Research Paper

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Serum Antibody Response Against *Listeria ivanovii* in Experimentally Infected Rabbits

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The research work wasconducted to investigate the serum antibody response against *Listeria ivanovii* in experimentally infected rabbits by inoculation method. It was found out that the blood monocyte count was increased, from 2-3% (prior injection) up to 15% to 72 hours post injection and the count declined after 120 hours. There was a significant rise in mononuclear cells after the repetition of doses except that the level was maintained during these intervals. The highest antibody level was observed at 14-15 days post-inoculation, which persisted for more than three months. These findings suggest that an association exists between the increased number of monocytes in the blood and humoral immune response in correlation with the resistance against listeric infection.

Key Words: antibody, *Listeria ivanovii*, rabbit

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Introduction

Listeriosis is an infection of animals and humans, which shows diversity of clinical symptoms. Human listeriosis has a short but deadly history. Until the early 1980, human cases of listeriosis were considered, an accidental infection with no relevant source found (Seeliger, 1988). Recent interest in many countries is mainly due to food borne outbreaks (Bille, 1990; Farbar and Peterkin, 1991).

Host susceptibility and survival of *L. monocytogenes* is a major factor in the epidemiology of Listeriosis, as most outbreaks and sporadic cases involve persons with impaired cell-mediated immunity due to disease process, medication, aging (Lammerding *et al.*, 1992). Pregnant women represents the high risk group *of L. monocytogenes* infection. Abortion, still birth or neonatal infection can be the serious out come of such an infection. (Abram and Doric, 1997). *L. monocytogenes* and *L. ivanovii* are considered the only pathogenic species of the genus Listeria (Rocourt *et al.*, 1993).

Much has been learnt and documented regarding the essential role of cell mediated immunity in listeric infections (Hahn and Kaufman, 1981; North 1973). The time course of antibody production and its relation to the course of naturally occurring listeriosis are poorly characterized in most of the animals and humans except those in mice. Listeric infection causes a considerable mononuclear reaction in most of the monogastric animals including men. The relationship between monocyte producing agent and virulence of *Listeria* strains is not very clear and remains to be studied.

Therefore, the research work was undertaken to study humoral immune response of rabbits experimentally infected with *L. ivanovii*. The relation between antibody levels and clinical characteristics were determined, the difference in monocyte counts in infected rabbits was also studied.

Materials and Methods

Animals: Set of five rabbits were used as test animals. And set of three rabbits were used as control; two of them received only sterile saline, while the third one was used as un inoculated control. Animals were kept under sanitary conditions in the animals house of the H. E. J Research Institute of chemistry, University of Karachi, fed conventional diet and given water ad libitum. Before inoculation all the animals were kept under observation for one week. During this period the blood samples were drawn and examined for any possible infection.

Strains: Serological reference type-5 strain No. 2379 of *L. ivanovii* was used.

Inoculation procedure: The purity of culture was checked by microscopy, cultural characteristics, motility and hemolysis on blood agar plates. For the further confirmation CAMP TEST was also performed with *Staphylococcus aureus* strain No. 25923.

Prior to experimental infection of rabbits, the virulence of infecting strain was checked in mice via intraperitoneal route. The culture was then grown in tryptone soy broth (TSB) (Oxoid) at 37°C for 24 hours. Cells were removed by centrifugation at 6000rpm for 20 minutes washed twice with sterile phosphate buffer saline (PBS, pH-7.0) to remove any trace of medium and finally the cells were re-suspended in PBS, the turbidity was matched with McFarland index. The number of viable organisms was also determined by duplicate platting on solid agar (Miles and Misra, 1938). Each animal was injected intravenously with 1×10^8 cells ml^{-1}.

Preparation of Antigen: Strain of *Listeria* type-5 have a tendency of autoagglutination. To avoid this and to get reliable results fresh antigen was prepared and mixed in small quantities according to the method of Miettinen *et al.*,(1990). For antigen preparation *L. ivanovii* was grown in TSB, cells were harvested and finally re-suspended in PBS followed by heat treatment at 60°C for 20 minutes.

Immunization and blood sample collection: Animals were given three doses with an interval of one week using intravenous route in marginal vein of ear. Control rabbits were injected with diluent (Sterile Saline). Animals were bled just before inoculation, then daily for the first week of post inoculation for determining monocyte count. Blood smears were prepared, stained with Giemsa's stain and monocyte count was determined. To determine the serum antibody titreblood samples were drawn weekly during the first month after primary inoculation and at two week intervals thereafter for three months. Antibody titre was determined by rapid slide agglutination test (Nichols and Nakamura, 1980), against respective antigen. Positive and negative controls were run simultaneously to exclude any possibility of autoagglutination. Results were evaluated by calculating Standard Error Mean(SEM) from arithmetic mean of values from each set of animals using statistical software STATISTIC for Windows, Release –5.

Results

After 24 hours, the animals developed only mild clinical symptoms, which persisted for 2-3 days. Complete recovery was observed in 4 days Table 1 showed blood monocyte count of rabbits after giving injection of *L. ivanovii* live cells. It is clear from the data that prior to injection blood monocytes count was found to be between 2-3%. After inoculation monocytes level increased up to 13% within 48 hours and it

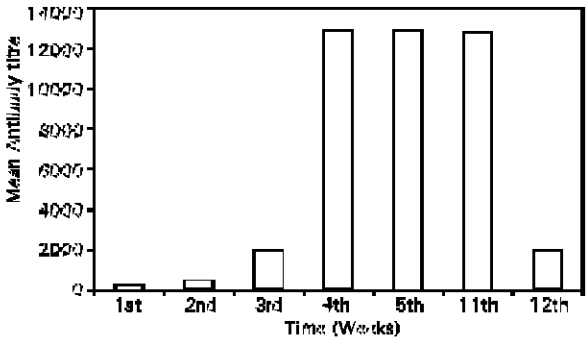

Fig. 1: Effect of *L. ivanovii* SLCC 2379 on the antibody titre under sustained monocytosis in rabbits
Dose 10^8 CFU ML^{-1}
Results are expressed as arithmetic mean of animals, n = 5
SEM ranges ± 0.7..... ± 1.05

Table 1: Monocyte producing ability of *Listeria ivanovii* Monocyte count (% hours)

Animals	Pre-injection	Post-injection					
Time (hours)	0	24	48	72	96	120	144
Test rabbits							
1st week	2-3	8	13	15	13	13	11
2nd week	11.0	13	12	12	12	11	10
3rd week	10.0	12	12	12	12	10	10
Control Rabbits (Inoculated with sterile saline)	2-3	2.5-3	2-3	2-3	2.5-3	2-3	2-3
Un-inoculated Control	2-3	2.5-3	2-3	2-3	2.5-3	2-3	2-3

Five rabbits were used as test animals
Two rabbits were used as sterile saline inoculated control.
SEM ranges ± 0.02 ..... ± 0.38
One rabbit was used as un inoculated control
Results expressed as arithmetic mean of values from each set of animals.
No of trials = 2

reached maximum 15% after 72 hours. A decrease in monocyte count was noticed after 120 hours. No further increase in monocytes count was found throughout the research work.

The serum antibody level was determined (Fig. 1). It was noted that there was an immediate response in the rabbits. The primary injection induced an eventual increase in the antibody titre reaching at maximum level in one week after booster inoculation. The antibody titre persisted for three months after 2nd booster inoculation and then started declining reached at the lowest level after 105 days post immunization.

There was no significant rise in monocyte count after the booster doses but the level of circulating blood monocyte was maintained to 11-13 % after the secondary dose.

Discussion

In this investigation, an experimental listeriosis was induced in rabbits by using an intravenous route of injection with *L. ivanovii* serovar-5. In the current series of experiments consisting of five rabbits, we recorded a markedly enhanced antibody response, which persisted in the serum for more than 3 months.

As evident from the results that there is a good correlation between inoculated dose and serological response. The first and second booster maintained the level of antibodies in the serum for longer periods of time. The pre-existing antibodies in serum were associated with rapid clearance of bacterial cells from blood streams and the absence of clinical symptoms, suggested that they were related to the resistance against the organism, as is evident from the experimental results. The present data indicated that antibodies producing cells in collaboration with circulating phagocytes mediate immunity by rapidly destroying *listeria*. As there is an evidence of progressive increase in the density of antibodies as the promonocyte matures into the circulating monocytes (Territo and Cline, 1975). Recognition of antigens by the T-lymphocytes is usually proceeded by phagocytosis. (Waldron, 1973).

Results are in partial agreement with Miettinen *et al.* (1990) who demonstrated the persistent high level of anti-listerial antibodies in serum of experimentally infected goat with *L. monocytogenes*. In our experimental conditions *L. invanovii* strains induced strong monocytosis. However, there is no explanation of this finding, it is clear that there is no strong relationship among monocyte producing activity and the presence of other virulence markers of Listeria strains. In conclusion, the data showed that, antibodies are important for the implications of resistance against listerial infections, along with the cell-mediated immune mechanisms.

More knowledge, regarding the antigenic specificity and determinants of the persistent antibody responses are required.

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