IMMUNIZATION OF MICE AGAINST *LISTERIA MONOCYTOGENES*

H. F. HASENCLEVER[1] AND W. W. KARAKAWA

Department of Bacteriology, College of Medicine, State University of Iowa, Iowa City, Iowa

Received for publication April 26, 1957

Listeria monocytogenes has a wide range of susceptible hosts. It has been isolated from numerous cases of listeriosis in sheep, cattle, hogs, chickens, and man (Hull, 1955). The disease occurs as a generalized septicemia, meningitis, and meningoencephalitis.

Several investigators have attempted to immunize animals against *L. monocytogenes*. Julianelle and Pons (1939) demonstrated that animals which recovered from a conjunctivitis due to this organism were refractile to subsequent conjunctival exposure, but were not immune to other routes of challenge. Their results also indicated that antiserum of high titer to Listeria did not confer any passive protection. Graham *et al.* (1940) were unable to find any increase in resistance to challenge in rabbits, chickens, guinea pigs, or sheep after immunization with formalinized suspensions of cells. Similar results were obtained using suspensions of viable Listeria. Likewise these workers were unable to demonstrate any protective ability of high titered antiserum to Listeria.

Our attempts to immunize mice with heat killed or formalinized suspensions of Listeria were also unsuccessful. Osebold and Sawyer (1957), however, reported that mice receiving sublethal doses of living virulent organisms subcutaneously were immune when subsequently challenged with a comparatively large number of virulent bacteria. Our investigation which was started independently before the aforementioned results were in print are confirmatory. In addition this report presents the results obtained with two routes of immunization, and several routes of challenge.

MATERIALS AND METHODS

The mice used in this study were an inbred Swiss white strain from a colony maintained in our department.

The strain of *L. monocytogenes* employed was one isolated in this laboratory, from a case of human listeriosis. The organism was cultivated on 5 per cent sheep's blood, beef infusion agar slants for 24 hr at 37 C. Growth from the slants was suspended in sterile 0.9 per cent physiological saline and diluted to a turbidity approximating that of a no. 3 MacFarlane nephelometer tube. Logarithmic dilutions, in sterile saline, using a separate, sterile pipette for each dilution, were then made from this suspension. To determine more accurately the number of viable cells present, duplicate pour plates, employing trypticase soy agar, were utilized. Experience indicated that reproducible results were consistently obtained by this procedure.

Virulence studies on *L. monocytogenes* indicated that a suspension of 2×10^2 to 5×10^2 cells was not lethal to mice. The immunization procedure consisted of injecting 0.1 ml of a sublethal dose of Listeria either intraabdominally or subcutaneously. Approximately 10 to 14 days later the mice being immunized were administered the next lower logarithmic dilution by the same route of inoculation. Ten days after the second immunizing dose the test animals were challenged. Nonimmune mice of the same age (8 weeks) were used as controls. Groups of 3 to 6 mice were used for each dilution of the organism. Death resulted in 1 to 7 days after challenge. Cultures from various tissues and organs showed that the animals died of generalized listeriosis.

RESULTS AND DISCUSSION

The results obtained from either intraabdominal or subcutaneous immunization with sublethal doses of *L. monocytogenes* are presented in tables 1 to 4.

As can be seen in table 1 an immunity exists with the magnitude of at least 1000-fold in mice immunized by the intraabdominal route, followed by intraabdominal challenge. Mice immunized intraabdominally and subsequently receiving 0.1 ml of virulent Listeria intravenously demonstrated approximately a 100-fold increase

[1] Present address: Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, Bethesda 14, Maryland.

in acquired resistance over the normal controls (table 2). Table 2 also presents the results obtained following intranasal instillation of 0.05 ml of the suspension to animals under light ether anesthesia. Results from intraabdominally immunized mice and controls injected intracerebrally are illustrated in table 3. The data shows that *L. monocytogenes* is extremely virulent when administered by this route.

The results of subcutaneous immunization are shown in table 4. Immunized animals injected intraabdominally displayed a considerably increased protection over the nonimmune group, whereas by intravenous challenge a greater than 10-fold protection was present.

Circulating antibodies could not be detected in serum from immune mice by agglutination, precipitation, complement fixation, or by passive immunization of normal animals.

Immunity to *L. monocytogenes*, as evidenced by these results, is quite unique in that a relatively high level of acquired resistance is observed, without the presence of a demonstrable antibody. The studies of Julianelle and Pons (1939), Graham *et al.* (1940), and Osebold and Sawyer (1957) illustrate that a high titered antiserum against Listeria has little or no protective ability. This evidence is highly suggestive that circulating antibodies are not responsible for immunity in mice to listeriosis.

This investigation illustrates that immuniza-

TABLE 1

Intraabdominal immunization followed by intraaabdominal challenge

Mice	Approximate Number of Organisms Injected				
	6×10^6	6×10^5	6×10^4	6×10^3	6×10^2
Immune	0/6*	0/6	0/6	—†	—
Normal	—	6/6	5/6	5/6	1/6

* Number of mice dead/number of mice inoculated.

† — = This dilution not done.

TABLE 2

Intraabdominal immunization followed by intravenous or intranasal challenge

Route of Challenge	Mice	Approximate Number of Organisms Injected				
		6×10^6	6×10^5	6×10^4	6×10^3	6×10^2
Intravenous	Immune	6/6*	4/6	0/6	—†	—
	Normal	—	6/6	6/6	5/6	0/6
Intranasal	Immune	4/6	5/6	2/6	0/6	—
	Normal	—	6/6	6/6	6/6	1/6

* Number dead/number inoculated.

† — = This dilution not done.

TABLE 3

Intraabdominal immunization followed by intracerebral challenge

Mice	Approximate Number of Organisms Injected			
	800	80	8	0.8
Immune	4/4*	4/4	1/3	0/3
Normal	3/3	4/4	3/3	1/3

* Number dead/number inoculated.

TABLE 4

Subcutaneous immunization followed by intraabdominal or intravenous challenge

Route of Challenge	Mice	Approximate Number of Organisms Injected				
		6×10^6	6×10^5	6×10^4	6×10^3	6×10^2
Intraabdominal	Immune	0/6*	0/6	0/6	—†	—
	Normal	—	6/6	4/6	0/6	0/6
Intravenous	Immune	6/6	5/6	3/6	0/6	—
	Normal	—	6/6	6/6	6/6	0/6

* Number of mice dead/number of mice challenged.

† —= This dilution not done.

tion with this organism can be accomplished, but it must also be recognized that the level of immunity varies with the route of challenge.

Studies on attempts to immunize animals with pasteurized infected organs and saline extracts of these organs are now in progress. Other investigation includes *in vitro* phagocytosis, and the passive effect of whole blood from immune mice.

SUMMARY

Active immunization of Swiss white mice against listeriosis with sublethal numbers of virulent *Listeria monocytogenes* has been demonstrated. The level of immunity varies with the route of challenge. Immune animals are most resistant to the organism given by intraabdominal administration, and least resistant to intracerebral infection.

Circulating antibodies could not be observed in the serum from immune donor mice by agglutination, precipitation, and complement fixation technique nor by passive transfer.

REFERENCES

GRAHAM, R., MORRILL, C. C., AND LEVINE, N. D. 1940 Studies on Listerella. IV. Unsuccessful attempts at immunization with living and dead Listerella cultures. The Cornell Veterinarian, **30**, 291–298.

HULL, T. G. 1955 *Diseases transmitted from animals to man*, 4th ed. Charles C Thomas, Springfield, Ill.

JULIANELLE, L. A. AND PONS, C. A. 1939 Immunological and serological reactions of *Listerella monocytogenes*. Proc. Soc. Exptl. Biol. Med., **40**, 364–365.

OSEBOLD, J. W. AND SAWYER, M. T. **1957** Immunization studies on listeriosis in mice. J. Immunol., **78**, 262–268.