEC/2021-22/28-28 dated 8/5/2021.

Chickens and virus: Zero-day old specific pathogen-free (SPF) white leghorn chickens (*Gallus gallus domesticus*) purchased from Kewal Ramani hatcheries private Ltd. Ajmer (Rajasthan) were housed in the Rajasthan University of Veterinary and Animal Sciences, Bikaner (Rajasthan) poultry farm isolators with water and feed freely available.

The all-vaccine virus strain [NDV ((R2B (10⁶ EID₅₀)) and Lasota, both live attenuated), AIBV @ dose rate 10^{3.5} EID₅₀ of massachusettsh120, Live attenuated virus), and IBDV (killed virus) were provided by the Venkateshwara hatcheries Pvt. Ltd. (Ventri Biologicals, Vaccine division dist. Pune, India). The birds were divided into four groups, each having six birds. Each group of birds was inoculated with the prescribed dose as mentioned above of different vaccines at 7 days of age. Blood was collected before inoculation (uninfected) and at the 3rd or 5th-day post-inoculation (dpi).

RNA isolation and cDNA synthesis: Total RNA was extracted from the blood by using the Trizol method (Sigma chemicals Pvt Ltd Mumbai India) as per the manufacturer protocol. The extracted RNA was checked for its concentration and purity by bio-spectrophotometer (Nenodrop, Thermo Scientific Pvt. Ltd, Mumbai, India).

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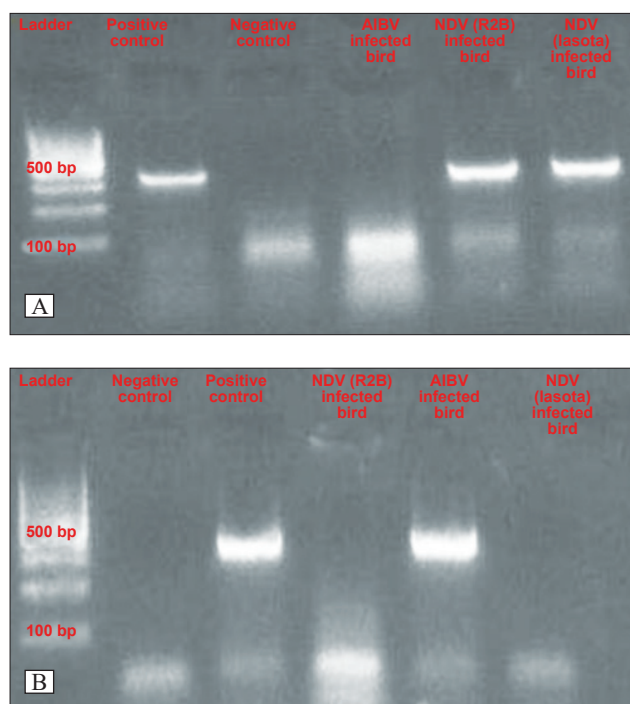


Fig. 1. Detection of viruses in peripheral blood at 3 dpi by PCR. A. NDV (RB2 and Lasota), B. AIBV.

The purified RNA was stored at -20°C for further use. The cDNA was synthesized from the isolated RNA using the RevertAid™ First Strand cDNA Synthesis kit (Thermo Scientific Pvt. Ltd, Mumbai, India) as per the manufacturer protocol.

Determination of the presence of virus in peripheral blood: The presence of virus, i.e. NDV, AIBV, and IBDV in peripheral blood were confirmed by performing PCR using previously described primers (Sumi *et al.* 2012, Jain *et al.* 2013 and Alsahami *et al.* 2018).

Real-time PCR: The amount of cytokine gene mRNA (cDNA) in peripheral blood was measured by quantitative real-time PCR (qRT-PCR) using gene-specific primer and house-keeping control gene (GAPDH) as previously described (Liu *et al.* 2010). The levels of cytokine genes expressed as threshold cycle (Ct) values, were normalized with the GAPDH housekeeping control gene. Relative fold-change in viral DNA copy number was determined by $\Delta\Delta\text{Ct}$ method (Liu *et al.* 2010).

Statistical analysis: The student's t-test was used to detect significant differences between infected and control groups. A P-value ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

In the present study, we evaluated the status of Th2 dependent (Humoral immunity) cytokine (*IL-4*, *IL-5*, *IL-10* and *IL-13*) and Th1 (Cell-mediated immunity) cytokine (*INF-gamma*, *IL-2* and *IL-12*) in chick during 3rd and 5th-day post-infection of the vaccine strain of four different poultry viruses.

The presence of target vaccine strain of viruses in peripheral blood at 3-day post-infection: Before cytokine expression evaluation we have determined the presence of viral genome in peripheral blood by performing PCR (Fig 1). In contrast, inactivated IBDV can't able to replicate in birds therefore we unable to detect the IBDV genome in IBDV inoculated birds. This is in agreement with the previous observation made by Abdul-Careem who studied virus replication and cytokine gene expression following virus infection and found a significant association between higher viral RNA levels and cytokine transcript concentration in various tissues (Abdul-Careem *et al.* 2008).

Th1-cytokines expression during the acute phase of different vaccine strain virus infection: The Infection of different viruses resulted in transcriptional changes of mRNA encoding, *IL-2*, *IL-12p40* and *INF-γ* during the acute phase of the disease. Differences in cytokine expression were given as fold-change using the chicken GAPDH gene for normalization.

IL-2 gene expression: R2B and AIBV up-regulated the expression of *IL-2* gene at 3 dpi whereas Lasota strain induced a marginal increase in *IL-2* mRNA transcripts level. In contrast to it, IBDV infection, down-regulated the expression of the *IL-2* gene in comparison with uninfected birds (Fig 2a; Table 1). *IL-2* stimulates the proliferation of chicken T lymphocytes and NK cells, which potentiate antiviral responses and decreased viral titers in blood, spleens, oral and cloacal secretions on 4–5 dpi (Susta *et al.* 2015).

IL-12p40 gene expression: All viruses markedly up-regulated the expression *IL-12p40* gene at 3 dpi and after that, they start to downtrend during the course (Fig. 2b; Table 1). Similar up-regulation of *IL-12p40* gene expression in the early phase of viral infection was reported in influenza virus and adenovirus (Jouanguy *et al.* 1999).

INF-γ gene expression: Following inoculation, the R2B, Lasota strains of NDV, AIBV and IBDV up-regulated the *INF-γ* gene expression at 5 dpi. In contrast, IBDV induced a strong increase in *INF-gamma* 3 dpi which got stabilize

Table 1. Th1 cytokine expression during acute phase of different vaccine strains

Th1/CMI response	3 Days				5 Days			
	NDV		AIBV	IBDV	NDV		AIBV	IBDV
	R2B	Lasota			R2B	Lasota		
<i>IL-4</i>	1.17±0.18	1.57±0.18	1.02±0.19	5.61±3.73	16.97±5.62	14.06±0.75	8.18±0.74	2.21±0.64
<i>IL-5</i>	3.09±0.61	1.47±0.18	3.03±0.02	0.94±3.73	2.75±3.73	1.37±5.73	1.35±7.73	0.5±6.73
<i>IL-10</i>	0.02±0.16	0.025±0.33	0.002±0.36	16.97±2.25	4.55±0.06	3.59±0.26	4.13±1.85	2.15±1.23
<i>IL-13</i>	2.17±0.18	1.27±0.18	2.02±0.19	3.77±0.43	2.47±0.18	1.47±0.18	3.59±0.26	

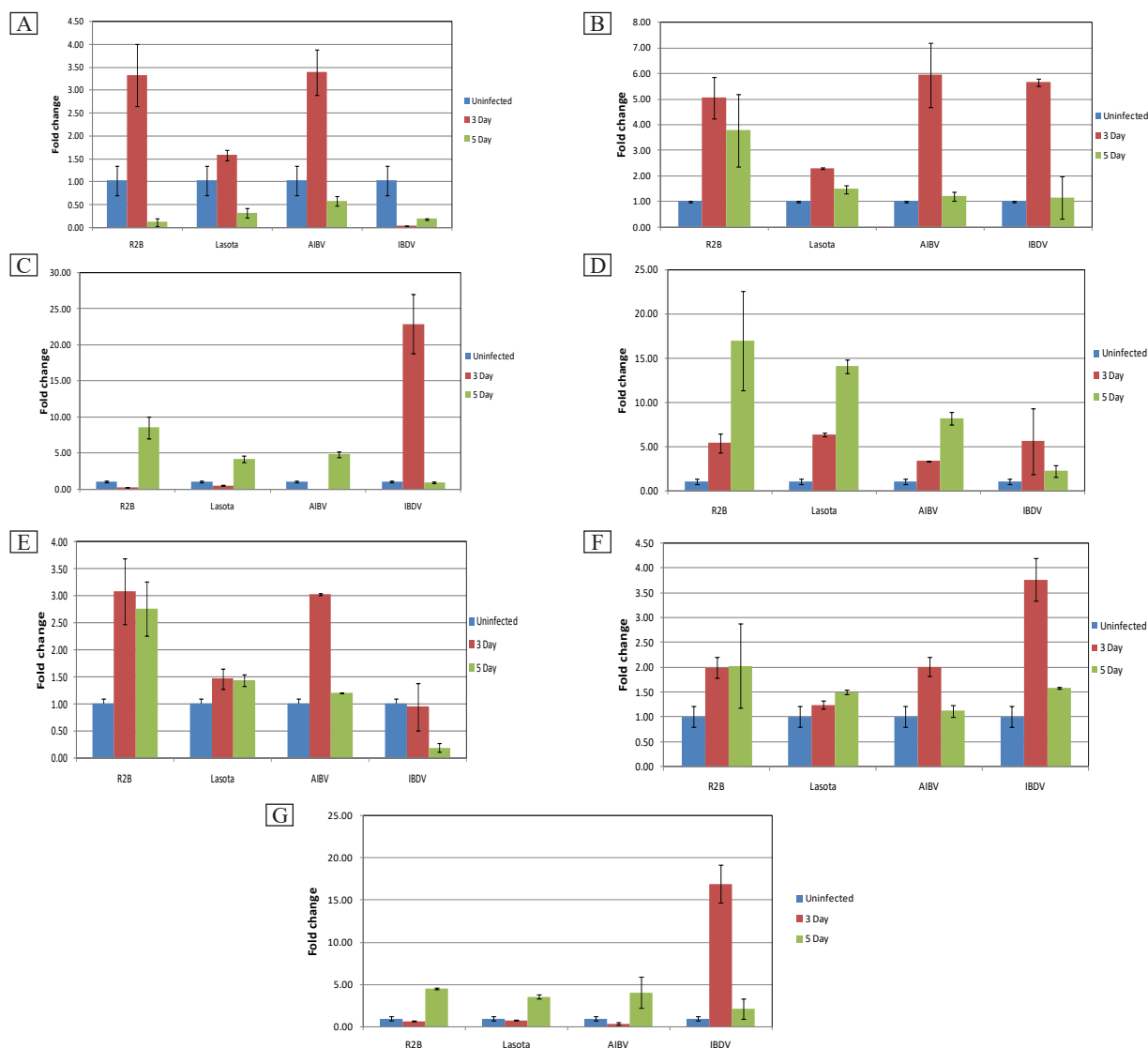


Fig. 2. Effect of R2B, Lasota, AIBV, IBDV, infection on Th1 and Th2 cytokine gene expression, in poultry birds. Relative fold-change (compared to mock-infected) in mRNA levels was determined by qPCR. IL-2(A) and IL-12(B), INF gamma (C), IL-4(D), IL-5(E), IL-13(F), IL-10(G) is shown. Error bars indicate SD.

at 5 dpi in expression in comparison with uninfected birds (Fig.2c; Table 1). Production of *IL-12* and *INF-gamma* is critical to host defense against intracellular pathogens (Jouanguy *et al.* 1999), indicating that it is possible to observe simultaneous up-regulation of *INF-gamma* and *IL-10* in response to IBDV infection. The observed increase in *IFN-γ* expression in IBDV-infected bursa presumably reflects the inflammatory response and it is consistent with earlier published results suggesting that cell-mediated responses are initiated to resolve infections (Li *et al.* 2009 and Eldaghayes *et al.* 2006).

Th2-cytokine expression during different virus infections: Temporal expression patterns of *IL-4*, *IL-5*, *IL-10*, and *IL-13* genes were evaluated in the peripheral blood of chickens infected with different viruses in comparison with uninfected birds.

IL-4 gene expression: The up regulation of *IL-4* gene expression following infection with R2B and Lasota strain

of NDV and AIBV after IBDV and AIBV inoculation (Fig. 2d; Table 2) was observed. *IL-4* has been shown to direct B cells to produce the anti-allergen IgE, to inhibit Th1 cell function, and to prevent the production of *IL-2*, *IL-12*, and *INF-gamma* that are necessary for the development of cytotoxic T cells (Becker 2004). However, our study observed the suppression of transcriptional activities of Th1 cytokines.

IL-5 gene expression: R2B and Lasota strain of NDV and AIBV up-regulated the *IL-5* gene expression, contrary to it in IBDV infected birds, it did not up-regulated (Fig. 2e; Table 2). In previous reports, expression levels of *IL-5* were significantly up-regulated in IBDV infection (Liu *et al.* 2012), whereas it was markedly decreased in REV infection (Xue *et al.* 2013).

IL-13 gene expression: Following inoculation with NDV (both R2B and Lasota strain) the expression of *IL-13* genes was up-regulated and peaked at 5dpi. In contrast in AIBV

Table 2. Th2 cytokine expression during acute phase of different vaccine strains

Th1/CMI response	3 Days				5 Days			
	NDV		AIBV	IBDV	NDV		AIBV	IBDV
	R2B	Lasota			R2B	Lasota		
<i>IL-2</i>	3.32±0.68	1.59±.11	3.38±0.50	0.23±0.28	0.2±0.72	0.30±0.13	0.58±.40	0.2±0.36
<i>IL-12p40</i>	5±0.68	2.29±0.	5±0.98	5±0.88	3.36±0.68	1.57±0.23	1.23±0.73	1.13±0.33
<i>IFN-γ</i>	0.2±0.16	0.25±0.33	0.02±0.36	22.86±4.10	8.51±1.49	4.81±0.37	4.16±0.42	0.94±0.11

and IBDV, it peaked at 3 dpi and then stabilized at 5 dpi. IBDV and AIBV up-regulated the *IL-13* gene expression at 3dpi (Fig. 2f; Table 2). Previously, expression levels of *IL-13* have significantly up-regulated in REV and IBDV infection (Liu *et al.* 2012, Xue *et al.* 2013).

IL-10 gene expression: IL-10 gene expression was up-regulated following inoculation with R2B and Lasota strain of NDV, AIBV at 5 dpi. In contrast, IBDV induced up-regulation of *IL-10* gene expression at 3dpi and it started to decline at 5 dpi (Fig. 2g; Table 2). *IL-10* is a potent stimulator of NK cells (Albert *et al.* 1998), a function that might contribute to the clearance of the pathogen and facilitate antigen acquisition from dead cells for cross-priming activated antigen-presenting cells (APCs), providing a link between the innate and the adaptive immune responses (Mocellin *et al.* 2003). The expression of *IL-10* in the bursa following IBDV infection has not been studied previously. In the present study, our results indicated that *IL-10* expression was markedly increased and similar to the extent of up-regulated expression of *IFN-γ* following infection by the H or T strain. This is consistent with the fact that *IL-10* plays a dual role in infectious diseases (Mocellin *et al.* 2003) and is in agreement with the observation made recently by Abdul-Careem (Abdul-Careem *et al.* 2008).

It was observed that inoculation with different vaccine strains of viruses in poultry induce up-regulation and down regulation of several Th1-cytokine expression (*IFN-γ*, *IL-2* and *IL-12p40* genes) and Th2-cytokines expression (*IL-4*, *IL-5*, *IL-10*, and *IL-13* genes). AIBV and NDV viruses markedly up-regulated *IL-2*, *IL-12p40*, *IL-4*, *IL-5* and *IL-13* whereas, IBDV induced prolonged up-regulation of *IFN-γ* and *IL-10* genes. Though the cytokines up-regulation and down-regulation are closely associated with virus replication, pathogenesis, and immunity, yet, further studies are necessary to elucidate their exact function in virus-induced pathogenesis and immunity.

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