Development and characterization of monoclonal antibodies specific for the genus *Listeria*

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Abstract

Monoclonal antibodies were obtained by the classic hybridoma technique with lymphocytes of BALB/c mice immunized with formalin killed *Listeria monocytogenes* cells. Among 1000 hybridomas issued from the fusion, four monoclonal antibodies (mAbs A6 A E4, Cl0 A F7, G4 A D6, G7 A D5) gave interesting results. By Western-blot analysis with various soluble extracts of different *Listeria* species, the four mAbs reacted with two major antigens of 38 and 41 kDa, with all *Listeria* species tested. The mAb A6 A E4 is an IgG2b with κ light chains and reacted only with *Listeria* antigens without any cross reaction with other organisms tested by ELISA, dot-blotting and Western-blotting. With the same conditions, the three other mAbs reacted with *Listeria* and with other genus extracts, particularly with *Streptococcus* and *Enterococcus*. mAb A6 A E4-reactive antigens are proteins, and glycoprotein immunoassay indicated that the epitope is devoid of carbohydrate moiety. This mAb A6 A E4-reactive protein was neither expressed on cell surface nor released outside the bacteria; immunogold electron microscopy showed that these antigens were localized in the cytoplasma area.

Keywords: *Listeria monocytogenes*; Monoclonal antibodies; Immunoenzymatic methods

1. Introduction

The genus *Listeria* is ubiquitous in nature and the species *Listeria monocytogenes* is the major pathogen encountered in both animals and humans. Individuals at greatest risk are pregnant women, newborns and immunocompromised patients. Outbreaks of listeriosis are often due to ingestion of *L. monocytogenes*-contaminated foods as dairy products, vegetables and raw or cooked meats [1]. The ability to grow at 4°C and resistance to preserving agents make this bacterium an ideal candidate for a post-process contaminated agent, especially for refrigerated foods [1,2]. In a recent (1992) outbreak in France, 279 cases of listeriosis were associated with consumption of pork tongue in aspic [3]. To monitor the incidence of *L. monocytogenes* in foods, several detection systems have been developed. Conventional culture methods are reliable but time-consuming. Development of new detection and identification techniques with monoclonal antibodies or nucleic acid probes are probably efficient methods which
could allow to detect rapidly the presence of \textit{L. monocytogenes} in food samples \cite{1}. The purpose of this study was to produce and characterize murine monoclonal antibodies (mAbs) which react specifically with genus-specific \textit{Listeria} antigen. Such mAbs would be adequate tools for the detection of \textit{Listeria} in food products.

2. Materials and methods

2.1. Bacterial strains and cultures

The strains were isolated from humans, animals or from the environment and were conserved in the bacterial collection of the Microbiology Laboratory, MLA: Microbiology laboratory, UFR Médecine Pharmacie, Angers, France.
UFR Sciences Médicales et Pharmaceutiques, Angers, France (Table 1). Some *Listeria* are ATCC strains and others were serotyped by the French National Center of Reference for *Listeria*, Nantes, France.

Bacterial organisms were grown at 37°C for 18 h in 2.5% protease peptone broth (Difco) supplemented with yeast extract (0.5%) (Difco, Detroit, USA), sodium bicarbonate (0.6%) (Meram) and glucose (0.5%) (Prolabo, Paris, France) and adjusted at pH 7.5. The cells were harvested by centrifugation (1800 × g, 20 min) and washed three times with phosphate-buffered saline, pH 7.2 (PBS: 10 mM sodium phosphate/140 mM NaCl) containing sodium azide (1%) and 2 mM phenylmethylsulfonylfluoride (PBS-AP). For immunogen preparation, pellets suspended in PBS containing formaldehyde (3.7%) were shaken 1 h at 37°C. Then the cells washed twice in PBS were resuspended in PBS to obtain an OD₆₉₀ of 1.3 to 1.4. Aliquots were kept at −20°C.

2.2. Mice immunization

For immunogen preparation, cells were suspended in PBS containing formaldehyde (3.7%) 1 h at 37°C. Then the cells were washed twice in PBS to obtain an OD₆₉₀ of 1.3 to 1.4. Aliquots were kept at −20°C. BALB/c mice (Iffa-Credo, PAbresle, France) were first immunized by subcutaneous injection of immunogen preparation (10⁸ to 2 × 10⁹ formalin-killed cells) of *Listeria monocytogenes* serovar 4b (MLA 619) with Freund’s complete adjuvant. The following intraperitoneal injections were done with the same immunogen at 3-week intervals: seven injections were made with Freund’s incomplete adjuvant and the two last without adjuvant. The best immune responder was determined by indirect immunofluorescence (IFI): 10 µl of formol-killed *Listeria* were spotted on wells of microscope slides (poly-Labo Paul Block, Strasbourg, France) and air dried. After incubation with 20 µl of mouse sera, (1 h at 37°C in damp box), slides were washed three times in PBS and 25 µl of anti-mouse FITC conjugate (1:300 in PBS) (Caltag, San Francisco, USA) were added. The slides were incubated and washed as described above. After drying, the slides were examined with a Nikon microscope equipped with a reflected light fluorescence. Mouse spleen was removed 3 days after the last intravenous booster injection.

2.3. Hybridoma production

Hybridoma cells were produced according to slightly modified Dippold procedure [4]. P3 × 63Ag8.653 mouse myeloma cells were maintained in RPMI 1640 Medium (Gibco laboratories, Grand Island, NY) containing 10% fetal bovine serum (FBS; J. Bio laboratories), 2 mM glutamine, 1 mg/ml ampicillin and 0.1 mg/ml gentamycin in a 5% CO₂ humidified atmosphere. Murine myeloma cells in logarithmic growth phase and spleen cells from immunized mice were fused at the ratio 1:5 in 1 ml of 41% (w/v) polyethylene glycol (molecular mass 1500); Serva, Heidelberg, Germany) in PBS with 15% (v/v) dimethyl sulfoxide (Sigma, St.Louis, MO). Fused cells were then distributed in 96-well tissue culture plates (2 × 10⁵ cells in each well) (Nunc, Kamstrup, Denmark) containing RPMI 1640/20% FBS at 37°C with selective agents, 100 µM hypoxanthine, 0.4 µM aminopterin and 15 µM thymidine (HAT medium; Sigma), and with a feeder layer of Balb/c mouse macrophages. When macroscopic growths were visible, cell supernatants were screened for anti-*Listeria* mAbs activity as described below. Selected positive hybrids were cloned in new 96-well culture plates by limiting dilution to obtain a single clone per well. Wells which contained a single colony were further propagated in RPMI/10% FBS medium and cells supernatants were tested by ELISA and immunoblotting.

2.4. Primary screening of hybridomas for anti-*Listeria* antibodies by ELISA and immunoblotting

First screening was performed by ELISA with soluble *Listeria* extract prepared as follows: washed cells suspended in carbonate buffer (50 mM carbonate buffer, pH 9.6) were sonicated for 1 min at 15 W with a Labsonic 1510 B Braun Sonifier and centrifuged at 20000 × g for 20 min; The supernatant was diluted with carbonate buffer to obtain a protein concentration of 10 µg/ml and used to coat 96-well flat-bottom microtiter plates for 1 h at 37°C (100 µl
Then, plates were washed three times with PBS buffer containing 0.5% Tween-20 (PBS-T) and blocked with the same buffer supplemented with 1% (w/v) bovine serum albumin (BSA-Sigma), overnight at 4°C. Washed plates were inoculated with hybridoma culture supernatants (100 µl of one supernatant per well) and incubated 1 h at 37°C. Plates were washed three times with PBS-T and a goat anti-mouse immunoglobulin (IgG) horseradish peroxidase conjugate (Caltag) (diluted at 1:1000 in PBS-T) was added (100 µl per well). Plates were incubated 1 h at 37°C. After three PBS-T washes, 200 µl of substrate solution (1 mg of O-phenylenediamine per ml of 150 mM sodium citrate buffer, pH 5, containing 30 µl of 0.1% H2O2) were added in each well. After 15 min incubation at room temperature, the reaction was stopped by addition of 0.1 N H2SO4 (50 µl per well) and the color reaction was measured with a plate reader (Titertek Multiscan, Flow Laboratories, France) at 492 nm.

Supernatants with positive reactivity (OD492 ≥ 0.035) were screened by Western-blotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of Listeria monocytogenes antigens were performed by the Laemmli method [5]. Antigens were prepared from supernatants of washed cells suspended in 25 mM Tris/192 mM glycine buffer containing 1% SDS and disrupted by sonication as described previously. After boiling 2 min, 800 µl of substrate solution (1 mg of O-phenylenediamine per ml of 150 mM sodium citrate buffer, pH 5, containing 30 µl of 0.1% H2O2) were added in each well. After 15 min incubation at room temperature, the reaction was stopped by addition of 0.1 N H2SO4 (50 µl per well) and the color reaction was measured with a plate reader (Titertek Multiscan, Flow Laboratories, France) at 492 nm.

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2.5. Secondary screening of hybridoma

Hybridomas supernatants which showed positive reactivity with Listeria monocytogenes serovar 4b in the primary screening were tested by ELISA and Western-blotting against a panel of Listeria and non-Listeria soluble extracts obtained as described before (either in carbonate or in Tris SDS buffer). We studied L. monocytogenes serovars 1/2a (MLA 81), 1/2b (MLA 618), 1/2c (MLA 593), L. innocua (MLA 192), L. ivanovii (MLA 429), L. seeligeri (MLA 297), L. grayi (MLA 326), Staphylococcus aureus, Enterococcus faecalis, Streptococcus pyogenes, Streptococcus agalactiae and Bacillus niger.

2.6. Isotyping of mAbs

Isotype analysis of mAbs chosen for their specificity after the secondary screening were conducted by ELISA immunocapture method (Gibco) and by a stick immunoenzymatic technique (Amersham, Les Ulis, France).

2.7. Subsequent study of four anti-Listeria mAbs

Four mAbs (A6 A E4, C10 A F7, G4 A D6, G7 A D5), which were reactive by immunoblotting to antigens with the same molecular mass of L. monocytogenes, were studied further.

mAbs were produced and purified as follows. Log-phase hybridoma cells were injected into pristane-primed mice. The ascites fluid containing mAbs were collected after the onset of ascites tumors, centrifuged (12,000 × g) and stored at −20°C. mAbs were purified by protein G-Sepharose affinity chromatography (Pharmacia) according to manufacturer’s instructions and stored at −20°C until use.

The activity spectrum of the four mAbs towards 29 strains including Listeria spp. and other genus (Table 1) were tested by ELISA and dot-blotting. ELISA was carried out as described above by using purified mAbs at 2 µg/ml.

For dot-blot, 10 µl of each soluble extract in PBS were spotted on nitrocellulose membranes (Bio-Rad, Richmond, USA). After 15 min at room temperature
the membranes were blocked 1 h at 37°C by 10% non-fat dry milk PBS-T before incubation (30 min at room temperature) with purified mAbs (2 μg/ml in PBS-T) followed by incubation with anti-mouse peroxidase conjugate (30 min at room temperature).

For Western-blotting (performed as described previously), 40 μl of each soluble extract of the 13 Listeria or strains other than Listeria were loaded into wells of the gel. After electrophoresis and electroblotting on Immobilon membrane, each mAb was tested with the different extracts.

2.8. Epitope characterization of the A6 A E4 reactive protein

For ELISA, coated antigens were treated with enzymatic or chemical procedures. They were incubated with 100 μl of proteolytic enzyme solutions: trypsin (Sigma) (at 1 or 0.1 or 0.01 or 0.001 mg/ml in a 67 mM phosphate buffer, pH 7) or pronase E (Merck, Darmstadt, Germany) (at 7.5 or 0.75 or 0.075 or 0.0075 mg/ml in a 67 mM phosphate buffer, pH 7) or α-chymotrypsin (Merck) (at 1 or 0.1 or 0.01 or 0.001 mg/ml in a 5 mM Tris-HCl buffer, pH 9) or proteinase K (Merck) (at 1 or 0.1 or 0.01 or 0.001 mg/ml in a 10 mM Tris-HCl buffer, pH 9) and pepsin (Merck) (at 1 or 0.1 or 0.01 or 0.001 mg/ml in a 60 mM HCl solution, pH 1.6). Antigens were also incubated in the same conditions with lysozyme (Boehringer, Mannheim, Germany) (at 0.2 or 0.02, or 0.002 or 0.0002 mg/ml in a 67 mM phosphate buffer, pH 7 or in a 5 mM Tris-HCl buffer, pH 9) and with sodium periodate (4.27 or 0.427 or 0.0427 or 0.00427 mg/ml in 150 mM acetate buffer, pH 4.5). Periodate oxidation treatment was performed for 1 h at room temperature in the dark, then the reaction was stopped by addition of 100 μl of glycine (1% in acetic acid buffer). After treatment, ELISA was carried out as described before.

For Western-blotting, fixed antigens on Immobilon membrane were treated by incubation in the same conditions with trypsin (0.1 mg/ml) or with pronase E (0.75 mg/ml) or proteinase K (1 mg/ml) or pepsin (0.1 mg/ml) or lysozyme (0.02 mg/ml) or sodium periodate (0.427 mg/ml). After treatment,
Western-blotting was done with mAb A6 A E4 as described previously.

2.9. Localization in L. monocytogenes serovar 4b cell of mAb A6 A E4 reactive structure

After a 24-h L. monocytogenes growth, this structure was searched by Western-blotting in the supernatants of 2.5% proteose peptone broth or of PALCAM broth (Merck) and in soluble extracts (detergent treatment: SDS) of cells grown in the broths, on blood agar or on PALCAM agar (Merck).

Cell surface expression of A6 A E4 specific antigens was determined as previously described by IFI by incubation with whole supernatant of mAb A6 A E4 hybridomas culture instead of mouse sera.

For immunogold electron microscopy, bacteria were prepared as described previously [7]. Listeria were fixed by addition of freshly prepared solution of paraformaldehyde (2%) and glutaraldehyde (0.2%) in sodium cacodylate buffer. Preparations stained with many1 acetate were dehydrated in alcohol solutions and embedded in Lowicryl (Chemise-Weake). Thin sections were picked on uncoated grids and incubated with mAbs (ascites dilutions 1:lO or 1:50 in PBS-0.1% BSA buffer) for 1 h and then for 30 min with protein A conjugated with 5 nm gold particles (diluted at 1:lOO in PBS). The sections were stained with uranyl acetate and examined on a 100 CX JEOL microscope.

3. Results

3.1. mAbs

More than 1000 hybridomas were generated after one fusion. 79 hybridomas showed positive ELISA

<table>
<thead>
<tr>
<th>Hybridomas</th>
<th>Analysis methods</th>
<th>Listeria</th>
<th>Staphylococcus aureus</th>
<th>Enterococcus faecalis</th>
<th>Streptococcus agalactiae</th>
<th>Streptococcus pyogenes</th>
<th>Bacillus niger</th>
<th>M, kDa</th>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
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<td>ND</td>
<td>38-41</td>
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<tr>
<td></td>
<td>WB</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
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<td>ND</td>
<td>38-41</td>
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<tr>
<td>A7 A C7</td>
<td>E</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>38-41</td>
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<td></td>
<td>WB</td>
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<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>38-41</td>
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<tr>
<td>A5 B B6</td>
<td>E</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>38-41</td>
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<tr>
<td></td>
<td>WB</td>
<td>+</td>
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<td>ND</td>
<td>-</td>
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<td>ND</td>
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<tr>
<td>A6 A E4</td>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>38-41</td>
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<td></td>
<td>WB</td>
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<td>ND</td>
<td>ND</td>
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<td>38-41</td>
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<tr>
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<td>-</td>
<td>+</td>
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<td>ND</td>
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<td></td>
<td>WB</td>
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<td></td>
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<td>B10 B C10</td>
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<td>E</td>
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<td>ND</td>
<td>+</td>
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<td>+</td>
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<td>ND</td>
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<td>ND</td>
<td>+</td>
<td>38-41</td>
</tr>
<tr>
<td>A11 B B10</td>
<td>E</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
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<td>+</td>
<td>ND</td>
<td>+</td>
<td>38-41</td>
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ND, not determined.
E = analysis of mAbs by ELISA methods: +, positive reaction; -, negative reaction.
WB = analysis of mAbs by Western blotting: +, detection of antigen band(s) on the blot; -, negative reaction: +, weak positive reaction.
M, molecular masses of antigens detected by mAbs.
reactivity. In primary screening, by immunoblotting, most of culture supernatants recognized two protein antigens in *L. monocytogenes* with molecular mass ($M_r$) of 38 and 41 kDa. Some mAbs reacted with few other bands of various $M_r$ range of 30 to 80 kDa. Four mAbs recognized a weak smear (Fig. 1).

22 hybridomas showing positive Western-blotting reactivity were cloned and analysed by ELISA and Western blotting. Then 11 mAbs were retained for their strong reactivity with *Listeria* antigens by immunodetection (Table 2). Among them, nine recognized proteins of 38 and 41 kDa (F4 A A4, A7 AC7.

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Monoclonal antibodies</th>
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<tr>
<td>Listeria sp.</td>
<td>A6 A E4</td>
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<tr>
<td>Bacillus sp.</td>
<td>C10 A F7</td>
</tr>
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<td>Erysipelothrix rhusiopathiae</td>
<td>G4 A D6</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>G7 A D5</td>
</tr>
<tr>
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<tr>
<td>Enterococcus faecalis</td>
<td>E DB WB</td>
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<td>Staphylococcus epidermidis</td>
<td>E DB WB</td>
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<td>Staphylococcus aureus</td>
<td>E DB WB</td>
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<tr>
<td>Salmonella sp.</td>
<td>Pseudomonas aeruginosa</td>
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<td>Clostridium perfringens</td>
<td>- , Negative reaction; + , positive reaction; ±, weak positive reaction.</td>
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Table 3
Specificity analysis of four mAbs (A6 A E4, C10 A F7, G4 A D6, G7 A D5) by ELISA (E), dot-blotting (DB) and Western-blotting (WB) with extracts of different bacterial species.
Fig. 3. Western-blot analysis of *Listeria* and non-*Listeria* soluble extracts after SDS-PAGE (12.5% polyacrylamide) with mAb G4 A D6. Lane M: Coomassie blue stained SDS-PAGE containing molecular mass standards. Lane 1: *L. monocytogenes* serotype 1/2a (MLA-618). Lane 2: *Staphylococcus epidermidis* (MLA-S5). Lane 3: *L. monocytogenes* serotype 4b (MLA-619). Lane 4: *Staphylococcus aureus* (MLA-S4). Lane 5: *Enterococcus faecalis* (MLA-E1). Lane 6: *L. innocua* (MLA-192). Lane 7: *Clostridium perfringens* (MLA-C1). Lane 8: *Bifidobacterium adolescentis* (MLA-B4). Lane 9: *Listeria murrayi* (MLA-326). Lane 10: *Bacillus subtilis* (MLA-B3). Lane 11: *Erysipelothrix rhusiopathiae* (MLA-E2). Lane 12: *Salmonella typhi* (MLA-S1). Lane 13: *Pseudomonas aeruginosa* (MLA-P3).

Table 4
mAb A6 A4 reactivity with *Listeria monocytogenes* antigens treated by enzymatic or chemical procedures; OD_{492 nm} obtained by ELISA methods

<table>
<thead>
<tr>
<th>Denaturing agents:</th>
<th>Trypsin</th>
<th>Pronase</th>
<th>α-Chymotrypsin</th>
<th>Proteinase K</th>
<th>Pepsin</th>
<th>Lysozyme pH = 7</th>
<th>Lysozyme pH = 9</th>
<th>Sodium periodate</th>
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<td>7.5</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.2</td>
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<td>Control 4</td>
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*Ag/PBS/mAb A6 A4/conjugate.*

*Ag/dilution buffer for denaturing agents/mAb A6 A4/conjugate.*

*Carbonate buffer/dilution buffer for denaturing agents/mAb A6 A4/conjugate.*

*Carbonate buffer/denaturing agents at 10^{-3}/mAb A6 A4/conjugate.*
A5 B B6, A6 A E4, G7 A D5, C3 B C6, C10 A F7, G4 A D6, B10 B C10), and two mAbs (B4 B F3, A11 B B10) detected a single protein band of 30 kDa present in *Listeria* and in other organism extracts.

Among the 11 mAbs, four (F4 A E4, A7 A C7, A5 B B6, A6 A E4) showed a specificity for *Listeria* genus and the others showed various reactivity profiles with non *Listeria* soluble extracts.

Isotype analysis of cloned hybridoma supernatants revealed that mAbs were IgG1 (G7 A D5, B4 B F3 and A11 B B10), IgG2a (G4 A D6 and B10 B C10) or IgG2b (A6 A E4, A7 A C7, F4 A E6, and C10 A F7) with κ light chains.

### 3.2. Study of four anti-Listeria mAbs

Four mAbs (A6 A E4, C10 A F7, G4 A D6, G7 A D5) which reacted with the two major proteins (38 and 41 kDa) were studied by ELISA, dot-blotting and Western-blotting. The results showed that mAb A6 A E4 reacted only with *Listeria* antigens (Fig. 2). Other mAbs (C10 A F7, G4 A D6 and G7 A D5) reacted with *Listeria* and with other genus extracts, particularly with *Streptococcus* and *Enterococcus* (Table 3). We observed more cross-reactions by Western-blotting than by ELISA or dot-blotting methods. *Staphylococcus aureus* gave a non specific reaction because of protein A-binding IgG (Fig. 3).

An image system analysis (Bioprofil system, Vilber-Lourmat, France) was used for determination of molecular masses of *Listeria* antigens detected by the four mAbs.

According to 12 experiments, molecular masses were in a range of 37.8 ± 1 and 40.8 ± 0.8 kDa.

### 3.3. Study of mAb A6 A E4

Treatment of antigen extracts from *L. monocytogenes* serovar 4b with various proteolytic enzymes interfered with the epitope binding of mAb A6 A E4 as we showed by ELISA (Table 4). Reduced epitope binding activity was not obtained after incubating extracts with lysozyme or sodium periodate (Table 4). Similar results were obtained by immunoblotting: no or weak reactivity was obtained after proteolytic enzyme treatment. However, the same reactivity was observed after lysozyme (Fig. 4, lane 6) or periodate incubation (data not shown).

A6 A E4 reactive proteins were not detected in either broth supernatants by immunoblotting or on the cell surface by IFI reaction. The proteins were not released outside bacteria or expressed on cell surface. Similarly, immunogold electron microscopy showed that the mAb A6 A E4 reactive protein is not on the cell surface, but colloidal gold spheres were localized in the cytoplasm area (Fig. 5). Only few particles were detected, but no significant labeling...
Fig. 5. Immunogold electron microscopy of *Listeria monocytogenes* 4b (MLA-619) after incubation with mAb and gold labelled protein A. The arrows indicate the localization of gold spheres. (A) incubation with anti-*Candida albicans* mAb. (B) incubation with anti-*Listeria monocytogenes* mAb A6 A E4.
was seen on cells probed either with PBS or with anti-Candida albicans mAb instead of Listeria mAb (Fig. 5).

4. Discussion

4.1. mAbs specificity

mAb A6 A E4 reacted with Listeria extracts without cross-reaction to any other Gram-positive or Gram-negative organisms by ELISA or dot blot analysis. Other mAbs showed particularly cross-reactivity particularly with Streptococcus extracts. Immunoblotting confirmed the specificity of mAb A6 A E4 for Listeria genus. In immunoblot, mAbs C10 A F7, G4 A D6 and G7 A D5 reacted with more non-Listeria species than in ELISA or dot-blot. To explain this non-specificity, one can speculate that SDS treatment that preceded SDS-PAGE, allowed the protein aggregates to unfold and expose some of the epitopes which were previously not recognized by the mAbs in the native state protein. SDS treatment would also increase the solubilization of antigens. These cross-reactions have been previously reported for other mAbs [8,9].


It appears that mAb A6 A E4 described in this study reacts only with antigens located inside the Listeria cell and shared in common with all Listeria species. Nevertheless, the mAb reactive antigens seem different from well known extracellular proteins [18–21] of Listeria or from enzymes located in cytoplasm or cytoplasmic membrane as catalase and superoxide dismutase whose M, are of 67 and 24.5 kDa [22,23]. It would be interesting to search for possible enzymatic activity of the 38 and 41 kDa proteins recognized by our mAb. This is currently under investigation in our laboratory.

4.2. mAb A6 A E4 reactive antigen characterization

mAb A6 A E4 detects two antigens of 38 and 41 kDa in all Listeria species, whatever are the serotypes.

mAb A6 A E4 reactive antigens were characterized as protein in nature according to their staining with Coomassie blue and as epitope-binding activity, considering their destruction by proteolytic enzymes. Association of carbohydrate moiety with the epitope is doubtful. Treatment with sodium periodate does not reduce the epitope-binding activity. These antigens seem to be located inside the bacteria cell. They can be extracted by sonication or by boiling, with or without SDS buffer.

It can be concluded therefore that the mAb A6 A E4 specifically recognize a heat-stable intracellular Listeria genus specific antigen. This mAb would be powerful tool for laboratory diagnostics, especially for listeria antigen detection in food products.

References


