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Effect of acute phase heat inactivated pseudomonas on mRNA level of cytokine in Broiler chickens

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Abstract
T cells have an important immunoregulatory and effector function through cytokine release. They are involved in the responses to Gram-negative bacterium. 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 heat inactivated pseudomonas culture and live attenuated culture with adjuvant given 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 pseudomonas vaccine canditure at 7 days of age. Blood was collected before inoculation (uninfected), at the 3rd and 5th day post-inoculation. Presence of pseudomonas culture 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 live attenuated pseudomonas culture modulates cytokine expression in order to elicit antibacterial immune responses with respect to killed culture. Live attenuated pseudomonas culture markedly up-regulate IL-4, IL-2 whereas prolonged up-regulation by IFN-γs genes.

Keywords: Ytokine, IFN-γ, IL-2, IL-4, IL-5,IL-12, IL-13, immunity, pseudomonas

Introduction
Pseudomonas aeruginosa is a gammaproteo bacterium, Gram-negative opportunistic pathogen capable of infecting humans and animlas and causing severe pulmonary disease. It grows in a biofilm, which is similar to intracellular bacteria like mycobacteria and may be thought of as a protective multicellular survival strategy. Th1-class cytokines, such as IFN-γ and TNF-α, are induces of a good immune response to intracellular bacteria; on the other hand, Th2-type cytokines, such as IL-4, iniate B cells to produce antibodies. al phagocytic response through cytokine release (Antonakos *et al*., 2017) [1]. T cells have been described as important inducers of TNF-α production by LPS-stimulated macrophages (Lenka *et al*., 2023) [8].
According to Bhuyan *et al.* (2021) [14], chickens can induce an immune response to a viral infection by Th1-cell-mediated immunity or Th2-dependent humoral immunity. However, these cells' release of cytokines has been linked to adaptive immune responses against several bacteria. Consequently, the goal of the current investigation was to ascertain the degree of Th1 dependent (Cell-mediated immunity) cytokine expression (IFN-γ) genes) and Th2 dependent (Humoral immunity) cytokines (IL-4, IL-5, IL-10 and IL-13 genes.) during the acute phase of infection against pseudomonas vaccine culture.

Materials and Methods
Chickens and Pseudomonas culture in adjuvant: 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. Pseudomas strain ATCC 27853 was culturre in microbiology lab and treated with heat inactivation of bacteria. Then this inactivated bacterial culture mixed with adjuvant and given to birds group for cytokine evaluation. 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 pseudomas culture canditure 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).

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.

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 pseudomonas culture canditure.

Th1-cytokines expression during the acute phase of heat inactivated pseudomonas culture canditure. The Infection resulted in transcriptional changes of mRNA encoding, IL-2 and IFN-γ during the acute phase. Differences in cytokine expression were given as fold-change using the chicken GAPDH gene for normalization.

IL-2 gene expression: No significanca change at 3 dpi whereas at 5dpi Up-regulated the expression of IL-2 gene and induced a increase in IL-2 mRNA transcripts level from 3dpi. In contrast to it down-regulated the expression of gene in uninfected birds (Table 2). IL-2 stimulates the proliferation of chicken T lymphocytes and NK cells, which trigger responses and decreased titers in different organ secretions on 5-7 dpi (Calum *et al*., 2020) [4].

IFN-γgene expression: Following inoculation of heat inactivated pseudomonas culture canditure up-regulated the IFN-γ gene expression at 3 dpi but down regulate at 5 dpi. In contrast, live vaccine culture induced a strong response at 3 dpi which got stabilize. Production of INF-gamma and IL-12 is essential for host defence against intracellular infections (Gurjar *et al*., 2023) [6], suggesting that INF-gamma can be shown to be upregulated simultaneously.

Th2-cytokine expression during pseudomonas culture canditure 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 pseudomonas inactivated culture in comparison with live culture and uninfected birds.

IL-4 gene expression: The up regulation of IL-4 geneexpression following infection with pseudomonas at 3dpi (Table 1) was observed. It has been demonstrated that IL-4 stimulates B cells to production of anti-allergen IgE, suppresses Th1 cell activity, and stops the synthesis of IL-2, IL-12, and INF-gamma, which are essential for the growth of cytotoxic T cells (Becker 2004) [2]. Moreover our research revealed that Th1 cytokine transcriptional activity was suppressed.

L-5 gene expression: live pseudomonas infection up-regulated the IL-5 gene expression at 3dpi, contrary to it in heat inactivated, it did not up-regulated (Table 1) In previous reports, expression levels of IL-5 were significantly up-regulated in pneumonic infection (Tiringer *et al*., 2013) [12], whereas it was markedly decreased in allergic inflammation (Tan *et al*., 2013) [11].

IL-10 gene expression: IL-10 gene expression was up-regulated following inoculation with live pseudomonas infection at 3 dpi. In contrast it down-regulation in heat inactivated at 3dpi and (Table 1). According to Penaloza *et al*. (2016) [10], IL-10 is a strong NK cell stimulator. This function may aid in the pathogen's removal and make it easier for antigen to be acquired from dead cells for cross-priming activated antigen-presenting cells (APCs), establishing a connection between the innate and adaptive immune responses (Garcia *et al*., 2018) [5]. This is in line with the finding that IL-10 has a dual function in infectious disorders (Murphey & Sherwood *et al*., 2008) [9] and supports a recent observation that pseudomonas could induce macrophage to senescence during the infection (Zhao *et al*., 2021) [13].

IL-13 gene expression: Following inoculation with live strain of pseudomonas the expression of IL-13 genes was up-regulated and peaked at 3dpi. In contrast in heat inactivated pseudomonas culture canditure was down-regulated in IL-13 gene expression at 3dpi (Table 1). Previously, expression levels of IL-13 have significantly up-regulated in anti-tumor activity (Kioi *et al*., 2004) [7].

Table 1: Th1 cytokine expression during acute phase of immune response

S no	Gene	Heat Inactivated		Live	
		3 dpi	5dpi	3dpi	5dpi
1	IL-4	3.32±0.68	1.59+-.11	1.35±7.73	0.5±6.73
2	IL-5	0.23±0.28	0.2±0.72	5±0.88	3.36±0.68
3	IL-10	1.57±0.23	1.23±0.73	2.17±0.18	1.27±0.18
4	IL-13	0.58±.40	0.2±0.36	3.38±0.50	0.23±0.28

Table 2: Th2 cytokine expression during acute phase of immune response

S no	Gene	Heat Inactivated		Live	
		3 dpi	5dpi	3dpi	5dpi
1	IFN-γ	5±0.68	2.29±0	2.75±3.73	1.37±5.73
2	IL-2	0.2±0.16	0.25±0.33	3.77±0.43	2.47±0.18

Conclusion

It was observed that inoculation with different pseudomonas culture canditure in poultry induce up-regulation and down regulation of several Th1-cytokine expression (IFN-γ, IL-2 genes) and Th2-cytokines expression (IL-4, IL-5, IL-10, and IL-13 genes). In heat inactivated pseudomonas culture markedly up-regulated IL-2, IFN-γ whereas, live culture induced prolonged up-regulation of IL-4, IL-5 and IL-13, IL-10 genes. Through the cytokines up-regulation and down-regulation are closely associated with mRNA replication, pathogenesis, and immunity, yet, further studies are necessary to elucidate their exact function in host-induced pathogenesis and immunity.

Conflict of Interest

Author says there is no conflict of interest.

Reference

1. Antonakos N, Tsaganos T, Oberle V, Tsangaris I, Lada M, Pistiki A, *et al*. Decreased cytokine production by mononuclear cells after severe gram-negative infections:

early clinical signs and association with final outcome. Critical Care. 2017;21(1):1-10.
2. Becker Y. The changes in the T helper 1 (Th1) and T helper 2 (Th2) cytokine balance during HIV-1 infection are indicative of an allergic response to viral proteins that may be reversed by Th2 cytokine inhibitors and immune response modifiers–a review and hypothesis. Virus genes. 2004;28:5-18.
3. Bhuiyan MSA, Amin Z, Rodrigues KF, Saallah S, Shaarani SM, Sarker S, *et al*. Infectious bronchitis virus (Gamma corona virus) in poultry farming: Vaccination, immune response and measures for mitigation. Veterinary Sciences. 2021;8(11):273.
4. Calum HP, Moser C, Jensen PO, Bjarnsholt T, Givskov M, Høiby N. Early IL-2 treatment of mice with Pseudomonas aeruginosa pneumonia induced PMN-dominating response and reduced lung pathology. Apmis. 2020;128(12):647-653.
5. Garcia M, Morello E, Garnier J, Barrault C, Garnier M, Burucoa C, *et al*. Pseudomonas aeruginosa flagellum is critical for invasion, cutaneous persistence and induction of inflammatory response of skin epidermis. Virulence. 2018;9(1):1163-1175.
6. Gurjar NR, Shringi BN, Kumar R, Miland M, Sharma DK. Cytokine profiling in the acute phase of viral vaccination in Poultry. The Indian Journal of Animal Sciences. 2023;93(6):561-565.
7. Kioi M, Kawakami K, Puri RK. Analysis of antitumor activity of an interleukin-13 (IL-13) receptor-targeted cytotoxin composed of IL-13 antagonist and Pseudomonas exotoxin. Clinical cancer research. 2004;10(18):6231-6238.
8. Lenka S, Bhuyan SK, Bhuyan R. Deregulation of cytokine affecting oral neutrophil subsets in oral cancer. Medical Oncology. 2023;40(11):307.
9. Murphey ED, Sherwood ER. Pre-treatment with the Gram-positive bacterial cell wall molecule peptidoglycan improves bacterial clearance and decreases inflammation and mortality in mice challenged with Pseudomonas aeruginosa. Microbes and infection. 2008;10(12-13):1244-1250.
10. Penaloza HF, Schultz BM, Nieto PA, Salazar GA, Suazo I, Gonzalez PA, *et al*. Opposing roles of IL-10 in acute bacterial infection. Cytokine & growth factor reviews. 2016;32:17-30.
11. Tan Y, Liu H, Yang H, Wang L, Qin X. An inactivated Pseudomonas aeruginosa medicament inhibits airway allergic inflammation and improves epithelial functions. The Journal of Physiological Sciences. 2013;63:63-69.
12. Tiringer K, Treis A, Fucik P, Gona M, Gruber S, Renner S, *et al*. A Th17-and Th2-skewed cytokine profile in cystic fibrosis lungs represents a potential risk factor for Pseudomonas aeruginosa infection. American journal of respiratory and critical care medicine. 2013;187(6):621-629.
13. Zhao Q, Luo YF, Tian M, Xiao YL, Cai HR, Li H. Activating transcription factor 3 involved in Pseudomonas aeruginosa PAO1-induced macrophage senescence. Molecular immunology. 2021;133:122-127.
14. Bhuyan A. Experts criticise India's complacency over COVID-19. The Lancet. 2021 May 1;397(10285):1611-2.