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SOME ASPECTS OF MURINE EXPERIMENTAL LISTERIOSIS

By

Raimo Pohjanvirta and *Tarja Huttunen*

POHJANVIRTA, R and T. HUTTUNEN: *Some aspects of murine experimental listeriosis.* Acta vet. scand. 1985, *26*, 563—580. — A set of experiments was carried out in order to approach the complex nature of L. monocytogenes infections from different aspects. Experiment 1 showed that Listeria are able to gain admission to body by numerous ways and both subcutaneous and oral entry can lead to fatal septicemia. It also gave slight support to the theory of direct neural transmission of Listeria to the brain and indicated the possibility that intestinal absorption after oral exposition at least partly occurs via lymphatic vessels. No inflammatory reaction could be caused to mice by ocular flushing with Listeria suspension.

The second trial proved that there are vast differences in the animal pathogenecity of Listeria strains — even among those of the same serotype. In experiment 3A the abolishing effect of dextran sulfate on the early resistance of mice to Listeria was confirmed and it turned out that cortisone at a therapeutic dose level did not bring about that phenomenon. Levamisole granted no conspicuous enhancement of resistance in this acute challenge; however, the results of the immunity test (3B) suggested that levamisole may be beneficial during the induction phase. On the other hand, starvation appeared to impair long-term immunity. Likewise, in experiment 4 starved mice were quite susceptible to acute challenge with Listeria. Raised ambient temperature, on the contrary, prominently increased the survival rate of the animals.

Owing to the fairly small number of animals these results should be regarded as preliminary starting points to further studies.

route of exposure; strain pathogenicity; dextran sulfate-500; cyclosporin-A; hydrocortisone; starvation; ambient temperature.

Cell-mediated immunological factors of Listeria monocytogenes infections have been subjects to extensive research during the past decade. However, from the veterinary standpoint many important stages of pathogenesis have still remained unresolved. For example, it is not known to what extent the arising lesions in listeriosis are dependent on the route of infection. The sig-

nificance of differences in pathogenicity and virulence among Listeria strains is also somewhat obscure. At least 4 distinct syndromes — meningoencephalitis, septicemia, abortion and keratoconjunctivitis — are associated with Listeria monocytogenes in ruminants. It is well established that various Listeria serotypes can be ranked according to their mouse pathogenicity (*von Koenig et al.* 1983), but it would be interesting to know, whether strains belonging to the same serotype but isolated from different syndromes in ruminants cause similar lesions in mice. Likewise, the effects of ambient temperature, starvation and some modern immunomodulating drugs on the course of listeriosis have not yet been sufficiently examined.

It was our aim to elucidate these problems by experiments made on mice.

MATERIALS AND METHODS

The mice used belonged to the strain DBA/2N/Kuo, which has been shown to be genetically susceptible to Listeria monocytogenes infection (*Skamene & Kongshavn* 1983). The mice were purchased from the National Laboratory Animal Center, Kuopio, Finland and were about 8 weeks old at the onset of the experiments. Males and females were used in equal numbers, excluding experiment 2, in which all animals were females. During the experiments the mice were housed in cages of 4—8 animals. They had free access to water and feed (Lab-3, Ewos, Sweden), except when they were being starved in experiments 3B and 4. With minor deviations the mice were monitored daily. The sacrifice was accomplished by cervical dislocation under ether anaesthesia.

The strains of Listeria monocytogenes were obtained from the National Veterinary Institute, Regional Laboratory in Kuopio. Usually we used a strain which had been isolated from bovine nonpurulent meningoencephalitis, in experiment 2 also strains isolated from bovine keratoconjunctivitis and abortion. All these strains belonged to serotype 1. The bacteria had been stored at —70°C. The melted suspension was cultured in bovine blood agar. A suitable number of colonies were diluted in physiologic (0.9 %) saline and the bacterial count was always checked by a serial 10-fold dilution.

The spleens were aseptically removed and homogenated in 10 ml of sterile 0.9 % saline. The number of Listeria in 1 ml of

spleen homogenate was determined by plating 0.1 ml portions of serial 1:100 or 1:10 dilutions on bovine blood agar and counting CFU after incubation at 37°C for 16 to 20 h.

A complete necropsy was performed on each animal that died or was sacrificed. Samples were taken from the brains, heart, lungs, liver and kidneys; in experiments 1 and 2 the right eye and uterus were also examined, respectively. The tissues obtained at necropsy were fixed in 10 % buffered formalin, the brains and eyes in Davidson's fixative. The tissues were embedded in paraffin, sectioned at 5 µm and stained in a routine manner with hematoxylin-eosin.

The statistical calculations in experiment 3A were made by using the t-test.

Experimental design

In each experiment the mice were randomly divided in groups of 4—8 animals.

Experiment 1. The aim of this study was to find out the possible dependence of histopathological lesions and animal survival on portals of entry of bacteria by administering Listeria suspension by different routes either once or for 5 consecutive days.

Group 1 received 8×10^6 CFU Listeria once in trial 1A and 8×10^5 CFU/day for 5 days in 1B epiconjunctivally on the right eye (the eye was flushed with 0.2 ml of the suspension).

The mice in group 2 were administered with a gastric tube either 8×10^6 or 8×10^5 CFU, respectively.

The animals in group 3 received Listeria suspension either 8×10^4 or 8×10^3 CFU subcutaneously (= sc.) on the back of the neck.

The mice in group 4 were challenged intraperitoneally (= i.p.) with the same doses of Listeria as their counterparts in group 3.

Group 5 comprised control animals and they received 0.9 % sterile saline, 1 mouse was exposed via each of the tested routes. Two days later they were inadvertently given low doses of Listeria (see Table 1).

In every instance the volume administered was 0.2 ml. The mice that did not die spontaneously were sacrificed after a period of about 2 weeks.

Experiment 2. The goal of this experiment was to compare the pathogenicity and virulence of 3 Listeria strains, all of which were of bovine origin and serotype 1.

Group 1 was challenged with a strain isolated from a case of bovine nonpurulent encephalitis with the dose of 5.7×10^3 CFU, group 2 with a strain cultured from bovine keratoconjunctivitis with the dose of 5.6×10^3 CFU and group 3 received a strain isolated from bovine abortion at the dose of 5.9×10^3 CFU. The volumes of the suspensions were 0.3 ml, 0.2 ml and 0.1 ml, respectively. All injections were given i.p. and the challenge took place only once.

Group 4 served as a control and these mice were administered 0.2 ml sterile 0.9 % saline i.p.

The mice were sacrificed either 3 or 7 days after the challenge.

Experiment 3A. This trial was designed to elucidate what kind of influence different immunomodulators have on mice survival and tissue lesions in listeriosis. All the animals (except the controls) were challenged with about 2×10^4 CFU Listeria after the following regimens:

Group 1 received cyclosporin-A (Sandimmun®, Sandoz, Switzerland) 1.5 mg/mouse/day with a gastric tube for 3 days and on the third day also Listeria suspension.

Group 2 was treated with dextran sulfate-500, 1 mg/mouse/day i.p. for 2 days and on the third day Listeria suspension was administered.

Group 3 obtained hydrocortisone acetate (Korti®, Lääkefarmos, Finland) 0.1 mg/mouse/day sc. on the back of the neck for 3 consecutive days, on the third day concurrently Listeria suspension.

Group 4 was treated in the same manner as group 3, but the drug given was levamisole (Levoripercol®, Leo, Denmark) with the dose of 0.1 mg/mouse/day.

Group 5 received only Listeria suspension once i.p.

The sixth group was a control group and these mice were injected with sterile 0.9 % saline i.p. for 3 consecutive days.

Experiment 3B. The aim of this experiment was to investigate the immunogenicity and latency of Listeria infection.

The mice were divided into 4 groups, all of which received 2.0×10^3 CFU Listeria i.p. for 5 consecutive days. Group 2 was concurrently treated with levamisole (0.1 mg/mouse/day) sc.

The challenge was followed by a pause of 3 weeks. After that period of time groups 1 and 2 were administered 2.2×10^4 CFU Listeria once i.p.; group 3 was starved for 2 days and sacrificed thereafter; group 4 was also starved for 2 days and then challenged with 2.2×10^4 CFU Listeria i.p.

Group 5 served as a control group. These mice were given sterile 0.9 % saline for 5 days i.p. After a 3-week pause half of the group was challenged with 2.2×10^4 CFU Listeria i.p., the other half received sterile 0.9 % saline i.p.

Only those mice which endured the entire treatment were included in the final results. Therefore the number of animals per group decreased to 2.

Experiment 4. In order to clarify the influence of environmental factors on the development and expression of Listeria infection the following trial was carried out.

The animals of the first group were kept under a lamp-heater (the temperature was above 30°C in the cage) during the test period, beginning 2 days before the administration of bacteria. One half of the group was challenged with 5.2×10^4, the other half with 5.2×10^3 CFU Listeria once i.p.

The control mice (= group 2) were also kept under a lamp-heater. They were administered once sterile 0.9 % saline i.p.

The third and fourth groups were deprived of food for 2 days. After that period of time half of the third group received 5.2×10^4, the other half 5.2×10^3 CFU Listeria once i.p. The fourth group was administered 5.2×10^3 CFU Listeria for 5 days after starvation.

The fifth group was again a control group, which was starved for 2 days and thereafter sterile 0.9 % saline was injected i.p. once or for 5 consecutive days.

The sixth group was a "null" group, which received 0.2 ml of sterile 0.9 % NaCl once i.p.

The mice were monitored 18—20 days before sacrifice.

RESULTS

The histopathological abnormalities detected in tissues were semiquantitatively graded from 0 to +++ according to severity.

In the liver 2 discrete changes were classified: the number and width of necrotic foci and the intensity of inflammatory reaction. Inflammation appeared in the form of microabscesses or granulomas, which mainly comprised variable numbers of

disintegrating neurophils often surrounded by monocytes and lymphocytes. Hepatocytes were in coagulation necrosis within and usually also around these foci. In some instances necrosis was not accompanied by inflammation at all. Kupffer cells had almost invariably proliferated.

In the lungs the inflammatory reaction was either intra-alveolar or interstitial. In the former neutrophils had infiltrated into the alveolar spaces, in the latter inflammatory cells — principally lymphocytes and macrophages — filled the interalveolar septa. Non-specific changes like congestion, edema and atelectasis were also observed but omitted because of the sacrificing procedure which could have contributed to their occurrence.

The histological lesions in the heart were fairly heterogeneous. When the inflammatory reaction was strictly confined to the outer rim of myocardium, it was designated as "epicarditis"; otherwise it was classified as "myocarditis". Most often there were either focal microabscesses or mononuclear cell infiltrations in the myocardium, only in 1 case (4A/3/7) was strong diffuse purulent inflammation detected.

Two different types of inflammatory reaction were distinguished in the brain too. The characteristic ruminant encephalitis — perivascular lymphocytic cuffing in the hind brain region — also occurred in mice. The other form was a purulent reaction, which could in most cases be defined as acute encephalitis or meningoencephalitis. True cerebral abscesses were very rare.

The inflammatory reactions in the kidney did not follow any particular pattern. Most were purulent nephritides, but 1 case of embolic glomerulonephritis (3B/3/1) and one of pyelonephritis (4/1/5) were also recarded as specific entities.

The eyes of the animals in groups 1A and 1B were examined both clinically (all) and histologically (4/8), but not a single sign of inflammation was observed. The same applies to the majority of uterus samples in experiment 2: just 1 animal had noticeable changes (2/1/1; purulent panmetritis).

The prevalence of the various histopathological tissue lesions was calculated for the entire material. The most numerous changes were in the liver (necrosis 54 %, moderate to severe inflammation 51 %), followed by the heart (23 %), lung (23 %), brain (11 %) and kidney (7 %).

The findings in the organs of each individual mouse are listed in Tables 1—6).

Table 1. Results of experiment 1 A.*

Group/ Animal	Dose of Listeria (CFU)	Life span** (days) s=sacrificed	Bacterial count of spleen (CFU)	Liver		Kidney	Lung		Heart		Brain	
				Necrotic foci	Inflammatory reaction	Inflammatory reaction	Interstitial pneumonia	Intra-alveolar granulocytic infiltration	Myocarditis	Epicarditis	Lymphocytic cuffing encephalitis	Purulent encephalitis or meningoencephalitis
I/1	8×10^6	17(s)	0	—	—	—	0	0	—	—	0	0
EC/2	„	17(s)	0	0	(+)	0	0	0	0	0	0	0
/3	„	17(s)	0	0	(+)	0	0	0	0	0	0	0
/4	„	17(s)	0	0	0	0	0	0	0	0	0	0
II/1	8×10^6	4	2×10^7	0	(+)	0	0	++(+)	0	0	0	0
IG/2	„	4	$7{,}5\times10^6$	0	(+)	0	0	+	0	++	0	0
/3	„	6	8×10^6	+++	++	0	0	+++	0	0	0	0
/4	„	18(s)	0	0	0	0	0	0	0	0	0	0
III/1	8×10^4	17(s)	0	0	(+)	0	0	0	0	0	0	0
SC/2	„	17(s)	0	0	+	0	0	0	0	0	0	0
/3	„	17(s)	2×10^1	0	+	0	0	0	0	0	+++	0
/4	„	17(s)	0	+++	+++	0	0	0	0	++	0	0
IV/1	8×10^4	5	1.5×10^7	+++	+++	0	—	—	0	0	0	0
IP/2	„	5	5.2×10^6	+	+++	0	0	0	0	0	0	0
/3	„	5	2.0×10^7	++	+++	0	+++	0	0	0	0	0
/4	„	5(s)x	1.3×10^7	+++	+++	0	++	0	0	++	0	0
V/1(EC)	$0+8\times10^5$†	17(s)	0	0	0	0	0	0	0	0	0	0
C/2(IG)	$0+8\times10^5$	18(s)	0	0	0	0	0	0	0	0	0	0
/3(SC)	$0+8\times10^3$	17(s)	3×10^1	0	+	0	0	0	+	0	0	0
/4(IP)	$0+8\times10^3$	18(s)	0	0	0	0	0	0	0	0	0	0

* — = Not examined
0 = No lesion
** From the beginning of the trial
† The challenge with Listeria took place two days subsequently
x In mortal agony

Table 2. Results of experiment 1B.

Group/ Animal	Dose of Listeria (CFU)	Life span (days) s=sacrificed	Bacterial count of spleen (CFU)	Liver		Kidney	Lung		Heart		Brain	
				Necrotic foci	Inflammatory reaction	Inflammatory reaction	Interstitial pneumonia	Intra-alveolar granulocytic infiltration	Myocarditis	Epicarditis	Lymphocytic cuffing encephalitis	Purulent encephalitis or meningoencephalitis
I/1	(5×)8×10⁵	17(s)	0	0	0	0	0	0	0	0	0	0
EC/2	„	17(s)	0	0	+	0	0	0	0	0	0	0
/3	„	17(s)	0	0	(+)	0	0	0	0	0	0	0
/4	„	17(s)	0	0	+	0	0	0	0	0	0	0
II/1	(5×)8×10⁵	8	1.5×10⁷	+++	+++	0	0	+++	+	0	0	++
IG/2	„	18(s)	2.2×10²	0	+(+)	0	+(+)	+(+)	(+)	0	0	0
/3	„	18(s)	0	(+)	++	0	++	0	0	0	+	++
/4	„	18(s)	0	0	+	0	+	0	0	0	0	0
III/1	(5×)8×10³	7	6×10⁶	+++	+++	0	0	0	++	0	0	0
SC/2	„	18(s)	0	0	+	0	0	0	0	0	0	0
/3	„	18(s)	0	0	+	0	+	0	0	0	+++	0
/4	„	18(s)	0	0	+	0	+	0	0	0	0	0
IV/1	(5×)8×10³	7	7×10⁶	+++	+++	0	+++	0	+	++	0	0
IP/2	„	18(s)	0	0	+	0	+	0	0	0	0	0
/3	„	18(s)	0	0	(+)	0	0	0	0	0	0	0
/4	„	18(s)	0	0	+	0	+	0	0	0	0	0
V/1	0	17(s)	0	0	(+)	0	0	0	0	0	0	0
C/2	0	18(s)	0	0	0	0	0	0	0	0	0	0
/3	0	18(s)	0	0	(+)	0	0	0	0	0	0	0

Table 3. Results of experiment 2.

Group/ Animal	Dose of Listeria (CFU)	Life span (days)	Bacterial count of spleen (CFU)	Liver		Kidney	Lung		Heart		Brain		Uterus
				Necrotic foci	Inflammatory reaction	Inflammatory reaction	Interstitial pneumonia	Intra-alveolar granulocytic infiltration	Myocarditis	Epicarditis	Lymphocytic cuffing encephalitis	Purulent encephalitis or meningoencephalitis	Inflammatory reaction
I/1	5.7×10^3	3*	2.8×10^7	+++	+	0	0	0	0	0	0	(+)	+++x
/2	„	3	1.1×10^5	+	+++	0	0	0	0	0	0	0	—
/3	„	7	4.3×10^2	+++	+++	0	0	0	0	0	0	0	0
/4	„	7	2.6×10^5	+++	++	0	0	+	++	0	0	0	—
II/1	5.6×10^3	3	0	0	0	0	0	0	0	0	0	0	0x
/2	„	3	0	0	(+)	0	0	0	0	0	0	0	0
/3	„	7	0	0	(+)	0	0	0	0	0	0	0	—
/4	„	7	0	0	(+)	0	0	0	+	0	0	0	—x
III/1	5.9×10^3	3	0	0	(+)	0	0	0	0	0	0	0	0x
/2	„	3	0	0	(+)	0	0	0	0	0	0	0	0
/3	„	7	0	0	0	0	0	0	0	0	0	0	0
/4	„	7	0	0	(+)	0	0	0	0	0	0	0	0
IV/1	0	3	0	0	(+)	0	0	0	0	0	0	0	0
/2	0	3	0	0	(+)	0	0	0	0	0	0	0	—
/3	0	7	0	0	0	0	0	0	0	0	0	0	0
/4	0	7	0	0	(+)	0	0	0	0	0	0	0	—

* = died spontaneously, all the others were sacrified

x = pregnant

Table 4. Results of experiment 3A.

Group/ Animal	Dose of Listeria (CFU)	Life span (days) s=sacrificed **	Bacterial count of spleen (CFU)	Liver		Kidney	Lung		Heart		Brain	
				Necrotic foci	Inflammatory reaction	Inflammatory reaction	Interstitial pneumonia	Intra-alveolar granulocytic infiltration	Myocarditis	Epicarditis	Lymphocytic cuffing encephalitis	Purulent encephalitis or meningoencephalitis
Cy-A/1	2×10^4	3	1.3×10^7	0	0	0	0	+	—	—	0	0
/2	„	3	3.1×10^7	+	(+)	0	0	0	0	0	0	0
/3	„	4	7.8×10^7	+++	+++	0	+	0	0	0	0	0
/4	„	5	1.7×10^8	+++	+++	+	++	0	0	0	0	0
DS/1	2×10^4	2	3.9×10^7	0	0	0	0	+	0	0	0	0
/2	„	2(s)x	1.6×10^7	+++	0	0	+	0	0	0	0	+
/3	„	3	6.0×10^7	++	(+)	0	++	0	0	0	0	0
/4	„	3	6.0×10^8	+++	+++	0	++	0	0	0	0	0
Cort/1	„	3(s)x	1.5×10^8	+++	+++	0	+	0	0	++	0	0
/2	„	4	1.9×10^8	+++	+++	0	0	0	0	0	0	(+)
/3	„	4	$>1\times10^7$	+++	+++	0	+	0	0	0	—	—
/4	„	4	1.4×10^8	—	—	0	++	0	0	0	0	0
Lev/1	2×10^4	4	8.0×10^6	+++	++	0	—	—	—	—	0	0
/2	„	4	8.0×10^6	+++	+++	0	0	0	0	0	0	0
/3	„	4	2.3×10^7	+++	+++	0	++	0	0	0	0	0
/4	„	6	1.1×10^7	+++	+++	0	+++	0	++	+++	0	(+)
Bact/1	2×10^4	3	6.6×10^7	+++	+++	0	(+)	0	0	0	0	0
/2	„	4	5.9×10^7	+++	+++	0	+	0	0	0	0	0
/3	„	4	1.7×10^8	+++	++	0	—	—	—	—	0	0
/4	„	5	7.0×10^7	+++	+++	0	+++	0	++	0	+	0
C/1	0	13(s)	0	0	+	0	+	0	0	0	0	0
/2	0	13(s)	0	0	+	0	+	0	0	0	0	0
/3	0	13(s)	0	0	(+)	0	+	0	0	0	0	0

x In mortal agony

** Group 2 (DS) differs from group 5 (Bact) significantly ($P < 0.05$)

Table 5. Results of experiment 3B.

Group/ Animal	Dose of Listeria (CFU)	Life span (days) s=sacrificed	Bacterial count of spleen (CFU)	Liver		Kidney	Lung		Heart		Brain	
				Necrotic foci	Inflammatory reaction	Inflammatory reaction	Interstitial pneumonia	Intra-alveolar granulocytic infiltration	Myocarditis	Epicarditis	Lymphocytic cuffing encephalitis	Purulent encephalitis or meningoencephalitis
1/1	$(5\times)2.0\times10^3$, 3 weeks later	30(s)	0	0	+	0	0	0	0	0	0	0
1/2	2.2×10^4	30(s)	0	+	++	0	(+)	0	0	0	0	0
2/1	the same as above	30(s)	0	0	+	0	0	0	0	+	0	0
2/2	+ levamisole	30(s)	0	0	(+)	0	0	0	0	0	0	0
3/1	$(5\times)2.0\times10^3$ + starvation after 3 weeks	30(s)	2.0×10^1	0	(+)	+++	0	(+)	0	0	0	0
3/2		30(s)	0	0	++	0	0	0	0	0	0	0
4/1	$(5\times)2.0\times10^3$, 3 weeks later	30(s)	1.8×10^2	++	++	0	+	0	0	0	0	0
4/2	2.2×10^4 after starvation	30(s)	2.0×10^1	++	++	0	0	0	0	(+)	0	0
5/1	2.2×10^4	5	3.4×10^6	+++	+++	0	+	0	0	0	0	0
5/2	„	5	2.0×10^7	+++	+++	0	+	0	0	0	0	0
5/3	0	9(s)	0	0	+	0	0	0	0	0	0	0
5/4	0	9(s)	0	0	(+)	0	0	0	0	0	0	0

Table 6. Results of experiment 4.

Group/ Animal	Dose of Listeria (CFU)	Life span (days) s=sacrificed	Bacterial count of spleen (CFU)	Liver		Kidney	Lung		Heart		Brain	
				Necrotic foci	Inflammatory reaction	Inflammatory reaction	Interstitial pneumonia	Intra-alveolar granulocytic infiltration	Myocarditis	Epicarditis	Lymphocytic cuffing encephalitis	Purulent encephalitis or meningoencephalitis
1/1	5.2×10^4	5	2.4×10^7	+++	+++	0	++	0	0	0	0	0
/2	„	6	2.3×10^6	+++	+++	0	++	0	++	++	—	—
/3	„	6	9.0×10^5	++	+++	0	++	0	0	0	0	0
/4	„	8—11	5.0×10^6	++	+++	0	0	0	++	0	+(+)	0
/5	5.2×10^3	20(s)	0	0	+	++	0	0	0	0	0	0
/6	„	20(s)	0	0	+	0	(+)	0	0	+	0	0
/7	„	20(s)	0	0	+	0	0	0	0	0	0	0
/8	„	20(s)	0	0	+	0	(+)	0	0	0	0	0
2/1	0	20(s)	0	0	(+)	0	0	+	0	+	0	0
/2	0	20(s)	0	0	+	0	0	++	++	+	0	0
3/1	5.2×10^4	5	8.0×10^6	—	—	—	—	—	—	—	—	—
/2	„	5	7.7×10^6	+++	+++	0	—	—	0	0	0	0
/3	„	5	8.0×10^7	+	++	0	+	0	0	0	0	+
/4	„	6	4.0×10^6	+++	+++	0	0	0	+	0	—	—
/5	5.2×10^3	7	2.0×10^6	++	+++	(+)	++	0	(+)	0	0	0
/6	„	7(s)x	2.0×10^5	+++	+++	0	+++	0	0	0	0	0
/7	„	8—11	6.5×10^4	+	0	0	0	+	+++	0	0	+++
/8	„	18(s)	0	0	(+)	0	+	0	+	+	0	0
4/1	0	18(s)	0	0	(+)	0	0	0	0	0	0	0
/2	0	18(s)	0	0	+	0	+	0	0	0	0	0
/3	0	18(s)	0	0	+	0	0	0	0	0	—	—
/4	0	18(s)	0	0	(+)	0	0	0	0	0	0	0
5/1	0	18(s)	0	0	(+)	0	0	+	—	—	0	0
/2	0	18(s)	0	0	+	0	0	+	0	0	0	0
6/1	(5×)5.2×10^3	7	2.2×10^6	+++	+++	0	—	—	—	—	0	0
/2	„	18(s)	6.4×10^2	++	+++	+++	0	0	0	0	0	0
/3	„	18(s)	0	0	+	0	0	0	0	0	0	0
/4	„	18(s)	0	0	+	0	+	0	0	0	0	0

x In mortal agony

DISCUSSION

The literature on histopathology of murine listeriosis is astonishingly scarce. Best described are the lesions in the liver but even there the exact role of the various cell types is still unresolved. The data on occurrence of lesions in other organs contain a few discrepancies. For example, *Mandel & Cheers* (1980) found no pathological changes in the brain, kidney, lung or heart after intravenous administration of Listeria, but *Silverman et al.* (1963) isolated Listeria from brain homogenates of about 25 % of mice challenged with these bacteria by various routes, and *Kautter et al.* (1963) detected lung lesions. In the present study we found fewer cases of lung affection (23 %) than *Miller & Burns* (1970) in their material after oral feeding of Listeria (60 %), but myocarditis and cerebral involvement were much more common than reported before. We also observed inflammatory reactions in the kidney, though they were quite rare and sporadic.

It is obvious that there exists large interspecies variability in the susceptibility to epiconjunctival inoculation of Listeria. A few spontaneuous cases of occular listeriosis in cattle have been reported (*Kummeneje & Mikkelsen* 1975, *Morgan* 1977, *Wadström et al.* 1982, *Pohjanvirta & Hedlund* 1984), in which fodder and just were suspected as sources of infection. Instillation of Listeria on the eye leads to purulent conjunctivitis in the rabbit and guinea pig (*Gillespie & Timoney* 1981). Mice appear to be a worse model in this respect, as no sign of inflammation was detected after repeated flushing of their eyes with a suspension of Listeria.

In search of natural routes of infection in listeriosis the possibility of wound infection has generally been overlooked. We included the subcutaneous route in our experimental schedule and made some interestiny discoveries. First, the ruminant-type cuffing encephalitis was encountered in its purest and most distinctive form in this group of mice. Second, the individuals with the aforementioned brain lesions did not have concomitant liver changes, which may point to transneural spreading of Listeria. It is considered probable that in ruminants Listeria seek their way to the brain by branches of the trigeminal nerve (*Rebhun & deLahunta* 1982). Our findings suggest that contaminated wounds on the head or back of the neck could lodge Listeria and seed them further on via nerves. — And third, the

sc. route turned out to be the most inconsistent and unpredictable way of exposure as far as the dose-response relationship is concerned.

It has been shown that compounds injected intraperitoneally are conveyed through the liver (*Lukas et al.* 1971). This obviously also accounts for the uniform distribution of liver lesions observed in mice exposed i.p. to Listeria. On the other hand, the correlation between liver lesions and the final outcome of infection was much poorer when the bacteria were administered into the stomach. Thus it is conceivable that these organisms — at least to some extent — gain entrance from the intestinal lumen to the general circulation by lymphatic vessels. This assumption is supported by the fact that the Peyer's patches appear to be the only site of tissue invasion and L. monocytogenes survival in the intestinal tissues (*MacDonald & Carter* 1980). The high mortality in the intragastric group was surprising, because other investigators have not recorded any deaths even at a dose of 10^9 viable Listeria per os (*MacDonald & Carter* 1980, *von Koenig et al.* 1983). The explanation is perhaps in the fact that our mice were conventionally raised and a number of them were affected by mild concurrent latent gastroenteritis, which may have contributed to the proliferation and invasion of Listeria in the intestine.

The difference in pathogenicity between the three tested Listeria strains of bovine origin and of the same serotype (1) was dramatic. One can naturally speculate that the repeated passage of strain 1 in animals had increased its virulence by species adaptation, but 2 things speak against this: first, during these experiments we passed the same strain through mice several times and did not notice any change in its virulence; and second, *von Koenig et al.* (1983) proved that only serotype 4d was able to adapt to mice. Moreover, *Kautter et al.* (1983) found no difference in the outcome of challenge with strains maintained on laboratory medium for some time and those freshly isolated from infections in animals. In accordance with our findings, *Kaufmann* (1984) recently showed that serotype 1 contains both persistent and nonpersistent strains.

In experiment 3A we compared the effects of some modern immunomodulators on the pathogenicity of Listeria to mice. It is well established that the defence against Listeria occurs in 2 phases. Bacterial multiplication is controlled by nonspecific

macrophages in the early phase (by 2—4 days after infection) and by acquired T cell-dependent immunity in the second phase (from 3 to 7 days after infection) (*Skamene & Kongshavn* 1983, *Hamada et al.* 1981, *Schaffner et al.* 1983). Antibodies play no significant role in this process (*Cheers & Ho* 1983, *Cooper et al.* 1983). DS-500 interferes with the function of macrophages (*Finger et al.* 1982) and Cy-A with that of T lymphocytes (*Häyry* 1984), while levamisole activates both of them (*Anderson* 1984).

Our results confirmed that DS-500 effectively abolishes the early resistance; animals treated with this compound died sooner than the others ($P < 0.05$). The bacterial count of the spleen was the lowest in the levamisole group, but no other beneficial effects of immunostimulation could be detected. In this context it must be noted, however, that we used the recommended anthelmintic dose rate (5.0 mg/kg), whereas the best immunomodulating properties are achieved at 2—3 mg/kg (*Anderson* 1984), and the bacterial inoculum was quite large. Owing to the latter fact we were also unable to demonstrate the late mortality in Cy-A group described by *Schaffner et al.* (1983).

The short life span of the mice in the DS-500 group evidently accounts for the apparent lack of inflammatory response in the liver, because *Lannigan et al.* (1979) and *Mandel & Cheers* (1980) have shown that in susceptible mice listeric microabscesses do not turn up in the liver until on the third or fourth day of infection, and because the infiltration of inflammatory cells into the lung parenchyma was not affected concurrently. Additionally, the same phenomenon was seen in the Cy-A group as well.

In principle, cortisone should have been detrimental to both macrophage and T lymphocyte function and thus in effect resemble DS-500. *Schaffner et al.* (1983) indeed reported that cortisone-treated mice lost their early resistance. The dose they administered, however, was about six-fold to the one used in the present experiment, in which it was selected from the therapeutic range. The most severe liver lesions (e.g. macroscopically visible, pale, net-like figures on surfaces) were detected in the cortisone group, but obviously the dose was too low for total inhibition of macrophage function.

It has been previously reported that the induction of immunity against Listeria requires a sufficient number of viable bac-

teria, which are not immediately eliminated from the host (*von Koenig et al.* 1983, *Kaufmann* 1984). We used a 5-day regimen in the immunization and were able to establish high level protection against a lethal challenge 3 weeks later. Levamisole enhanced immunity in terms of overall lesions. On the contrary, starvation seemed to impair mice's ability to get rid of Listeria, this being reflected in both the pathological changes in the liver and the bacterial count of the spleen. The group of mice that did not receive the second challenge proved that Listeria can persist alive for a considerable period of time as a latent infection in the body.

Acute nutritional deprivation has been suggested to shield mice against Listeria (*Wing & Young* 1980, *Wing et al.* 1983), apparently by enhanging macrophage function. Nevertheless, in trials 3B and 4 we did not notice any protective influence of starvation; conversely, starved mice proved at least as susceptible as regularly fed animals to the first challenge and possessed weaker immunity to the second one.

As ambient temperature has often been proposed to be of importance in listeriosis, we kept one group of mice under a lamph-heater. *Kutter et al.* (1963) observed no difference in mortality among mice kept at the temperature range of 15—25.7°C; however, they thus excluded the critical extreme temperatures. Because L. monocytogenes is a facultatively intracellular pathogen (*Gillespie & Timoney* 1981) and raised ambient temperature is a routine treatment in viral infections, we considered it justifiable to test the effect of heat on listeriosis. And indeed, extra heat appeared to give marked protection. At the higher dose of Listeria the mice in this group lived longer than their starved counterparts; at the lower one there were no deaths in this group versus the mortality rates of 27—75 % in starved or conventionally housed (trial 2, group 1) mice, the lesions in tissues also being fewer in number and less in severity.

Our material was too small for strict conclusions, but the results hopefully awaken further interest in examining the presented topics of the diverse interactions between host, agent and environment in listeriosis.

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SAMMANFATTNING

Vissa aspekter på experimentell listerios hos möss.

En serie på fyra experiment om listerios hos möss utfördes. Det blev visat, att Listeria (L.)-organismer kan inträngа i djuret via olika vägar och att både subkutant och oralt inträde kan leda till letal septikemi. Det fanns också tecken på neural spridning och tarmabsorption via lymfkärl av L. Ingen inflammatorisk reaktion kunde vållas med okular spolning med L.-suspension. Patogeniteten av L.-stammar (som tillhörde samma serotyp) varierade mycket. DS-500 fördärvade mössens tidiga resistens emot L., men kortison på terapeutisk dosnivå åstadkom det inte. Någon nyttig effekt av levamisol observerades inte vid akut blottställning, men resultaten tydde på att den skulle kunna vara gynnsam vid produktion av immunitet. Fastan tycktes försämra mössens immunitet och motståndsförmåga; förhöjd temperatur däremot ökade antalet överlevande djur.

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Reprints may be requested from: Raimo Pohjanvirta, the National Veterinary Institute, P. O. B 92, 70701 Kuopio, Finland.