Virulence of *Listeria* spp.: course of infection in resistant and susceptible mice

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Summary. The virulence of representative strains of the five species of *Listeria monocytogenes sensu lato* was compared in C57BL/6 and BALB/c mice in terms of LD50 values and of bacterial growth kinetics and histological changes in mouse livers. *L. monocytogenes sensu stricto* and *L. ivanovii* showed relatively low LD50 values and much bacterial growth for 2–3 days before viable counts declined. Histological changes in *L. ivanovii* infection resembled those caused by *L. monocytogenes*, with early development of neutrophil-rich micro-abscesses and hepatocyte necrosis followed by macrophage infiltration and formation of granulomas. By contrast, *L. innocua*, *L. welshimeri* and *L. seeligeri* were almost entirely avirulent as shown by high LD50 values, early elimination of viable bacteria and no evidence of growth. Histological changes consisted only of slight, transient infiltration of the liver with neutrophils. Both groups of bacteria were seen infrequently in Kupffer cells early in infection, but only the highly virulent species appeared to replicate. LD50 values for *L. monocytogenes* and *L. ivanovii* were (10–20)-fold greater, and for the less virulent bacteria at least two-fold greater, in C57BL/6 than in BALB/c mice. This difference in host susceptibility was not reflected in detectable histological differences between the two mouse strains.

Introduction

Organisms of the genus *Listeria* are widespread in nature; they are found in animals, birds and man (Seeliger and Jones, 1986). *Listeria monocytogenes sensu lato* has been classified into five species on the basis of DNA homology (Rocourt *et al.*, 1982) and contains at least 16 serovars. The genomic distinction correlates with phenotypic differences based on pathogenicity for mice (Rocourt *et al.*, 1983a), haemolytic activity and carbohydrate fermentation reactions (Rocourt *et al.*, 1983b). *L. monocytogenes sensu stricto* (hereafter referred to as *L. monocytogenes*) causes severe opportunistic infections in man and animals (Ralovich, 1984), *L. ivanovii* is preferentially pathogenic for sheep, and *L. innocua*, *L. welshimeri* and *L. seeligeri* are generally considered to be avirulent (Seeliger and Jones, 1986).

The facultative intracellular pathogen, *L. monocytogenes*, has been widely used for the study of cell-mediated immunity in infection (North, 1981; Bortolussi *et al.*, 1984; Kaufmann,

1984). Three clear-cut stages are seen when this organism is injected intravenously into mice (Mitsuyama *et al.*, 1978). Firstly, 90% of the inoculum is cleared from the blood by liver Kupffer cells and, within 6 h, 90% of these bacteria are killed. During the next 2–3 days, residual organisms grow abundantly, despite pronounced focal accumulations firstly of neutrophil polymorphs (PMNs) and then of mononuclear phagocytes (Mandel and Cheers, 1980). In surviving animals, immune T cells quench the infection in its final phase through macrophage (MP) activation. Despite their early prominence, PMNs appear to have no role in bacterial killing *in vivo* (Mitsuyama *et al.*, 1978; Tatsukawa *et al.*, 1979) and, indeed, bacteria are eliminated in the third stage of the hepatic infection as PMNs are replaced by MP-rich granulomas.

In recent years, genetically determined differences in innate resistance to *L. monocytogenes* have been found in inbred strains of mice (Cheers and McKenzie, 1978; Kongshavn, 1985) that can be attributed neither to PMNs nor to natural killer (NK) cells (Skamene and Kongshavn, 1983). After similar uptake of inoculum bacteria by Kupffer cells of both resistant and susceptible mice (Cheers

et al., 1978), relative susceptibility is expressed during the second stage of infection, before the onset of acquired immunity, by impaired mobilisation of inflammatory MPs (Gervais *et al.*, 1984; Czuprynski *et al.*, 1985) or by reduced listericidal capacity of otherwise adequate cellular infiltrates (Czuprynski *et al.*, 1985; Wood *et al.*, 1986).

This general description of listerial pathogenicity was extended by Kaufmann (1984) in a study which determined the capacity of different serotypes of intravenously-inoculated listeriae to persist for several days in the spleens of C57BL/6 mice. Persisters were able to induce strong, cross-reactive, T cell-mediated immunity. By contrast, organisms that were rapidly eliminated (non-persisting strains) were unable to induce effective cellular immune responses, although they expressed antigenic epitopes that were recognised by immune T cells.

No studies have yet compared patterns of infection by representatives of all five *Listeria* spp. in genetically resistant and susceptible mice. Accordingly, and as a preliminary to detailed studies of listerial virulence determinants in relation to mechanisms of early host resistance, we have performed such experiments in terms of bacterial growth kinetics and histology in the livers of intravenously-infected C57BL/6 and BALB/c mice.

Materials and methods

Sources and characterisation of bacteria and tests for haemolysis

Known serotypes of *Listeria* were obtained from Professor H. P. R. Seeliger (Institute of Hygiene and Microbiology, University of Würzburg, Federal Republic of Germany), Dr A. G. Taylor (Central Public Health Laboratory, Colindale, London) and Dr J. Rocourt (Institut Pasteur, Paris, France). Other strains were isolated in the Department of Bacteriology, University of Aberdeen, and serotyped at the Central Public Health Laboratory, Colindale, London. All were confirmed or initially identified as *L. monocytogenes sensulato* (Rocourt *et al.*, 1982) by the criteria of Seeliger and Höhne (1979). They were further examined for species-specific characteristics—in particular for fermentation of D-xylose, L-rhamnose and α -methyl-D-mannoside (Rocourt *et al.*, 1983b), for the presence (and zone diameter) of β -haemolysis around colonies after incubation for 24 h at 37°C on 5% sheep-blood agar, and by the CAMP test. The CAMP test was performed according to adaptations for listeriae (Groves and Welshimer, 1977; Fraser, 1962) of a test originally described for Group-B streptococci by Christie *et al.* (1944); it detects *Listeria* species-related accentuated haemolysis in the neighbourhood of growth of β -lysin-producing *Staphylococcus aureus* or *Rhodococ-*

cus equi. Based on these tests, 13 strains of *Listeria*, of different serotypes and representative of all five species, were selected for virulence studies.

Cultures of the Oxford strain of *S. aureus* and of *R. equi* NCTC 1621 were obtained from Dr D. R. Fenlon (Bacteriology Division, The North of Scotland College of Agriculture, Aberdeen).

Mice

BALB/c and C57BL/6 mice, raised under specified pathogen-free conditions, were used shortly after delivery to a conventional animal house when 8–12 weeks old and weighing 22–25 g. They were fed pellets of CRM mouse diet (Labsure, Manea, Cambridgeshire) and water *ad libitum*.

Virulence studies

For LD50 experiments, bacteria were cultured directly from freeze-dried stocks. However, for strain AB1 of *L. monocytogenes* in these experiments, and for all bacteria in the growth experiments, cultures were prepared from infected spleen homogenates after a single passage in BALB/c mice. Suspensions of bacteria grown in tryptose phosphate broth at 37°C for 18 h were assayed spectrophotometrically and viable counts were confirmed retrospectively by the method of Miles *et al.* (1938). LD50 values were determined by the method of Reed and Muench (1938) on the basis of mortality scored 10 days after the intravenous inoculation of five groups of five mice with 10-fold decreasing doses of viable bacteria. Patterns of bacterial growth in mouse livers were determined after injection into the tail vein of doses of viable bacteria approximating the LD50 for BALB/c mice. At intervals after infection, livers from groups of four or five mice were removed aseptically, individually homogenised in Tris buffer (0.05 M, pH 7.4) and the numbers of viable bacteria in the homogenates determined, after inoculating 10-fold dilutions on to blood-agar plates, by the method of Miles *et al.* (1938).

Histological evaluation

After gross examination, portions of mouse livers were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were cut at 5 μ m and stained with haematoxylin and eosin and by Gram's method.

Results

Cultural and biochemical features of Listeria species

The 13 strains of *Listeria* chosen for virulence studies were gram-positive, non-spore-forming bacilli which were motile at room temperature but not at 37°C. They produced catalase but not oxidase or urease, hydrolysed aesculin, and gave positive

Voges-Proskauer and methyl red reactions. None reduced nitrate to nitrite. All produced acid but not gas from glucose, salicin and trehalose and none produced acid from mannitol. All were agglutinated by *Listeria* polyvalent O antiserum (Difco). Accordingly they were considered *L. monocytogenes sensu lato* (Seeliger and Höhne, 1979; Rocourt *et al.*, 1982) and speciated by tests for haemolysis and biochemical reactions (table) according to the criteria of Rocourt *et al.* (1983b).

LD50 values

There was no sex-related difference in LD50 values of *L. monocytogenes* SLCC 2540, type 3b, for either strain of mouse (5.7×10^5 cfu for both male and female BALB/c and 6.2×10^6 and 6.1×10^6 cfu for male and female C57BL/6 mice respectively). Consequently, mixed-sex populations were used for the ensuing experiments.

LD50 values for the 13 *Listeria* strains (table) showed a clear distinction between those of the highly virulent species, *L. monocytogenes* and *L. ivanovii*, on the one hand and those of the almost avirulent species, *L. innocua*, *L. welshimeri* and *L. seeligeri*, on the other. *L. monocytogenes* serotypes showed the greatest capacity to kill and also showed variations in lethality of up to 16-fold or 6-fold, depending on whether or not they had previously been passaged in mice. For all *Listeria* spp. the BALB/c mice were more susceptible than the C57BL/6 mice, by (10–20)-fold for *L. monocytogenes* and *L. ivanovii* and by at least (2–4)-fold for the other species.

Course of infection in mouse liver

Two strains of *L. monocytogenes* and one each of the other species were selected to study the course of infection in livers of both mouse strains, and two distinct patterns emerged (fig. 1). For both strains of *L. monocytogenes* and for *L. ivanovii*, low recoveries of bacteria 6 h after infection were followed by (500–10 000)-fold increases during the next 30–50 h. Thereafter, there was a more or less rapid decline in viable counts. As defined by LD50 values (table), virulence correlated with more abundant growth in the liver in relation to the inoculum dose (fig. 1) and, for *L. ivanovii* as for *L. monocytogenes* (Cheers *et al.*, 1978), the more resistant host showed earlier onset of acquired immunity as well as lower peak levels of bacteria. By contrast, there was no apparent growth of bacteria in livers of mice infected intravenously with *L. innocua*, *L. welshimeri* or *L. seeligeri*. Rather, from initially

Fig. 1. Course of infection by *Listeria* species in livers of C57BL/6 (○—○) and BALB/c (●—●) mice: (a) *L. monocytogenes* SLCC 2540; (b) *L. monocytogenes* AB1; (c) *L. ivanovii* W5379; (d) *L. innocua* AB6; (e) *L. welshimeri* CIP 8149; (f) *L. seeligeri* AB2. Bar represents 1 SEM of counts from four or five mice per group (Hodge and Seed, 1977); ▲ = bacterial inoculum.

low yields 6 h after infection, viable counts declined, at first gradually and then, in some instances and after ≥ 40 h, apparently more steeply. In accordance with expectations from the LD50 values, fewer organisms were recovered from C57BL/6 than from BALB/c mice. However, apart from the infections with *L. welshimeri*, the differences were not marked until late in infection.

Histopathological evaluation

Gross examination revealed small white foci in livers from mice infected with *L. monocytogenes* and *L. ivanovii*, but not after infection with the other *Listeria* spp. Microscopic appearances (fig. 2) also distinguished infections with the pathogens from those with the non-pathogens, but no clear-cut differences were noted between the two mouse strains. Mice infected with *L. monocytogenes* and *L. ivanovii* developed an intense focal inflammatory response, as previously noted for *L. monocytogenes* (Mandel and Cheers, 1980). Sparse infiltrations of

Table. Identity, sources, haemolytic and biochemical properties, and LD50 values of *Listeria* strains

<i>Listeria</i> spp.	Strain designation (obtained from)*	Serotype	Haemolysis on sheep blood agar (zone in mm)	CAMP test† with		Acid production from			LD50 (cfu × 10 ⁶)	
				<i>S. aureus</i>	<i>R. equi</i>	D-xylose	L-rhamnose	α-methyl D-mannoside	C57BL/6	BALB/c
<i>L. monocytogenes</i>	NCTC 7973(T) (ATCC 19111)	1/2a	0·8–1·0	+	—	—	+	+	4·4	0·44
	SLCC 2540(S)‡	3b	0·5–0·8	+	—	—	+	+	6·2	0·57
	NCTC 10527(S)	4b	0·5–0·8	+	—	—	+	+	3·0	0·14
	AB3 (L)	4b	0·5–0·8	+	—	—	+	+	1·7	0·10
	AB1 (P)‡	1/2a	0·8–1·0	+	—	—	+	+	0·76	0·035
<i>L. ivanovii</i>	ATCC 19119(S) (SLCC 2379)	5	1·0–1·2	—	+	+	—	—	20	0·90
	NCTC 1100(T)	5	1·0–1·2	—	+	+	—	—	20	0·95
	W 5379(T)‡	5	1·0–1·2	—	+	+	—	—	40	2·6
<i>L. innocua</i>	NCTC 11299(S)	6a	0	—	—	—	+	+	> 1000	330
	AB6 (L)‡	6b	0	—	—	—	+	+	> 1000	290
<i>L. welshimeri</i>	CIP 8149 (R)‡	6b	0	—	—	+	—	+	> 1000	240
<i>L. seeligeri</i>	CIP 100100(R)	1/2b	0·4–0·6	+	—	+	—	—	> 1000	420
	AB2 (L)‡	6	0·4–0·6	+	—	+	—	—	> 1000	470

* ATCC, American Type Culture collection; CIP, Collection Institut Pasteur; NCTC, National Collection of Type Cultures; SLCC, Special *Listeria* culture collection, Institute of Hygiene and Microbiology, University of Würzburg; AB1, AB2, AB3 and AB6, isolates from infected neonate (AB1) or from healthy faecal carriers (AB2, 3 and 6), Department of Bacteriology, University of Aberdeen; T, Dr A. G. Taylor; S, Professor H. P. R. Seeliger; R, Dr J. Rocourt; L, Dr R. J. Lamont; P, Professor R. Postlethwaite.

† CAMP test, accentuation of haemolysis on sheep-blood agar in the vicinity of *S. aureus* or *R. equi*.

‡ Strains used to study course of infection in mouse liver.

Fig. 2. Histology of mouse livers infected by *Listeria* spp. at indicated hours after infection: (a) *L. seeligeri* AB2, 6 h—mild PMN infiltration; (b) *L. monocytogenes* SLCC 2540, 30 h—micro-abscess and hepatocyte degeneration; (c) *L. ivanovii* W 5379, 30 h—micro-abscess and hepatocyte degeneration; (d) *L. ivanovii* W 5379, 9 days—residual granuloma. (Haematoxylin and eosin; bar=50 µm).

liver sinusoids with PMNs were seen 6 h after infection and these developed into randomly distributed focal collections by 21 h and into large micro-abscesses by 31–72 h. Both PMNs and surrounding hepatocytes began to degenerate from 21 h and areas of hepatocyte necrosis were prominent around degenerate micro-abscesses by 44–55 h. Macrophages accumulated peripherally 31–44 h after infection and, by 55–72 h, inflammatory

foci became granulomatous in character, consisting predominantly of MPs and lymphocytes with central degenerate PMNs. Granulomas were still present 9 days after infection, being composed of MPs, lymphocytes, plasma cells, fibroblasts and occasional PMNs. By contrast with these florid histological manifestations of infection, mice infected with *L. innocua*, *L. welshimeri* and *L. seeligeri* showed no consistent abnormality apart from mild

infiltration with PMNs 6 h after infection and this resolved within 21 h. Control mice given diluent alone showed no significant abnormality.

Gram-positive bacilli were seen in livers of mice of both strains after infection with all *Listeria* spp. except *L. welshimeri*. In infections with the almost avirulent bacteria, they were present only in resident Kupffer cells, in small numbers, and up to 31 h after infection (72 h for *L. innocua*). By contrast, and additionally, clusters of apparently replicating bacteria were seen in the developing inflammatory foci of livers infected with the virulent species *L. monocytogenes* and *L. ivanovii* and, in the more severe infections, also inside some hepatocytes.

Discussion

These experiments extend earlier findings (Cheers *et al.*, 1978; Mitsuyama *et al.*, 1978; Mandel and Cheers, 1980; Rocourt *et al.*, 1983a; von Koenig *et al.*, 1983) by relating virulence and histological features for representative strains of all five *Listeria* spp., and by showing that *L. ivanovii*, like *L. monocytogenes*, is highly pathogenic for both C57BL/6 and BALB/c mice, but that *L. innocua*, *L. welshimeri* and *L. seeligeri* are not. Furthermore, C57BL/6 mice were more resistant than BALB/c mice to infection by *L. ivanovii*, as previously reported and confirmed here for *L. monocytogenes* (Kongshavn, 1985). Differential sensitivity of the two mouse strains to the less pathogenic listeriae was less clear-cut than for the virulent bacteria. Although the weak pathogens appeared not to grow in the livers of either strain of mouse, they were cultured in consistently greater numbers from livers of BALB/c than of C57BL/6 mice, and LD50 values were consistently lower in BALB/c mice.

A precise immunochemical basis for serotypic differences in mouse virulence amongst listeriae is not established. Variations in sugar substituents of cell-wall teichoic acids and possibly lipoteichoic acids (Seeliger and Jones, 1986), that appear partly to determine O-antigenic specificities, probably do not account for the serotypic differences in virulence amongst strains of *L. monocytogenes* reported here and by others (Rocourt *et al.*, 1983a; von Koenig *et al.*, 1983). Thus, listeriae of high and low virulence can have such O-antigen specificities in common, as exemplified by the existence of serotype 3 strains both of the virulent *L. monocytogenes* and of the largely avirulent *L. innocua* (Knorz and Hof, 1986), and similarly by the serotype 1/2 strains of both *L. monocytogenes* and *L. seeligeri* examined in this study. Conversely, strains with similar (high or low)

levels of virulence may have different O-antigenic specificities, as represented in this study by the highly virulent serotypes 4b (AB3) and 3b (SLCC 2540) of *L. monocytogenes* and the almost non-pathogenic *L. seeligeri* serotypes 1/2b (CIP 100100) and 6(AB2). Other structural components of *L. monocytogenes*, including a lipopolysaccharide, endotoxin-like component and a high molecular weight, carbohydrate-containing, immunosuppressive agent have been studied as immunomodulating agents (Galsworthy, 1984) but not as inter- or intra-species-related virulence determinants. A virulence-determining role for cell-wall proteins was recently suggested in a study of smooth and rough variants of haemolytic type 1/2a strains of *L. monocytogenes* (Hof and Chatzipanagiotou, 1987), despite the demonstration by Lamont *et al.* (1986) of rather similar profiles, in SDS-PAGE gels, of whole-cell polypeptides from serotypes of all species of *L. monocytogenes sensu lato*. Whether protein differences might explain the minor intraspecific variations in virulence amongst strains of *L. ivanovii* (type 5) reported here and by Rocourt *et al.* (1983a) remains to be shown. The reason for the higher virulence of our type 1/2a *L. monocytogenes* strain AB1, compared with strain NCTC 7973, is not clear; but enhancement of virulence by mouse passage is well known (von Koenig *et al.*, 1983). It may also be relevant that *L. monocytogenes* NCTC 7973 was amongst the non-persisting strains in the study of Kaufmann (1984). The predominance of serotypes 1/2a and 4b in natural human and animal infections (Ralovich, 1984) is as yet unexplained.

Histological examination showed no qualitative differences between the two mouse strains in the evolution of the hepatic infection, whether induced by *L. ivanovii* or *L. monocytogenes*. Mandel and Cheers (1980) described quantitative differences in the development of inflammatory foci in the livers of C57BL/10 and BALB/c mice as a consequence of infection with high, but not low, doses of *L. monocytogenes*. Such differences were not seen with the lower doses used here and our results agreed with aspects of previous histological descriptions for *L. monocytogenes* infections in single strains of mice (North, 1970; Tatsukawa *et al.*, 1979; Mandel and Cheers, 1980; von Koenig *et al.*, 1983), in which, overall, early pyogenic foci with abundant bacilli and developing hepatocyte necrosis were replaced by MP-rich granulomas devoid of bacilli. These histological features accord with the capacity of both bacterial species to induce homotypic or heterotypic protective immunity (Seeliger *et al.*, 1982; von Koenig *et al.*, 1983) and it seems likely that the highly virulent strains of

L. monocytogenes SLCC 2540 (type 3b) and AB1 (type 1/2a) and of *L. ivanovii* W5379 (type 5) belong to the immunologically-active persisting category defined by Kaufmann (1984).

Genetic differences in innate resistance to *L. monocytogenes*, which distinguish C57BL/6 from A/J mice, have been attributed mainly to a deficiency in the latter of the C5 component of complement (Gervais *et al.*, 1984; Czuprynski *et al.*, 1985) with consequent impairment in the accumulation of inflammatory cells during the second stage of infection. However, to account for different degrees of bacterial growth in susceptible mice, Gervais *et al.* (1984) postulated the expression of a second gene. Such a gene might account for the relative susceptibility of C5-sufficient BALB/c mice to both *L. monocytogenes* and *L. ivanovii*; and, indeed, inflammatory peritoneal MPs from C57BL/6 mice have superior listericidal capacity compared with those from BALB/c mice (Wood *et al.*, 1986) but not with those from A/J mice (Gervais *et al.*, 1986). Also relevant is the membrane-derived monocytoysis-producing activity (MPA) of *L. monocytogenes*, to which A/J mice do not respond (Galsworthy, 1984) and which is not produced by *L. innocua* (Galsworthy, 1987), and the variable monocytopoietic responses of different mouse strains to soluble factors produced by the host during inflammatory responses (Wing *et al.*, 1985).

Apart from a slight and transient infiltration with PMNs, there was no other morphological response by either host in the livers infected by the three less pathogenic listeriae. Although apparently at variance with the demonstration by Patočka *et al.* (1979) and Manev *et al.* (1981) of intracerebral and intra-oesophageal pathogenicity of *L. innocua* for suckling mice and for adult mice and rats, respectively, this finding accords with the failure of von Koenig *et al.* (1983) to demonstrate a granulomatous response in mouse liver. However, these workers were able to induce a weak protective immunity against a serotype 4b strain of *L. monocytogenes* with large doses of *L. innocua* serotype 6b. By contrast, avirulent variants of *L. monocytogenes* (Berche *et al.*, 1987) and a recently-described serotype 3 strain of *L. innocua* (Knorz and Hof, 1986) were entirely lacking in protective immunogenicity, though capable of boosting a pre-existing immunity. Whilst histological examination may have limited sensitivity for the detection of acquired cell-mediated immunity, these seeming discrepancies call into question the possible immunopathogenic significance of the consistent, but inadequately-evaluated, differences between the lethality figures obtained with the two

different strains of mice for all three of the weakly pathogenic listeriae, as of the more variable differences in bacterial recoveries after 6 h of infection.

Listeriae are not killed by mouse serum and the rapid decline in viable bacteria during the first 6 h of liver infection with *L. monocytogenes* is generally attributed to the bactericidal action of resident Kupffer cells. This might also hold for the weakly virulent listeriae studied here, although the proportion of inoculum bacteria taken up by the liver, and thus the absolute amount of intra-hepatic killing, was not determined. Moreover the fate of such bacteria in cultured MPs has not been adequately studied (Watson and Lavizzo, 1973) and, *in vivo*, the eradication of a serotype 1/2a strain of *L. seeligeri* and of a serotype 3a strain of *L. innocua* was little affected by prior elimination of MPs (Hof and Chatzipanagiotou, 1987; Knorz and Hof, 1986). It is probably significant that, whilst *L. monocytogenes* and *L. ivanovii* were recently shown to invade and grow in a cultured line of human colon carcinoma cells, *L. innocua*, *L. welshimeri* and *L. seeligeri* were unable even to invade these cells (Gaillard *et al.*, 1987). For *L. monocytogenes*, early *in-vivo* decline is not always great (Newborg and North, 1980) and cultured MPs showed substantial inhibitory activity only if previously activated by inflammatory or immunological stimuli (Godfrey *et al.*, 1983; Czuprynski *et al.*, 1984; Krishnan and Humphrey, 1986). Indeed, immunologically naïve Kupffer cells (Krishnan and Humphrey, 1986) and otherwise non-activated MPs (Godfrey *et al.*, 1983; Kathariou *et al.*, 1987a) permitted early growth of *L. monocytogenes in vitro*, and peritoneal MPs from BALB/c mice supported bacterial growth better than those from C57BL/6 mice. Moreover Kupffer cells failed to generate significant amounts of hydrogen peroxide during the course of murine listeriosis (Lepay *et al.*, 1985), although killing of *L. monocytogenes*, especially of organisms in the logarithmic phase of growth, may not necessarily occur by oxidative mechanisms (Godfrey and Wilder, 1984; Bortolussi *et al.*, 1987). Whether non-oxidative mechanisms and cells other than Kupffer cells are important in early listerial killing needs further investigation. Possible roles for NK cells early in infection (Kearns and Leu, 1984) and of cytotoxic Lyt 2⁺ immune T cells later (Kaufmann *et al.*, 1986) have been suggested, although NK activity appeared not to account for genetically-determined innate resistance (Skamene and Kongshavn, 1983) and was not stimulated by an avirulent strain of *L. monocytogenes* (Kearns and Leu, 1984).

The role of PMNs in murine listeriosis, despite their early prominence, is generally considered irrelevant (North, 1970; Cheers and McKenzie, 1978; Mitsuyama *et al.*, 1978; Tatsukawa *et al.*, 1979). North (1970), but not Mandel and Cheers (1980), showed that most bacteria in the liver foci of 24 h-infected mice were in PMNs, some being extra-cellular and others in parenchymal cells; but bacterial killing began only with the later appearance of bactericidal inflammatory MPs. However, Czuprynski *et al.* (1984), using opsonised bacteria, showed that inflammatory peritoneal PMNs killed *L. monocytogenes* *in vitro* better than did MPs, and those from C57/BL/6 mice somewhat more effectively than those from A/J mice (Czuprynski *et al.*, 1985). Nevertheless, C57BL/6J MPs were not intrinsically more listericidal than those from A/J mice (Gervais *et al.*, 1986). Whether non-persisting strains of *Listeria* (Kaufmann, 1984) are especially susceptible to killing by PMNs remains to be shown for, if so, any consequent impairment of antigen presentation could affect the demonstrably deficient development of cell-mediated immunity. As confirmed by MacGowan *et al.* (1983), cultured human PMNs are strikingly listericidal.

The use of bacterial variants and genetically-defined hosts permits increased understanding of the pathogenesis of infection, and this study emphasises the importance of early events and areas of present uncertainty. However, natural variants are of undefined genotype and may differ in ways which are neither recognised nor understood. Recent studies with the technique of transpo-

son mutagenesis (Gaillard *et al.*, 1986; Kathariou *et al.*, 1987b; Mengaud *et al.*, 1987), which can eliminate such uncertainties, have discounted the importance of listerial catalase and re-emphasised the role of listerial haemolysin as a major virulence determinant. It could operate early in the initial encounter with host phagocytes (Kingdon and Sword, 1970), thus permitting escape from the phagocytic vacuole and intracytoplasmic bacterial growth which, in turn, is required for the induction of T cell-mediated immunity (Berche *et al.*, 1987). The heterogeneity of listerial haemolysins (Parrisius *et al.*, 1986) could readily explain the observed differences in CAMP test results between strains of *L. monocytogenes* and those of *L. ivanovii*. The apparent paradox that strains of *L. monocytogenes* and *L. seeligeri* show similar CAMP test reactions, but vary greatly in virulence, may reflect an inability of *L. seeligeri* to produce a virulence-related α -haemolysin, whilst sharing with *L. monocytogenes* the capacity to produce an oxygen-stable haemolysin, responsible for the visible zone of β -haemolysis on a blood-agar plate. Whether listerial lipolysin might contribute to the PMN and hepatocyte necrosis described here, as shown for cultured MPs by Watson and Lavizzo (1973), remains to be shown. Further molecular genetic studies will undoubtedly add to our understanding of these and other virulence determinants of this bacterial genus.

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