DETECTION OF IMMUNE RESPONSE OF LIPOPOLYSACCHARIDE (LPS) OF PASTEURELLA MULTOCIDA B: 2 (P52) IN RABBITS BY DOT-ELISA*

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ABSTRACT

Present study detects the nature of immune response of LPS of Pasteurella multocida (P52) in 4 groups of rabbits that were challenged with P. multocida on 21st, 14th, 7th and 5th DPI. The results of dot-ELISA from collected serum samples manifested gradual increase of antibody titre from 5th DPI to 21st DPI. However, in contrast, this rising antibody titre was adjudged unprotective against challenge with P. multocida.

INTRODUCTION

Haemorrhagic Septicaemia (HS) is considered as one of the most economically important disease of dairy industry in India (Bhalla, 1997). HS is caused by B: 2, B: 2.5 and B: 5 serotypes of Pasteurella multocida in Asia and by E: 2 serotypes in African countries. Since after the progressive control of rinderpest, HS accounts for the highest proportion of reported mortality, reaching upto 50-60% in cattle and buffaloes (Singh et al., 1996). Humoral immunity plays an important role in protection of the disease (Confer, 1993). Vaccination of cattle and buffalo against HS is an effective practice to control the disease in livestock. Commonly used vaccines like oil adjuvant vaccine (OAV) is unpopular among livestock practitioners due to its high viscosity (DeAlwis, 1992) and duration of protection afforded by aluminium gel bacterin is very short. The continuing efforts for search of better vaccine products capable of inducing a long lasting immunity, present study was conducted in rabbit model using lipopolysaccharide (LPS) of P. multocida as antigen. This study also elucidated to understand the role of a specific class of immunoglobulin in protection against HS.

MATERIAL AND METHODS

P. multocida (P52) strain was procured from the Type Culture Laboratory, Division of Standardization, IVRI, under freeze-dried condition. The freeze-dried culture was rejuvenated on blood agar (HiMedia Ltd, India), brain heart infusion agar (BHI, HiMedia Ltd, India) and the purity of the culture was checked. The rejuvenated culture was passaged through a buffalo calf and the organism was reisolated on blood agar. LD₅₀ of P. multocida (P52) in rabbits was detected as per the method of Reed and Muench, (1938).

LPS was extracted from phase-1 culture of P. multocida (P52) by phenol water method of Westphal and Jann, (1965). LPS immunizing agent was prepared in the composition containing LPS 100 µg/ml of vaccine with 10 parts sterile liquid paraffin (HiMedia, India) and 1 part sterile lanoline. Viscosity of the preparation was reduced by mixing with equal volume of formal saline containing 2% Tween-80 (HiMedia, India). Sterility and safety of LPS immunizing agent was checked by the method described in Drug and Cosmetics Act, India.

In immune response experiment, 22

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healthy albino rabbits (*Lepus cuniculus*) of either sex, weighing not less than 1 kg were procured from Laboratory Animal resources (LAR) section, IVRI. Rabbits were screened to detect anti-*P. multocida* antibody by slide agglutination test (SAT) using *P. multocida* antigen. They were divided in 4 groups i.e., each of 5 rabbits and 2 controls. During the 21 day of immune response experiment, all the rabbits of 4 groups were immunized with 2 ml LPS immunizing agent in such a schedule so that, immunized animal of group-I was challenged with 10 LD$_{50}$ dose of 18 hr old *P. multocida* (*P$_{52}$*) grown in BHI broth by the S/C route on 21$^{st}$ day post immunization (DPI), group-II on 14$^{th}$ DPI, group III on 7$^{th}$ DPI and group IV on 5$^{th}$ DPI. Serum samples were collected from immunized animals on 0 day and just before challenge. Serum from control animals was collected on 0 and 21$^{st}$ day, just before challenge infection.

Immune response was monitored by modified method of dot-ELISA (Srivastava *et al.*, 2000). Antigen for the test was prepared by sonicating the *P. multocida* (*P$_{52}$*) cell suspensions at 10 $\mu$m for 2 min in 5 cycles. The material was then centrifuged at 10000 x g for 20 min and the supernatant was collected as antigen.

**RESULTS AND DISCUSSION**

Present study showed that, dot-ELISA was a rapid and cost effective method to detect the systemic immune response. Result revealed a gradual increase in the intensity of dots with HRPO conjugated anti-rabbit goat globulins (Fig. 1) from 5$^{th}$ DPI to 21$^{st}$ DPI. But in contrast, protection study in rabbits immunized with LPS immunizing agent revealed 20%, 20%, 0%, 0% protection, respectively on 21$^{st}$, 14$^{th}$, 7$^{th}$ and 5$^{th}$ DPI against the challenge of *P. multocida*. This unprotective nature of LPS is consistent with the earlier reports of various workers (Ramdani *et al.*, 1991; Muniandy *et al.*, 1993; Adler *et al.*, 1996). As LPS is a thymus-independent antigen (TI antigen, Kuby, 2000) it predominantly produces Ig-M type of immunoglobulin (Muniandy *et al.*, 1998) in mice, which is insufficient to check the exponentially growing organisms in the tissue (Kuby, 2000).

![Fig. 1. dot-ELISA of LPS immunized rabbit showing decreased intensity of dot from 21st DPI to 5th DPI (left to right including control at the extreme right)](image-url)
REFERENCES