



## STUDY ON HUMORAL AND CELL MEDIATED IMMUNE RESPONSES FOLLOWING *PASTEURELLA MULTOCIDA* LIPOPOLYSACCHARIDES IMMUNIZATION IN RABBITS

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### ABSTRACT

*Pasteurella multocida* infection is a major disease in most of animal species. The lipopolysaccharides (LPS) and outer membrane protein (OMP) are the main surface antigens responsible for the immunization, microbiological significance and for pathogenesis of the disease. For this reason a local strain of *Pasteurella multocida* was isolated, their lipopolysaccharides LD50 was 300 $\mu$ /ml. Fifteen rabbits were S/C immunized with LPS 30  $\mu$ / Kg / rabbit, 2 doses and at 2 weeks intervals . The other 15 rabbits were injected with phosphate buffer saline (PBS) as a control group. High level of antibodies were detected in immunized LPS group with the meant  $\pm$  SE (140  $\pm$  31.35) whereas, in control group (2  $\pm$  0.063). Also the LPS group showed high level of cell mediated immune response detected by delayed type hypersensitivity skin test and high concentration level of interferon gamma (INF - ) and tumor necrosis factor – alpha (TNF - ) comparable to the control group

**KEYWORDS:** Outer membrane protein, immunization, hypersensitivity, interferon.

### INTRODUCTION

*Pasteurella multocida* lipopolysaccharides (LPS) membrane proteins (OMP) are the main major surface antigen, possessing both the microbiological and immunological significance and mediate the direct interaction between the microbial agents and their surrounding environments [1,2]. As soon as bacteria enters the body, it is comforted with two lines of defense a humoral line and cellular line. The humoral factors was included complement, antibodies, and a cute phase proteins whereas, the cellular lines of defense included the mononuclear cells and neutrophils through which release of pro-inflammatory cytokines such as tumor necrosis factor- alpha, interleukins–1, IL6, IL8 and platelets activating factor and other chemokine's[3,4]. Activated neutrophils expressed CD14, CD11 and CD8 and several complement and FC receptors and are thus able to recognize and phagocyte the lipopolysaccharides and outer membrane protein's (LPS and OMP)[5]. The inflammatory mediators secreted by the different cell populations attract and activate B and T lymphocytes, in turn, it release the IL2 and IFN-  $\gamma$  and granulocytes and macrophage colony stimulating factor (GM – CSF). Through the Importance of LPS in induction of humoral and cellular immune response, this study aimed at to estimate both humoral and cell mediated immune response against the LPS Ag of *Pasteurella multocida* in rabbits using passive hemagglutination test, DTH– skin test , INF  $\gamma$  and TNF- $\alpha$  test.

### MATERIALS & METHODS

*Pasteurella multocida* strain was supplied by AL Kindi Company for veterinary drugs and vaccine production, Baghdad Iraq. This bacterial agent was re-identified to be sure *Pasteurella multocida* using cultural, biochemical, API- 20 K.t (Biomérieux, USA[6] and their LPS were extracted and purified[7,8]. LD50 for LPS was 300 $\mu$ /ml[9]. Fifteen rabbits were S/C injected with 30 $\mu$  of LPS /Kg / rabbit; other group 15 rabbits were injected with phosphate buffer saline (PBS) as a control group. The injection occurred at two doses and 2 weeks intervals. At 15<sup>th</sup> and 27<sup>th</sup> days, both humoral and cell mediated immune responses estimated using passive hem agglutination test and delayed type hypersensitivity skin test [10]. Tumor necrosis factor – alpha and interferon gamma using Enzyme linked immunosorbant Assay (Alisa)[11].

### RESULTS & DISCUSSION

Humoral immune response; passive hemagglutination test: The mean of antibodies titers were increased significantly (P< 0.05) in the immunized group with LPS compared to the control non immunized group (140 $\pm$ 31.35) and 2 $\pm$ 0.63 respectively .

#### Cellular immune response

1. Delayed type hypersensitivity skin test there is gradual increase in the skin fold thickness in rabbits at the site of Ag injection during 24hr and 48 hrs ( P<0.05) comparable to control group skin thickness (table -1).

**TABLE -1** Delayed type hypersensitivity (DTH – skin test) in immunized LPS and control groups of rabbits

Group	Zero hr	24 hr	48 hr	72 hr
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Immunized LPS	2.22±0.11	4.98±0.28	5.9±0.32	3.96±0.05
Control PBS	2.1±0.134	2.3±0.178	2.22±0.18	2.16±9.09

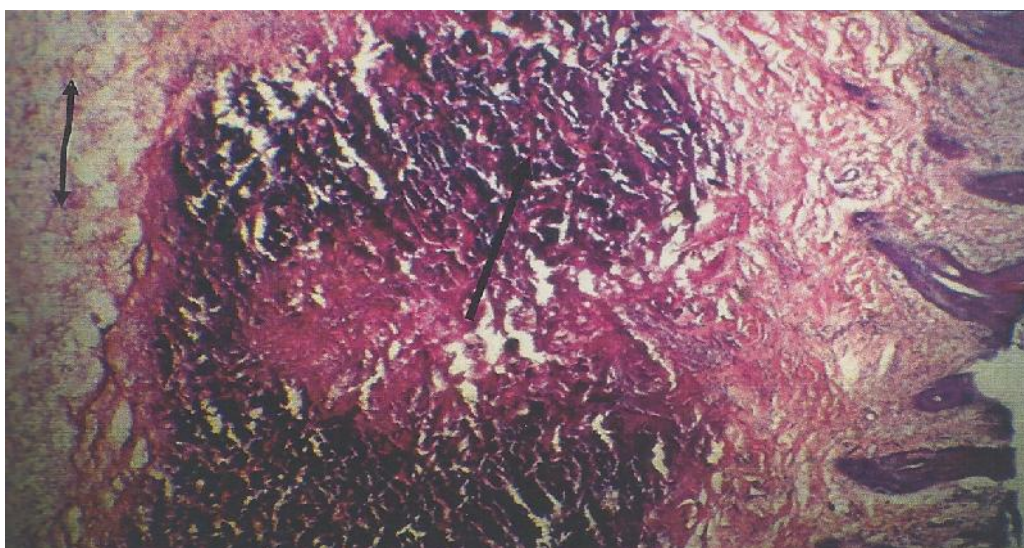
(P< 0.05 )

## 2. Gamma interferon – IFN - $\gamma$

There is increase in the concentration of IFN -  $\gamma$  in the immunized LPS group compared with the control group with high significant mean concentration in the immunized LPS group comparable to the control PBS group ( $387 \pm 25.51$  Pg/ml) in immunized LPS group and below  $< 62$  Pg/ml in control PBS group.

3. Tumor necrosis factor alpha (TNF-  $\alpha$ ) there is significant increase (P< 0.05) in the concentration of the TNF-  $\alpha$  in the immunized LPS group compared with control PBS group. The mean level of TNF-  $\alpha$  concentration was  $450.9 \pm 37.59$  Pg/ml. In immunized LPS group whereas, in control PBS group was below  $< 78$  Pg/ml). The high level of antibody response in the group of immunization indicates that the LPS has a pivotal role in enhancement of antibody response<sup>[12]</sup>, which detected in this study and the LPS has a mitogenic effect of B cell region, through their activation of macrophages to secrete IL1 which in turn enhance TH2 to release IL4 and IL5 to provoke B-lymphocytes differentiation and proliferation to plasma cells and producing antibodies<sup>[13,14]</sup>. Similarly the LPS a potent mutagen stimulate cellular immune response detected by the skin test<sup>[15]</sup>, that the ability of the TH1 cells to recognize the LPS and secrete IL2 as a

chemotactic factor to attract macrophages around the area of activated T cells which also secrete IFN- a good activator for the macrophages at the site of Ag injection in the skin causing skin thickness and erythema and central necrosis which more evident in this study, together with edema, congestion and neutrophils infiltration (Fig-1). The inflamed cellular reaction began with neutrophils during 24hrs and lymphocytes and macrophages later on 48 hrs, similarly observed by<sup>[16]</sup>, and these inflammatory cells occurred as a result of previous exposure for similar Ag<sup>[17]</sup>. This study revealed that increase in the level of TNF-  $\alpha$  in immunized LPS group comparable to the control. TNF-  $\alpha$  generated by macrophages in a response to LPS<sup>[18,19]</sup>. The IFN- $\gamma$  also produced as a response of the killer cells, TH1CD4 and cytotoxic cells CD8 against the LPS stimulation. IFN -  $\gamma$  called the later activator for macrophages, increase Ag presentation and the lysosomal activity of macrophages and promotes adhesions and binding required for leukocytes migration in response to LPS<sup>[20]</sup>. A similar response to porins of outer membrane proteins that stimulate releasing of proinflammatory and immunomodulatory cytokines, such as tumor necrosis factor-  $\alpha$ , IL1, IL6 by monocytes and IFN-  $\gamma$  and IL4 by lymphocytes an essential cytokines in immunity against the pathogens<sup>[21]</sup>.



**FIGURE-1:** Skin section of rabbit in LPS group after 47hr post injection showing infiltration of neutrophils, edema and congestion in epidermis (→) and dermis infiltrated with mononuclear cells, edema and necrosis (↔) (H & E x40)

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