The *inlA* gene required for cell invasion is conserved and specific to *Listeria monocytogenes*

Claire Poyart, Patrick Trieu-Cuot and Patrick Berche

Author for correspondence: Patrick Berche. Tel: +33 1 40 61 53 79. Fax: +33 1 40 61 55 92.

INSERM U.411, Faculté de Médecine Necker-Enfants Malades, 156 rue de Vaugirard, 75724 Paris Cedex 15, France

The Gram-positive bacterium *Listeria monocytogenes* can actively induce its own uptake by epithelial cells and fibroblasts through a surface-exposed 80 kDa protein, internalin (InlA), encoded by *inlA*. We studied the distribution and the DNA polymorphism of *inlA* sequences in a wide variety of wild strains of *L. monocytogenes* as compared to other *Listeria* species. This was done by PCR-amplifying *inlA* sequences encoding the fifteen repeats A and the three repeats B of InlA. *inlA*-repeated sequences were only found in *L. monocytogenes*. The amplified fragment of *inlA* encoding the repeats A displayed an *AluI* DNA polymorphism which arises from point mutations. These results indicate that *inlA* required for cell invasion is specific to *L. monocytogenes* and that the intragenic repeats only exhibit a genetic heterogeneity due to point mutations and not to recombinations.

Keywords: *Listeria monocytogenes*, *inlA* gene, internalin

INTRODUCTION

Listeria monocytogenes is a Gram-positive bacterium which is widely distributed in the environment and causes severe food-borne infections in humans and most warm-blooded animals (Gray & Killinger, 1966). Its pathogenicity is due to its capacity to survive and to multiply in a wide variety of cell types, including macrophages, fibroblasts, epithelial cells, hepatocytes and enterocytes (Mackness, 1962; Racz *et al.*, 1972; Havell, 1986; Gaillard *et al.*, 1987; Woods *et al.*, 1993). Like other intracellular bacteria, including the Gram-negative *Yersinia pseudotuberculosis* (Isberg *et al.*, 1987; Isberg & Leong, 1990), *Shigella flexneri* (Hale & Bonventre, 1979) and *Salmonella typhimurium* (Elsinghorst *et al.*, 1989; Galan & Curtiss, 1989), *L. monocytogenes* can actively induce its own uptake by host cells through a specific interaction between a surface-exposed 80 kDa protein, termed internalin, and an unidentified receptor (Gaillard *et al.*, 1987; Gaillard *et al.*, 1991). The role of internalin has been established by isolating insertion mutants, which are non-adherent and non-invasive (Gaillard *et al.*, 1991). Gene complementation with *inlA* restores the invasive phenotype of these *L. monocytogenes* mutants and enables the non-invasive species *Listeria innocua* to penetrate enterocytes (Gaillard *et al.*, 1991). However, *inlA* has only been characterized in two *L. monocytogenes* reference strains, EGD and LO28 (Gaillard *et al.*, 1991), and almost nothing is known about

the distribution of the gene in clinical isolates of this species.

The gene *inlA* encodes a protein of 800 amino acids composed, from its amino- to its carboxy-terminus, of a signal peptide, 15 successive repeats of 22 amino acids (repeats A), three successive repeats of 70, 70 and 49 amino acids (repeats B), a proline/glycine-rich segment, a hexapeptide (LPTTGD) preceding a stretch of 20 hydrophobic amino acids, and a short positively charged tail (Gaillard *et al.*, 1991; Dramsi *et al.*, 1993) (Fig. 1). Sequence analysis of the segments of *inlA* encoding regions A and B revealed that they are made up of DNA repeats with an identity of 33–69% for repeats A, and 61–71% for the repeats B (Table 1) (Gaillard *et al.*, 1991; Dramsi *et al.*, 1993). Several pairs of segments encoding repeats A have in common a region of identity larger than 10 bp, and the two contiguous segments coding for repeats B2 and B3 share an identical 25 bp motif (Table 1). Moreover, Southern blot analysis indicates that *inlA* is also part of a gene family (Gaillard *et al.*, 1991). One of them, designated *inlB*, is located immediately downstream of *inlA* and displays 56% identity with *inlA* (Gaillard *et al.*, 1991). These structural data suggest that *inlA* might generate variants by intragenic recombination between repeated segments or by extragenic recombination with *inlA*-related sequences, as previously described for the *emm* genes of *Streptococcus pyogenes* encoding the M protein (Fischetti, 1989).

Fig. 1. Structural organization of internalin (a) and partial restriction map of *inlA* (b). The position of the peptide cleavage site (LPTTGD) is indicated by an open vertical arrow. The hatched and black segments represent the proline/glycine-rich and the hydrophobic membrane-anchored regions, respectively. Repeats A and B are represented by numbered open squares. The positions of the primers O1, O2, O3, O4 and O5 used to amplify *inlA* repeated sequences are indicated by horizontal black arrows. Only relevant restriction sites are shown: *AluI*, *Accl*, *HindIII* and *RsaI* restriction sites are indicated by black circles, black squares, black stars and open squares, respectively. Numbering begins at the first base of the initiation codon of *inlA* (Gaillard *et al.*, 1991; Dramsi *et al.*, 1993).

The aim of this work was to investigate the distribution and DNA polymorphism of *inlA* in wild, pathogenic and nonpathogenic strains of *L. monocytogenes* and related *Listeria* species.

METHODS

Bacterial strains. Sixty-eight strains of *L. monocytogenes* from various origins were used (Table 2): (i) 19 strains from several reported epidemics, 16 in humans [three from Los Angeles, CA (Linnan *et al.*, 1988), two from the Canton de Vaud, Switzerland (Bille, 1990), three from Nova Scotia, Canada (Fleming *et al.*, 1985), three from Angers, France (Carbonnelle *et al.*, 1978), three from Boston, MA (Schlech *et al.*, 1983) and two from the last epidemic in France in 1992 (Rebière & Goulet, 1993)] and three in sheep, obtained from Dr Vasquez-Boland, Madrid; (ii) 38 sporadic strains, including 36 strains isolated from human listeriosis cases from various French hospitals during 1987 and 1993 (personal collection) and two strains, one from a human case and one from listeriosis in turkey (Oklahoma, USA), obtained from Dr Swaminathan, Atlanta, GA; (iii) nine strains isolated from cheese or dairy products (INRA, Tours, France) (Tabouret *et al.*, 1991); (iv) the reference strains EGD (Gaillard *et al.*, 1986) and LO28 (Vicente *et al.*, 1985). Most strains belonged to serovar 4b (68%) and 1/2a (28%), the other serovars being 1/2b, 1/2c, 3a, 3b, 3c and 7. All strains isolated from human or animal specimens were virulent in Swiss mice, as tested after intravenous infection (LD₅₀ values 10^{4.5}–10^{5.2} bacteria per mouse (unpublished results), whereas the environmental strains were avirulent (Tabouret *et al.*, 1991). We used as controls 10 strains from other *Listeria* species: *L. innocua*, four strains (CIP8011^T, SLCC6462, SLCC4202, SLCC4213); *Listeria ivanovii*, two strains (SLCC4121, CIP7842^T); *Listeria seeligeri*, three strains (CIP100100^T, SLCC3503, SLCC4152); *Listeria welshimeri*, one strain (SLCC5334^T). The following clinical isolates from unrelated bacterial species, isolated from patients (personal collection), were used as controls: *Enterococcus faecalis*, *Streptococcus pyogenes*,

Streptococcus pneumoniae, *Staphylococcus aureus*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Escherichia coli* and *Pseudomonas aeruginosa*. Bacterial strains were grown on brain heart infusion (BHI) broth and BHI agar (Difco), and stored at –80 °C.

Characterization of *inlA* repeats A and B by PCR of *inlA*. PCR was used to amplify the repeats A and B of *inlA*. Total DNA was extracted from each of these strains as described previously (Poyart-Salmeron *et al.*, 1992). For repeats A, we used the pair of primers O1 (5'-GAATGTAACAGACACG-3') and O2 (5'-ATCGCTAATAGTAGC-3') to amplify a 1157 bp DNA fragment. For repeats B, we used the pair of primers O3 (5'-AGCCACTTAAGGCAAT-3') and O4 (5'-AGTTGATGTGTTAGA-3'), to amplify a 760 bp DNA fragment (Fig. 1). The PCR reactions were carried out in a final volume of 100 µl containing 50 ng of genomic DNA, 0.5 µM of each primer, 200 µM of each dNTP (Pharmacia Biotech) and 2.5 U of *Taq* DNA polymerase (Technomama) in a 1 × amplification buffer [10 mM Tris/HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂]. The PCR mixtures were denatured (5 min at 95 °C), then subjected to 40 cycles of amplification (1 min of annealing at 55 °C, 90 s of elongation at 72 °C and 1 min of denaturation at 94 °C). PCR products were separated by electrophoresis on 1% (w/v) agarose gel. Molecular markers (1 kb ladder) were used (Gibco, BRL). The amplified DNA fragments were transferred to Nytran membranes (Amersham), as described by Sambrook *et al.* (1989). Prehybridization and hybridization under stringent conditions were carried out for 5 h and 18 h, respectively, at 65 °C in 6 × SSC (1 × SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate), 0.5% SDS/0.005% non-fat dry milk, followed by two washings in 2 × SSC/0.1% SDS at room temperature for 30 min, and by two washings in 0.2 × SSC/0.1% SDS at 68 °C for 45 min. Southern blot analysis was performed with two probes intragenic to *inlA*: a 487 bp *DraI*–*HindIII* DNA fragment for repeats A, and a 454 bp *RsaI* DNA fragment for repeats B (Gaillard *et al.*, 1991). Labelling of DNA probes with [α -³²P]dCTP (Amersham) was carried out by using the Random Priming kit (Amersham). The DNA

Table 1. Pairwise comparisons of *inlA* repeated sequences A1–A15 and B1–B3.

For each comparison, the percentage of identity is indicated in the first line and the size, in bp, of the longest stretch of identity is indicated in parentheses in the second line. Segments of identity equal to, or larger than, 10 bp are indicated in bold.

	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12	A13	A14	A15
A1	42 (3)	40 (4)	42 (7)	43 (3)	40 (2)	42 (7)	40 (5)	50 (5)	36 (4)	36 (4)	45 (4)	39 (4)	35 (4)	44 (5)
	A2	32 (3)	60 (6)	52 (9)	53 (7)	51 (5)	62 (8)	50 (6)	51 (5)	49 (6)	49 (5)	56 (7)	45 (5)	53 (5)
		A3	58 (8)	49 (6)	62 (10)	56 (5)	58 (11)	56 (6)	49 (6)	51 (6)	64 (6)	56 (7)	45 (3)	55 (8)
			A4	52 (5)	65 (6)	51 (7)	62 (10)	58 (8)	55 (9)	49 (4)	51 (6)	58 (7)	51 (4)	55 (6)
				A5	41 (6)	48 (10)	63 (8)	54 (7)	57 (6)	57 (10)	49 (8)	66 (8)	55 (5)	49 (6)
					A6	56 (5)	55 (8)	61 (8)	55 (6)	48 (6)	51 (5)	61 (8)	47 (5)	64 (13)
						A7	45 (5)	45 (5)	33 (4)	41 (6)	47 (9)	53 (8)	56 (9)	42 (5)
							A8	61 (9)	61 (8)	56 (9)	33 (5)	64 (13)	55 (7)	68 (11)
								A9	65 (7)	53 (7)	58 (7)	62 (11)	53 (5)	61 (7)
									A10	61 (8)	47 (4)	51 (8)	47 (4)	61 (8)
										A11	42 (5)	53 (7)	45 (6)	62 (11)
											A12	59 (6)	39 (3)	53 (4)
												A13	56 (7)	59 (8)
													A14	49 (5)
B1	B2	B3												
	66 (10)	61 (15)												
	B2	71 (25)												

polymorphism of the amplified DNA fragments was studied by enzymic restriction. Amplified fragments (0.2 µg) were incubated with 2–3 U of *AluI*, *HindIII*, *AclI* or *RsaI* (New England Biolabs) for 2 h at 37 °C and the resulting DNA fragments were separated by electrophoresis on 3.5 % (w/v) metaphor agarose gel (FMC Bioproduct).

Sequence analysis of *inlA* repeats A. The 406 bp amplified fragments of *inlA* repeats A were amplified by using primers O2 and O5 (5'-GGTTTAACCGCACTCAC-3') and purified from agarose gels by using the QIAE XII kit (Qiagen), treated with DNA polymerase I (Pharmacia) to convert ragged ends to blunt ends, and cloned in both orientations into a *SmaI*-linearized and dephosphorylated replicative form of M13mp18. For each cloning, three clones were sequenced on both strands (Sanger *et al.*, 1977).

RESULTS AND DISCUSSION

Amplification of *inlA* repeats A and B in *Listeria* species

The presence of *inlA* was studied by amplifying intragenic fragments of repeats A and B in 68 strains of *L. monocytogenes*, as compared to 10 strains of other *Listeria* species (*L. innocua*, *L. seeligeri*, *L. ivanovii* and *L. welshimeri*) and eight strains of unrelated Gram-positive and Gram-negative species (*E. faecalis*, *Strep. pyogenes*, *Strep. pneumoniae*, *Staph. aureus*, *H. influenzae*, *N. meningitis*, *E. coli* and *P. aeruginosa*). For each pair of primers specific for intragenic fragments of repeats A and B of *inlA*, a single DNA fragment of the expected size was observed for the 68

Table 2. Origin of *L. monocytogenes* strains

Strains	Origin
Reference strains	
LO28	Madrid, Spain (Vicente <i>et al.</i> , 1985)
EGD	Trudeau Institute, New York (Gaillard <i>et al.</i> , 1986)
Epidemic strains*	
850190	} Los Angeles, USA, 1988 (Linnan <i>et al.</i> , 1988)
850203	
850212	
NEM405	} Switzerland, 1990 (Bille, 1990)
NEM 406	
820163	} Nova Scotia, Canada, 1985 (Fleming <i>et al.</i> , 1985)
820162	
820160	
C93	} Angers, France, 1978 (Carbonnelle <i>et al.</i> , 1978)
C42	
C38	
830335	} Boston, USA, 1983 (Schelch <i>et al.</i> , 1983)
830336	
830337	
NEM296	} Paris, France, 1992 (P. Berche, Necker Hospital)
NEM295	
NEM263	} Madrid, Spain, 1992, from sheep (Dr J. A. Vasquez-Boland)
NEM264	
NEM265	
Sporadic strains*	
F6963	Oklahoma, USA (Dr B. Swaminathan, Atlanta)
F6953	Oklahoma, USA, from turkey (Dr B. Swaminathan, Atlanta)
NEM152-180	France, 1987-1993 (Dr J. Croizé, Grenoble)
NEM261-262	} France, 1987-1993 (P. Berche, Necker Hospital)
NEM266-269	
Environmental strains	
INRA36	} Dr M. Tabouret, France, 1992
INRA49	
INRA50	
INRA64	
INRA85	
INRA35	
INRA41	
INRA61	} Dr A. Audurier, Tours, France, 1987
NEM270	

* All isolates of human origin except where indicated.

strains of *L. monocytogenes*, including sporadic, epidemic and environmental isolates. These fragments were hybridized under high stringency conditions with intragenic probes of repeats A and B of *inlA*. This is illustrated for 12 representative strains in Fig. 2, including four epidemic, four sporadic and three environmental strains. In contrast, no amplification product was detected with any strain belonging to other *Listeria* species or to unrelated bacterial species, either by ethidium bromide staining or hybridization with the *inlA* probe under stringent con-

ditions (data not shown). The PCR assay did not detect *inlA* repeats in the other *Listeria* species or in various unrelated bacterial species. These results indicate that *inlA* is constantly present in *L. monocytogenes*, regardless of the origin, serovar and virulence of the isolates. It should be noted that sequences related to *inlA* have been detected in the chromosomes of *L. ivanovii* and of the noninvasive species *L. innocua* by DNA-DNA hybridization under low stringency conditions (Gaillard *et al.*, 1991). The gene *inlA* and the related sequences present in

Fig. 2. Analysis by agarose gel electrophoresis (left) and by hybridization (right) of PCR products obtained by using primers amplifying the segments of *inlA* in *L. monocytogenes* total DNA encoding the repeats A and B of the corresponding protein. PCR products were separated by electrophoresis on 1% agarose gel and probed with intragenic probes specific for repeats A or B. Lanes: 1, reference strain EGD; 2–5, epidemic strains C93, NEM296, 850190 and 820163; 6–9, sporadic strains NEM152, NEM153, NEM169 and NEM170; 10–12, environmental strains INRA36, INRA49, INRA50; L, 1 kb ladder used as molecular size standard.

Fig. 3. Analysis by agarose gel electrophoresis (3.5% agarose) of *AluI* restriction patterns of PCR products corresponding to the amplified repeats A of *inlA* in total DNA of 20 strains of *L. monocytogenes*. Lanes: 1 and 2, reference strains EGD and LO28; 3–8, epidemic strains C93, NEM296, 850190, 850203, 820163, 830335; 9–14, sporadic strains NEM152, NEM171, NEM153, NEM169, NEM170 and NEM155; 15–20, environmental strains INRA36, INRA49, INRA50, INRA64, INRA85 and NEM270.

these phylogenetically closely related species are likely to be derived from a common ancestor. This hypothesis implies that these sequences have sufficiently diverged from each other not to allow the detection of *inlA*-related sequences with our PCR assay.

The molecular organization of internalin is analogous to

that of certain cell wall proteins from Gram-positive bacteria, especially that of the M protein of *Strep. pyogenes*. It is known that, in serotype M6, M proteins of different sizes appear to result from intragenic recombination between central repeat blocks of the *emm6* gene (Hollingshead *et al.*, 1986). Recombination between the central repeat blocks results in deletions producing changes in the primary amino acid sequence, which modify antigenic determinants or lead to the loss of an opsonic epitope in the A-repeat region (Jones *et al.*, 1988). Another mechanism involving extragenic recombination between adjacent *emm*-like genes has also been reported (Haanes & Cleary, 1989). It is unlikely that such recombinations do occur in *L. monocytogenes* in view of our results. Indeed, the size of the amplified DNA fragments of repeats A and B of *inlA* is constant in all 68 isolates of *L. monocytogenes*. Part of this analysis is illustrated in Fig. 2. In fact, antigenic variation has only been described for extracellular pathogens where it allows them to escape the immune system (Robertson & Meyer, 1992). As a facultative intracellular pathogen, *L. monocytogenes* is slightly exposed to the selective pressure of antibodies, at least at the early phase of infection. Entry into host cells through ligand–receptor interaction might require that internalin be structurally conserved.

Genetic polymorphism of *inlA* repeats A and B

The DNA polymorphism of the *L. monocytogenes* amplified DNA fragments of repeats A and B of *inlA* was then studied by enzymic restriction, using *AluI*, *HindIII* or *RsaI*. According to the sequence of *inlA* (Gaillard *et al.*, 1991; Dramsi *et al.*, 1993), there are 10 restriction sites cleaved by these enzymes in this DNA fragment (Fig. 1). The *HindIII* and *RsaI* restriction patterns obtained were indistinguishable (data not shown), whereas *AluI* restriction analysis discriminated five different profiles for the 68 *L. monocytogenes* strains studied. This is illustrated for representative strains in Fig. 3. The sizes of the *AluI* fragments used for reference purposes were those of *inlA* from *L. monocytogenes* EGD (Gaillard *et al.*, 1991; Dramsi *et al.*, 1993) and the corresponding electrophoretic pattern was arbitrarily chosen as defining the type I restriction profile (Fig. 1). Size increase (approx. 27 bp) or decrease (approx. 15 bp) of the 180 bp *AluI* restriction fragment containing 5 bp of repeats A14 and A15, and 109 bp downstream of repeat A15 (Fig. 1) defines the restriction profiles of types II (Fig. 3, lanes 2, 14–17) and III (lanes 5–8, 12, 13, 19), respectively. Types IV (Fig. 3, lanes 3, 4, 9–11, 18) and V (lane 20) are related to types II and III, respectively, but exhibit a size increase (approx. 12 bp) of the 102 bp *AluI* fragment which contains 57 bp of A12 and 45 bp of A13 (Figs 1 and 3). The two *AluI* segments involved in the observed polymorphism are located in the same region of *inlA*, i.e. that encoding the carboxylic part of the repeats A. This might simply be due to the fact that five out of the eight *AluI* restriction sites present in the segment of *inlA* coding for the repeats A are clustered within this region (Fig. 1). A specific profile cannot be associated with the epidemic or sporadic behaviour of the strains, either with their serovars or their virulence.

Table 3. Differences between the amplified 406 bp DNA fragment of *inlA* encoding the repeats A11–A15 from *L. monocytogenes* strains representative of the five *AluI* restriction profiles

Base no.*	Codon† and corresponding amino acid					
	Strain ...	EGD‡ (type I)§	L028 (type II)§	850190 (type III)§	C93 (type IV)§	NEM270 (type V)§
966	TTa/L					TTt/F
972	CTt/L				CTa/L	CTa/L
985/987	cTg/L			cTa/L	tTa/L	tTa/L
1035	ACg/T		ACt/T	ACt/T		
1143	AAc/N				AAt/N	
1149	ACc/T		ACa/T	ACa/T		
1167	TCa/S			TCg/S	TCg/S	
1170	GCt/A			GCc/A		
1221	ATc/I				ATt/I	
1222	aCC/T			gCC/A		
1228	cTA/L				tTA/L	
1233	GGg/G				GGa/G	
1242	GAt/D				GAc/D	
1247	GcA/A				GaA/E	
1258	gCA/A				cCA/P	
1266	GTa/V				GTg/V	
1277	GcA/A				GtA/V	
1281	AAt/N					AAc/N
1283	GtA/V			GcA/A		
1314	ACt/T			ACg/T	ACg/T	
1317	GGc/G					GGt/G
1323	TTg/L		TTa/L			
1332	CCt/P	CCa/P	CCa/P	CCa/P	CCa/P	CCa/P

* Numbering refers to the first base of the ATG codon of the revised sequence of *inlA* (Gaillard *et al.*, 1991; Dramsi *et al.*, 1993) and indicates the position of nonidentical bases.

† Identical and nonidentical nucleotides in the codons are written in upper and lower case, respectively.

‡ Data are from Gaillard *et al.* (1991).

§ Data are based on the sequence determination of the amplified 409 bp DNA fragments. Only the codons which differ from those of the revised sequence of *inlA* (Gaillard *et al.*, 1991; Dramsi *et al.*, 1993) are shown.

The DNA polymorphism of the 760 bp amplified DNA fragment encoding the repeats B was studied similarly following digestion with *AccI*, *AluI* or *RsaI* endonucleases. There are six restriction sites cleaved by these enzymes in this DNA fragment (Fig. 1). The restriction patterns obtained with each of the three enzymes were indistinguishable for all 68 *L. monocytogenes* strains (data not shown).

Characterization of *inlA* variants

In an attempt to elucidate the molecular basis of the *AluI* polymorphism, we amplified a 406 bp DNA fragment of *inlA* encoding the repeats A11–A15 from *L. monocytogenes* strains (EGD, L028, 850190, C93, NEM270) representative of the five types of *AluI* restriction profiles (I, II, III, IV and V, respectively) by using the primers O5

and O2 (Fig. 1). The amplified DNA fragments were purified from each amplification mixture, cloned into M13mp18 and sequenced entirely. Comparison of their nucleotide sequences with that of the corresponding segment of *inlA* from *L. monocytogenes* EGD (Gaillard *et al.*, 1991; Dramsi *et al.*, 1993) taken as a reference, revealed that they differ at 24 positions which are located in 23 codons (Table 1). Surprisingly, the amplified DNA fragment obtained from *L. monocytogenes* EGD differed at one position from the previously published sequences (Gaillard *et al.*, 1991; Dramsi *et al.*, 1993). However, since the same mutation was found in the four other sequences determined (Table 3), it is unlikely that it results from misincorporation of nucleotides during the PCR assay. The amplified DNA fragments representative of the five restriction profiles characterized have the same size and their nucleotide sequences are highly related (the level of identity is $\geq 96\%$ depending on the sequences con-

sidered). Thus, the observed DNA polymorphism most likely results from point mutations and not from intragenic recombination within *inlA* or from extragenic recombination between *inlA* and the highly divergent *inlA*-related sequences. Among the 13 mutations located in the region encoding A11–A15, 11 were silent and the remaining two at positions 966 and 1222 maintained a similarly charged residue at the same place (Table 1). Eleven mutations were located in the 109 bp sequence downstream of the region coding for A15. Of these changes, six mutations were silent and the substitutions at positions 1258, 1277 and 1283 maintained a similarly charged residue at the same place (Table 1). Only the change at position 1247 corresponds to a nonequivalent amino acid replacement: alanine (EGD, LO28, 850190 and NEM270) versus glutamic acid (C93). Since strain C93 was isolated from an epidemic and is therefore virulent, it is assumed that this mutation has not led to modification of the functionality of InlA. In conclusion, our results show that *inlA* required for invasion is specific to *L. monocytogenes* and that the intragenic repeats only exhibit a genetic heterogeneity due to mutations and not to recombinations.

ACKNOWLEDGEMENTS

We are grateful to Dr Croizé (Grenoble), Dr Swaminathan (Atlanta), Dr Audurier (Tours), Dr Bille (Genève), Dr Tabouret (Tours) and Dr Vasquez-Boland (Madrid) for the gift of *L. monocytogenes* strains. R. Fournier is also thanked for typing the manuscript. This work was supported by INSERM, the University Paris V and the Fondation pour la Recherche Médicale.

REFERENCES

- Bille, J. (1990). Epidemiology of human listeriosis in Europe, with special reference to the Swiss outbreak. In *Foodborne Listeriosis*, pp. 71–74. Edited by A. J. Miller, J. L. Smith & G. A. Somkuti. New York: Society for Industrial Microbiology/Elsevier Science Publishing.
- Carbonnelle, B., Cottin, J., Parvery, F., Chambreuil, G., Kouyoumdhan, S., Le Lirzin, M., Cordier, G. & Vincent, T. (1978). Epidémie de listériose dans l'ouest de la France (1975–1976). *Rev Epidemiol Sante Publique* **26**, 451–467.
- Dramsi, S., Deboux, P. & Cossart, P. (1993). Common features of Gram-positive bacterial proteins involved in cell recognition. *Mol Microbiol* **9**, 1119–1122.
- Elsinghorst, E. A., Baron, L. S. & Kopecko, D. J. (1989). Penetration of human intestinal epithelial cells by *Salmonella*: molecular cloning and expression of *Salmonella typhi* invasion determinants in *Escherichia coli*. *Proc Natl Acad Sci USA* **86**, 5173–5177.
- Fischetti, V. A. (1989). Streptococcal M protein: molecular design and biological behavior. *Clin Microbiol Rev* **2**, 285–314.
- Fleming, D. W., Cochi, S. L., MacDonald, K. L., Brondum, J., Hayes, P. S., Plikaytis, B. D., Holmes, M. B., Audurier, A., Broome, C. & Reingold, A. L. (1985). Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *New Engl J Med* **312**, 404–407.
- Gaillard, J. L., Berche, P. & Sansonetti, P. (1986). Transposon mutagenesis as a tool to study the role of hemolysin in the virulence of *Listeria monocytogenes*. *Infect Immun* **52**, 50–55.
- Gaillard, J.-L., Berche, P., Mounier, J., Richard, S. & Sansonetti, P. (1987). *In vitro* model of penetration and intracellular growth of *Listeria monocytogenes* in the human enterocyte-like cell line Caco-2. *Infect Immun* **55**, 2822–2829.
- Gaillard, J.-L., Berche, P., Fréhel, C., Guoin, E. & Cossart, P. (1991). Entry of *L. monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci. *Cell* **65**, 1127–1141.
- Galan, J. E. & Curtiss, R., III (1989). Cloning and molecular characterization of genes whose products allow *Salmonella typhimurium* to penetrate tissue culture cells. *Proc Natl Acad Sci USA* **86**, 6383–6387.
- Gray, M. L. & Killinger, A. H. (1966). *Listeria monocytogenes* and listeric infections. *Bacteriol Rev* **30**, 309–382.
- Haanes, E. J. & Cleary, P. P. (1989). Identification of a divergent M protein gene and an M protein-related gene family in *Streptococcus pyogenes* serotype 49. *J Bacteriol* **171**, 6397–6408.
- Hale T. L. & Bonventre, P. F. (1979). *Shigella* infection of Henle intestinal epithelial cells: role of the bacterium. *Infect Immun* **24**, 879–886.
- Havell, E. A. (1986). Synthesis and secretion of interferon by murine fibroblasts in response to intracellular *Listeria monocytogenes*. *Infect Immun* **54**, 787–792.
- Hollingshead, S. K., Fischetti, V. A. & Scott, J. R. (1986). Complete nucleotide sequence of type 6 M protein of the group A streptococcus: repetitive structure and membrane anchor. *J Biol Chem* **261**, 1677–1686.
- Isberg, R. R. & Leong, J. M. (1990). Multiple β_1 chain integrins are receptors for invasins, a protein that promotes bacterial penetration into mammalian cells. *Cell* **60**, 861–871.
- Isberg, R. R., Voorhis, D. L. & Falkow, S. (1987). Identification of invasins: a protein that allows enteric bacteria to penetrate cultured mammalian cells. *Cell* **50**, 769–778.
- Jones, K. F., Hollingshead, S. K., Scott, J. R. & Fischetti, V. A. (1988). Spontaneous M6 protein size mutants of group A streptococci display variation in antigenic and opsonogenic epitopes. *Proc Natl Acad Sci USA* **85**, 8271–8275.
- Linnan, M. J., Mascola, L., Lou, X. D., Goulet, V., May, S., Salminen, C., Hird, D. X., Yonekura, M. L., Hayes, P., Weaver, R., Audurier, A., Plikaytis, B. D., Fannin, S. L., Kleks, A. & Broome, C. V. (1988). Epidemic listeriosis associated with Mexican-style cheese. *N Engl J Med* **319**, 823–828.
- Mackness, G. G. (1962). Cellular resistance to infection. *J Exp Med* **116**, 381–406.
- Poyart-Salmeron, C., Trieu-Cuot, P., Carlier, C., MacGowan, A., McLauchlin, J. & Courvalin, P. (1992). Genetic basis of tetracycline resistance in clinical isolates of *Listeria monocytogenes*. *Antimicrob Agents Chemother* **36**, 463–466.
- Racz, P., Tenner, K. & Mero, E. (1972). Experimental *Listeria enteritis*. I. An electron microscopic study of the epithelial phase in experimental *Listeria* infection. *Lab Invest Meth Cell Biol* **26**, 694–700.
- Rebière, I. & Goulet, V. (1993). La listériose: revue générale et référence à l'épidémie française de 1992. *Lett Infectiol* **8**, 130–135.
- Robertson, B. D. & Meyer, T. F. (1992). Genetic variation in pathogenic bacteria. *Trends Genet* **8**, 422–427.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular cloning: a laboratory manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**, 5463–5467.

Schlech, V. F., Lavigne, P. M., Bortolussi, R. A., Allen, A. C., Haldane, E. V., Wort, A. J., Hightower, A. W., Johnson, S. E., King, S. H., Nicholls, E. S. & Broome, C. V. (1983). Epidemic listeriosis – evidence for transmission by food. *N Engl J Med* **308**, 203–206.

Tabouret, M., Rycke, J., Andurier, A. & Poutrel, B. (1991). Pathogenicity of *Listeria monocytogenes* isolates in immunocompromised mice in relation to listeriolysin production. *J Med Microbiol* **34**, 13–18.

Vicente, M. F., Baquero, F. & Perez-Diaz, J. C. (1985). Cloning and expression of the *Listeria monocytogenes* haemolysin in *E. coli*. *FEMS Microbiol Lett* **30**, 77–79.

Woods, S., Maroushek, N. & Czuprynski, C. J. (1993). Multiplication of *Listeria monocytogenes* in a murine hepatocyte cell line. *Infect Immun* **61**, 3068–3072.

Received 23 May 1995; revised 3 August 1995; accepted 23 August 1995.