First case of *Listeria innocua* meningitis in a patient on steroids and etanercept

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**Introduction:** *Listeria innocua* is widespread in food and the environment and is considered to be a non-pathogenic bacterium in healthy subjects. To date, this species has only been associated with human diseases in a fatal case of bacteraemia in an elderly patient. Here, we describe a case of acute meningitis infection caused by this bacterium.

**Case presentation:** Our patient had an increased risk of infection because of treatment with etanercept and a corticosteroid given for rheumatoid arthritis. Etanercept use has been described previously as the possible cause of multiple *Listeria monocytogenes* infections (to date, four cases have been described, of which two were cases of arthritis and two of meningitis), but etanercept has never been associated with *L. innocua* meningitis. In our case, despite rapid identification of the pathogen and proper antibiotic treatment, the patient had an unfavourable outcome.

**Conclusion:** To the best of our knowledge, this report constitutes the first documentation of a case of meningitis due to *L. innocua*, and our experience serves as a warning to microbiologists and clinicians that *L. monocytogenes* is not the only *Listeria* sp. that can cause human meningitis.

**Keywords:** etanercept; listeriosis; meningitis.

**Introduction**

*Listeria innocua* is considered a non-haemolytic saprophyte that is widely distributed in the natural environment. *L. innocua* is able to survive in various extreme conditions (high pH, high and low temperatures, and high salt concentrations) and has been isolated from soil, surface water, decaying vegetables, sewage and foodstuffs (Moreno et al., 2012). It is most closely related to *Listeria monocytogenes* but is generally considered non-pathogenic. In fact, *L. innocua* does not seem to carry the virulence-associated genes described in *L. monocytogenes* and *Listeria ivanovii*. Nevertheless some atypical *L. monocytogenes*-like haemolytic *L. innocua* strains have been described (Johnson et al., 2004; Moreno et al., 2012). Some genetic characteristics of these *L. innocua* strains, such as possession of the LIPI-1 virulence cluster and part of the *inlAB* operon, can justify the ability to replicate intracellularly in mammalian cells and so be causative agents of disease, especially in immunocompromised mammals (Buchrieser et al., 2003; Glaser et al., 2001; Johnson et al., 2004; Milillo et al., 2012; Perrin et al., 2003; Volokhov et al., 2007; Walker et al., 1994; Welch, 2007). Perrin et al. (2003) described a fatal bacteraemia caused by a *L. innocua* strain and this was the first case reported in humans. Corticosteroids increase the risk of listeriosis (Schuchat et al., 1992), and etanercept use is associated with the possible occurrence of *L. monocytogenes* infection (La Montagna, G., Valentini, G., 2005; Pagliano et al., 2004; Rachapalli & O’Daunt, 2005; Salmon-Ceron et al., 2011; Schett et al., 2005). Here, we describe the first case of *L. innocua* meningitis, to the best of our knowledge, in a patient treated with etanercept and corticosteroids given for rheumatoid arthritis (Pagliano et al., 2004; Rachapalli & O’Daunt, 2005; Schett et al., 2005; Slifman et al., 2003).

**Case report**

A 72-year-old woman was admitted to our hospital in June 2010 with a high fever (up to 39.5 °C) and confusion. The patient had a 10-year history of rheumatoid arthritis, and had been treated in the last 3 years with methotrexate 10 mg once a week, and with intravenous infliximab...
100 mg every 6 weeks. In the last 5 months, the therapy for rheumatoid arthritis changed to 25 mg prednison once a day and 25 mg etanercept once a week. It is worth noting that, 5 months prior to the admission, the patient had a 2-week episode of meningomyeloradiculitis with Epstein–Barr virus-positive cerebrospinal fluid (CSF). At the time of admission, on objective examination, the patient appeared stuporous and presented neck stiffness and fever (39.5 °C). A lumbar puncture was performed again.

Investigations

The CSF appeared subclear and the white blood cell count was 3848 mm

−3 [the lymphocytes were numerous (60 %) and several were activated, and a discrete number of monocytes were present as well as some red blood cells], protein 499.1 mg dl

−1 (reference value 10–45), white blood cell count was 73 mm

−3, CSF glucose was 0 mg dl

−1, serum glucose was 118 mg dl

−1 and lactate was 66.6 mg dl

−1. Microscopic examination of the CSF showed Gram-positive rods with a coryneform appearance, suggesting a diagnosis of L. monocytogenes. An overnight culture of the CSF on sheep blood agar plates facilitated the growth of small, white, non-haemolytic colonies. The absence of haemolysis was unusual for the expected L. monocytogenes but is otherwise common for other Listeria spp. (such as L. innocua, Listeria welshimeri and Listeria seeligeri) (Rocourt & Grimont, 1983; Seeliger, 1981). Our isolate also exhibited characteristic tumbling motility when viewed under a light microscope (by hanging drop). On Listeria selective agar (PALCAM; bioMérieux Italia), a very faint blackening of the medium (due to the aesculin hydrolysis) was only obtained after 48 h of incubation at 37 °C in aerobic conditions. Catalase production, aesculin hydrolysis (albeit very slow) and the production of acid from glucose, maltose, trehalose and lactose were all positive. Bacterial identification was performed using a GP card on a VITEK 2 system (bioMérieux) and by nucleotide sequencing. In particular, the bacterial DNA was extracted and then amplified as described previously by Turner et al. (1999). The sequencing analysis was performed using an ABI Prism 310 Genetic Analyser (Applera). The sequence obtained was compared with all of the bacterial sequences available in GenBank, using BLAST (http://blast.ncbi.nlm.nih.gov/BLAST.cgi). The analysis showed 99 % nucleotide identity between the isolate and three published L. innocua sequences (GenBank accession nos HM007562.1, S55473.1 and AL596173.1) and 98 % nucleotide identity between the isolate and L. monocytogenes (GenBank accession no. EU090894.1). Additional auxiliary identification tests were performed, and the absence of haemolytic activity was confirmed by performing a CAMP test (named from Christie, Atkins and Munch-Petersen), which was negative for our isolate (Christie et al., 1944). Detection of phosphatidylinositol phospholipase C (PlcA) activity, which is useful in distinguishing L. innocua from L. monocytogenes, was performed on an agar plate and was also negative (Restaino et al., 1999). Serotyping indicated that our isolate was serotype 4 (Johnson et al., 2004). To better characterize our isolate, L. innocua gene tests were performed. These gene tests are known to be able to identify species of Listeria isolates on the basis of the presence (or absence) of 12 virulence genes, as well as on the detection of some L. innocua-specific genes. The virulence genes were: prfA, hly, plcA, plcB, mpl, actA, inlA, inlB, clpE, iap, daaA and inIC (Johnson et al., 2004; Volokhov et al., 2002), while the L. innocua-specific genes, detectable by PCR assays, were lin02693, lin1074, lin1068, lin0558, lin1073, lin0198, lin2454, lin0372 and lin0419, and for the non-coding intergenic regions were lin0454–lin0455–lin2134 (Glaser et al., 2001; Johnson et al., 2004). We also detected and sequenced the 16S–23S intergenic region (Johnson et al., 2004). The primer sequences and the PCR conditions were the same as those reported by Johnson et al. (2004). The results of the PCR assays are shown in Table 1. Our isolate contained the following virulence genes: prfA, inlA, inlB and the virulence cluster genes hly and plcA (not plcB), which encode, respectively, the L. monocytogenes haemolysin (listeriolysin O) and inositol-specific phospholipase C. The strain had mpl, iap, clpE and daaA but not actA. Moreover, our strain contained some of the L. innocua-specific genes namely: lin0372, lin0454–lin0455, lin0558 and lin1068 (Table 1). The majority of these genes had the expected sizes, particularly when our isolate was compared with the isolate reported by Johnson et al. (2004): L. innocua PRL/NW 15B95. The sizes of the amplicons are shown in Table 1. Only a few exceptions were observed and concerned the following genes: plcA, mpl, lin0454–lin0455 and lin1068. However, these differences could be explained by the extreme variability of this species regarding gene assortment, as already described by several authors (Glaser et al., 2001; Johnson et al., 2004; Volokhov et al., 2007). The sequence analysis of the 16S–23S intergenic region showed 99 % nucleotide identity with the L. innocua genome deposited in GenBank. The accession number of the sequence is NCBI accession no. HM007562.1.

Our isolate was tested for antimicrobial susceptibility and was resistant to penicillin and oxacillin but susceptible to all other antimicrobials tested (ampicillin, ciprofloxacin, levofloxacin, moxifloxacin, tetracycline, clindamycin, streptomycin, gentamicin quinupristin/dalfopristin, rifampicin, vancomycin, teicoplanin and linezolid).

L. monocytogenes is an invasive opportunistic, foodborne pathogen and is one of the leading causes of mortality from foodborne infections. The complete genome sequence of L. monocytogenes demonstrates that it is closely related to other non-pathogenic species such as L. innocua. The most striking features of the L. monocytogenes genome are an exceptionally large number of surface proteins and an abundance of transport proteins (in particular, proteins dedicated to carbohydrate transport and an extensive regulatory repertoire). Surface proteins have important roles in the interactions of the micro-organism with its...
environment, in particular during host infection. Thus, the presence/absence of such proteins could be a valuable determinant of virulence. Particularly, among the surface proteins, internalins and InlB, are necessary to enter eukaryotic cells, while ActA plays a key role in the movement of \textit{L. monocytogenes} in the host cell (Volokhov et al., 2007; Welch, 2007). Another fundamental feature of \textit{Listeria} spp. characterization is the presence/absence of some genes, particularly those that belong to the LIPI1 pathogenicity island of \textit{L. monocytogenes} (Volokhov et al., 2002). Data obtained using the PCR assays confirmed the potential pathogenicity of our isolate, which had some genes typical of \textit{L. monocytogenes} (\textit{prfA}, \textit{hly}, \textit{plcA}, \textit{plcB}, \textit{iap}, \textit{clpE} and \textit{daaA}). In contrast, the absence of \textit{pclB} and \textit{actA} (typical of \textit{L. monocytogenes}) confirmed the difference of our isolate from \textit{L. monocytogenes}.

The gene asset, \textit{plcA}+\textit{plcB}, of our isolate could justify the absence of the phosphatidylinositol phospholipase C activity that was shown on the agar plate. Other features such as the presence of some \textit{L. innocua}-specific genes (\textit{lin0372}, \textit{lin0454–lin0455}, \textit{lin0558} and \textit{lin1068}) as well as the sequence analysis of the 16S–23S intergenic region confirmed that our isolates was a \textit{L. innocua} strain and is not a variant of \textit{L. monocytogenes}. Moreover, our strain appeared to be different from previously reported \textit{L. innocua} strains. This evidence was particularly clear when we compared our isolate with those reported by Johnson et al. (2004), particularly with the \textit{Listeria} sp. strain PRL/NW 15B95, which was the main subject of their study (see Table 1). This \textit{L. innocua} strain was capable of invasive disease in the absence of cell-to-cell spreading, as suggested by the absence of the \textit{actA} gene product (an important virulence determinant for \textit{L. monocytogenes}).

**Diagnosis**

A diagnosis of acute meningitis and late obstructive hydrocephalus was made.

**Treatment**

At the time of CSF-positive culture, a therapy based on 3 g ampicillin every 6 h and 80 mg gentamicin every 8 h was started. The common dosage for ampicillin is 2 g every 4 h, but some clinical experience has shown that ampicillin given in daily doses of 6 g or more is probably equally effective against \textit{L. monocytogenes} (Jones et al., 1997). In our case, the dose of ampicillin used was intermediate between the two and this may have negatively influenced the patient’s clinical course.

**Outcome and follow-up**

As the microscopic examination of CSF was positive, the patient was transferred to an Infectious Disease Centre

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**Table 1. Comparison of the gene assortment of \textit{L. monocytogenes}, \textit{L. innocua} PRL/NW 15B95 and our isolate**

<table>
<thead>
<tr>
<th>Gene</th>
<th>\textit{L. monocytogenes}*</th>
<th>\textit{L. innocua} (our isolate)</th>
<th>\textit{L. innocua} PRL/NW 15B95†</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{prfA}</td>
<td>Present</td>
<td>Present (470 bp)</td>
<td>Present (479 bp)</td>
</tr>
<tr>
<td>\textit{inlA}</td>
<td>Present</td>
<td>Present (1400 bp)</td>
<td>Not present (1423 bp)‡</td>
</tr>
<tr>
<td>\textit{inlB}</td>
<td>Present</td>
<td>Present (110 bp)</td>
<td>Not present (1107 bp)‡</td>
</tr>
<tr>
<td>\textit{inlC}</td>
<td>Present</td>
<td>Not present</td>
<td>Not present (1861 bp)‡</td>
</tr>
<tr>
<td>\textit{hly}</td>
<td>Present</td>
<td>Present (490 bp)</td>
<td>Present (496 bp)</td>
</tr>
<tr>
<td>\textit{plcA}</td>
<td>Present</td>
<td>Present (690 bp)</td>
<td>Present (798 bp)</td>
</tr>
<tr>
<td>\textit{plcB}</td>
<td>Present</td>
<td>Not present</td>
<td>Present (2691 bp)</td>
</tr>
<tr>
<td>\textit{mpl}</td>
<td>Present</td>
<td>Present (820 bp)</td>
<td>Present (674 bp)</td>
</tr>
<tr>
<td>\textit{iap}</td>
<td>Present</td>
<td>Present (610 bp)</td>
<td>Present (619 bp)</td>
</tr>
<tr>
<td>\textit{clpE}</td>
<td>Present</td>
<td>Present (1020 bp)</td>
<td>Present (1021 bp)</td>
</tr>
<tr>
<td>\textit{daaA}</td>
<td>Present</td>
<td>Present (1600 bp)</td>
<td>Present (1671 bp)</td>
</tr>
<tr>
<td>\textit{actA}</td>
<td>Present</td>
<td>Not present</td>
<td>Present (209 bp)</td>
</tr>
<tr>
<td>\textit{lin0372}</td>
<td>Not present</td>
<td>Present (384 bp)</td>
<td>Present (384 bp)</td>
</tr>
<tr>
<td>\textit{lin0419}</td>
<td>Not present</td>
<td>Not present</td>
<td>Present (429 bp)</td>
</tr>
<tr>
<td>\textit{lin2134}</td>
<td>Not present</td>
<td>Not present</td>
<td>Present (2159 bp)</td>
</tr>
<tr>
<td>\textit{lin0454–lin0455}</td>
<td>Not present</td>
<td>Present (1200 bp)</td>
<td>Present (470 bp)</td>
</tr>
<tr>
<td>\textit{lin0558}</td>
<td>Not present</td>
<td>Present (500 bp)</td>
<td>Present (586 bp)</td>
</tr>
<tr>
<td>\textit{lin1068}</td>
<td>Not present</td>
<td>Present (490 bp)</td>
<td>Present (1092 bp)</td>
</tr>
<tr>
<td>\textit{lin1073}</td>
<td>Not present</td>
<td>Not present</td>
<td>Present (1098 bp)</td>
</tr>
<tr>
<td>\textit{lin1074}</td>
<td>Not present</td>
<td>Not present</td>
<td>Present (1100 bp)</td>
</tr>
<tr>
<td>\textit{lin2693}</td>
<td>Not present</td>
<td>Not present</td>
<td>Present (2711 bp)</td>
</tr>
<tr>
<td>\textit{lin0198}</td>
<td>Not present</td>
<td>Not present</td>
<td>Present (189 bp)</td>
</tr>
<tr>
<td>\textit{lin2454}</td>
<td>Not present</td>
<td>Not present</td>
<td>Present (2473 bp)</td>
</tr>
</tbody>
</table>

*\textit{L. monocytogenes} from our strain collection.
†\textit{L. innocua} strain described by Johnson et al. (2004).
‡The gene was not present in the \textit{L. innocua} PRL/NW 15B95 strain and the amplicon size refers to that of \textit{L. monocytogenes}.
and, shortly thereafter, to an intensive care unit, continuing treatment with the following specific antibiotic therapy: 3 g ampicillin every 6 h for 1 week and 80 mg gentamicin every 8 h for 2 weeks. After a transient clinical improvement associated with an almost normal CSF examination, the consciousness of the patient worsened. Upon magnetic resonance imaging, the brain showed enlarged lateral ventricles and periventricular oedema, whereas the mesencephalic duct was of a small calibre. The obstructive hydrocephalus was treated with a ventricular shunt. A short improvement of consciousness was interrupted again 2 weeks later by the occurrence of *Acinetobacter baumannii* pneumonia, which was unresponsive to different cocktails of antibiotics (vancomycin, levofloxacin, linezolid, colistin, tigecycline and fluconazole), and death occurred in approximately 10 days. An autopsy was not performed.

**Discussion**

*L. innocua* is widespread in the environment and food. It causes animal infections but was not considered to be a human pathogenic bacterium until the publication by Perrin et al. (2003) who presented a case of fatal sepsis. Due to the strong similarities between *L. monocytogenes* and *L. innocua*, the correct identification of *L. innocua* could be a challenge. Some of the most common biochemical and/or serological tests are often not sufficient to discriminate between the two species. In our case, the automatic identification system correctly identified our strain within 7 h. The subsequent sequence analysis of the DNA extracted from the isolate as well as the PCR assays helped us to confirm the initial identification. This evidence suggests that, to produce a report in a short time, particularly in a case of meningitis, it is not strictly necessary to use a molecular-based test for confirmation. On the contrary, the latter could be considered a second-choice tool that is just necessary to confirm the pathogen identification.

The present report is the second description of a human invasive infection caused by *L. innocua* (Perrin et al., 2003) and the first case, to our knowledge, in a patient being treated with etanercept. The rapid identification of the pathogen and the prompt institution of a specific effective antibiotic therapy were effective in treating the acute meningitis. However, the occurrence of obstructive hydrocephalus as a late complication of *L. innocua* infection should be taken into account and, in our case, could explain the poor outcome.

**Acknowledgements**

Due to loss of the patient, a generic ethical approval document was obtained from our institutional ethical committee and a written consent letter was obtained from the patient’s relatives. A copy of these documents is available for review by the Editor-in-Chief of this journal. The authors declare that they have no competing interests.

**References**


