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Monoclonal Antibodies Against LipL32, the Major Outer Membrane Protein of Pathogenic *Leptospira*: Production, Characterization, and Testing in Diagnostic Applications

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ABSTRACT

Pathogenic serovars of *Leptospira* have a wide antigenic diversity attributed mainly to the lipopolysacharide present in the outer membrane. In contrast, antigens conserved among pathogenic serovars are mainly represented by outer membrane proteins. Surface exposure of a major and highly conserved outer membrane lipoprotein (LipL32) was recently demonstrated on pathogenic *Leptospira*. LipL32 in its recombinant form (rLipL32) was used to immunize BALB/c mice to develop murine monoclonal antibodies (MAbs). Three MAbs against rLipL32 were produced, isotyped, and evaluated for further use in diagnostic tests of leptospirosis using different approaches. MAbs were conjugated to peroxidase and evaluated in a native protein enzyme-linked immunosorbent assay (ELISA) with intact and heat-treated leptospiral cells, conjugated to fluorescein isothiocyanate (FITC) for indirect immunofluorescence with intact and methanol fixed cells and were used for LipL32 immunoprecipitation from leptospiral cells. rLipL32 MAbs conjugated to peroxidase or used as primary antibody bound to intact and heat-treated cells in ELISA, proving that they could be used in enzyme immunoassays for detection of the native protein. In immunofluorescence assay, MAbs labeled bacterial cells either intact or methanol fixed. Two MAbs were able to immunoprecipitate the native protein from live and motile leptospiral cells and, adsorbed onto magnetic beads, captured intact bacteria from artificially contaminated human sera for detection by polymerase chain reaction (PCR) amplification. Results of this study suggest that the MAbs produced can be useful for the development of diagnostic tests based on detection of LipL32 leptospiral antigen in biological fluids.

INTRODUCTION

LEPTOSPIROSIS, A ZOONOTIC DISEASE widespread in the world, is caused by pathogenic bacteria of the genus *Leptospira*.[1] There are over 230 pathogenic leptospiral serovars identified and this antigenic diversity is mainly attributed to the lipopolysacharide (LPS) covering bacterial surface.[2] Diagnostic approaches based on detection of antibodies generated during infection or on detection of antigens using antibodies generated against *Leptospira* strains have poor sensitivity because LPS is the dominant antigen and antibody reaction is serovar specific. Because of the LPS diversity, recent research focused on the use of outer membrane proteins (OMPs) conserved among pathogenic *Leptospira* species to increase sensitivity of diagnostic tests.

A small number of leptospiral outer membrane lipoproteins are exposed on the cell surface, among which are LipL32, LipL21, and LipL41.[3] Expression of the major OMP LipL32 has been demonstrated both in culture and in host infections,[4] and its surface exposure on the bacterial membrane has recently been proven.[3] Sera from patients with leptospirosis react strongly with the recombinant form of LipL32[5] and an enzyme assay using this antigen was able to detect human[6] and animal cases of leptospirosis.[7,8]

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In this study we report on the production and characterization of three monoclonal antibodies (MAbs) against recombinant LipL32 (rLipL32) and their use in different approaches to demonstrate their potential for diagnosis of leptospirosis. The MAbs recognized the native protein on the surface of pathogenic *Leptospira* cells and did not react with saprophytic strains or other microorganisms, suggesting they could be a useful diagnostic tool for leptospirosis.

MATERIALS AND METHODS

Leptospira *strains and culture conditions*

L. interrogans serovar Copenhageni strain FIOCRUZ L1 130 used in this study was provided by A.I. Ko (Centro de Pesquisa Gonçalo Moniz, FIOCRUZ, Salvador, BA, Brazil). Other *Leptospira* serovars used were obtained from the Center for Zoonosis Control (Universidade Federal de Pelotas, Brazil). Leptospires were grown at 30°C in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium (Becton Dickinson Co., MD), supplemented with 8% of bovine serum albumin.[1]

Antigen preparation

The *lipL32* gene, obtained by polymerase chain reaction (PCR) using the DNA from *L. interrogans* L1 130 as template, was cloned into the pAE expression vector[9] that allows fusion of the protein with a 6×His tag. This plasmid was used to transform *Escherichia coli* BL21 (DE3). Purification of the protein was accomplished by affinity chromatography with Ni-NTA resin using the QIA EXPRESSIONIST Kit (Qiagen Corporation, Valencia, CA) following the manufacturer's instructions. The eluate was then dialyzed against phosphate-buffered saline (PBS) and glycine 0.1%, pH 8.0, for approximately 16 hours at 4°C. Protein in the final preparation was quantified by the Bradford method.[10]

Generation and purification of MAbs

Two 6-week-old BALB/c female mice were immunized intraperitoneally on day 1 with 100 μg of the recombinant protein LipL32 (rLipL32) mixed with Freund's complete adjuvant (Sigma Aldrich Co., St. Louis, MO). This was followed by three intraperitoneal injections of rLipL32 mixed with Freund's incomplete adjuvant (Sigma Aldrich) on days 14, 21, and 28. Three days before fusion the mouse with the highest titer of serum antibodies against rLipL32 in an indirect ELISA was boosted with 20 μg of the protein intravenously. Hybridoma cells were obtained by established procedures.[11] Splenic lymphocytes were fused to murine Sp2/O-Ag14 myeloma cells in the presence of PEG 1450 (Sigma Aldrich). Fused cells were cultivated in Dulbecco's modified Eagle's medium (DMEM; Sigma Aldrich) containing 20% fetal calf serum (FCS; Cultilab, Campinas, Brazil) and supplemented with hypoxanthine, aminopterin, and thymidine (HAT; Sigma Aldrich). Hybridomas were primarily screened for specific antibodies by indirect ELISA with immunizing antigen. Supernatants showing at least 40% of the positive control (sera from rLipL32 immunized mouse) OD were considered positive and the hybridomas cloned twice by limiting dilution. A secondary screening was performed by immunoblotting and indirect ELISA using supernatant from cloned cells and heat-treated *Leptospira* from different serogroups as antigen. Specific hybrid cell lines identified in this second screening were expanded and stored in liquid nitrogen. For ascites production the hybridomas were removed from liquid nitrogen, cultivated on DMEM with 10% FCS, collected by centrifugation, washed five times in DMEM without FCS, and injected into pristane-primed BALB/c mice. MAbs were purified from ascitic fluid by affinity chromatography on a protein A-Sepharose CL-4B column (GE Healthcare Company, Piscataway, NJ) according to the manufacturer's instructions. Purification efficacy was evaluated by SDS-PAGE and final concentration was measured by spectrophotometry at 280 nm. Purified MAbs were stored at −20°C. The MAbs were isotyped by ELISA with a mouse subisotyping kit following manufacturer instructions (Sigma Aldrich).

Gel electrophoresis and immunoblotting

Proteins immunoprecipitated from outer membrane or heat-treated leptospiral cells were solubilized in final sample buffer 50 mM Tris-HCl (pH 6.8), 100 mM dithiothreitol, 0.1% bromophenol blue, 2% SDS, and 20% glycerol and separated in a 12% polyacrylamide gel using a discontinuous buffer system.[12] After electrophoresis, the gels were stained with 0.2% Coomassie brilliant blue R-250 (in 10% acetic acid—45% methanol) or transferred electrophoretically to a polyvinylidene difluoride (PVDF) membrane (GE Healthcare) at 25 volts for 60 minutes. The membranes were blocked with 0.1 M PBS containing 0.1% Tween 20 (PBS-T, pH 7.4) and 5% skim milk for 30 minutes, washed twice in PBS-T and incubated for 1 hour with the MAbs diluted 1/1000 in PBS-T. After washing the membrane three times with PBS-T, a rabbit anti-mouse immunoglobulin (Ig) horseradish peroxidase conjugate (Sigma Aldrich) diluted 1:2000 in PBS-T or a MAb anti-LipL32 peroxidase conjugate was added for 1 hour and the membrane was again washed three times with PBS-T. The MAb-peroxidase conjugate was prepared according to established protocol.[13] Bands were visualized after the addition of the substrate/chromogen solution (H_2O_2/4-chloro-1-naphtol).

ELISA with intact and heat-treated leptospiral cells

Seven-day cultures of *L. interrogans* serovar Copenhageni strain Fiocruz L1 130, and of saprophytic *L. biflexa* serovar patoc (strain Patoc I) were harvested by centrifugation (15,000*g*, for 30 minutes) at 4°C and washed once in PBS (0.01 M, pH 7.2). The cells were resuspended in PBS, counted in a Petroff-Hausser chamber and the concentration was adjusted to approximately 10^9 cells per milliliter. For ELISA with intact leptospira cells, microtiter plates (Nunc Polysorp, Nalge Nunc International, Rochester, NY) were first coated overnight at 4°C with 100 μL of a 10 times diluted 0.1% poly L-lysine solution and then with 100 μL of the bacterial suspension in PBS for 2 hours at 30°C. Wells were washed 3 times with leptospira culture medium (LCM) and 100 μL of twofold dilutions of each ascites MAb in LCM was added to the wells for 2 hours at 30°C. Washing was repeated and 100 μL of rabbit anti-mouse Ig-POase conjugated was added for 2 hours at 30°C. After 2

washes with LCM and 3 washes with PBS, 100 μL of enzyme substrate/chromogen solution (H_2O_2/ortophenylenediamine) in citrate-phosphate buffer, pH 5.0, was added and the reaction was allowed to take place in the dark for 10 minutes. Optical density was read at 450 nm in an ELISA reader (Multiskan MCC/340, Titertek Instruments, Huntsville, AL). ELISA with heat-treated leptospiral cells was performed in the same way except that leptospiral cultures were first inactivated overnight at 56°C and stored at −20°C until use. As control of cell integrity, rat serum against cytoplasmatic protein GroEL was included in both tests. To investigate how protein conjugation would affect MAb performance in ELISA, a conjugate of MAb 1D9 and POase was also used in this experiment.

Direct and indirect immunofluorescence

Slide chambers (ICN Biomedicals Inc., Costa Mesa, CA) were coated with a 0.01% Poly L-lysine solution (Sigma Aldrich) and dried for 1 hour at room temperature. A 7-day culture of *L. interrogans* L1-130 was washed once in PBS, resuspended to a density of 10^8 cells per milliliter in PBS, and incubated in the slide chamber for 2 hours at 30°C. The slides were washed twice with LCM and coated with MAb ascites diluted 1:10 in LCM. After incubating for 1 hour at 30°C, the slides were washed again twice with LCM and a 1:100 dilution of rabbit anti-mouse FITC conjugate was added and incubated for 1 hour in a dark humid chamber at 30°C. After washing with LCM a drop of mounting medium was added and a coverslip was sealed with acrylic. In experiments with permeabilized membrane, slides were incubated in 5 mL of methanol for 10 minutes at 4°C followed by washing twice with LCM. The following controls were used in this experiment: (1) MAb against *Salmonella* OMP was used as primary antibody, (2) rabbit anti-mouse FITC conjugate was applied to slides without primary antibody, and (3) normal mouse serum was used as primary antibody. For direct immunofluorescence MAb 1D9 FITC conjugate was produced following established procedures[14] and used together with a conjugate of rabbit antibodies against whole *Leptospira* and FITC (Ames Laboratories, National Veterinary Services Laboratory, Ames, IA) as positive control. Labeling was visualized by fluorescence microscopy (Olympus BX 51) with excitation wavelength of 450 nm.

Immunoprecipitation of native LipL32 and immunomagnetic separation of leptospiral cells

Surface immunoprecipitation of native LipL32 was performed according to Shang et al.[15] with modifications. A 2-mL volume of each heat-inactivated MAb ascites was mixed with 30 mL of a culture of *L. interrogans* L1-130 containing 3×10^{10} actively motile bacteria. After shaking the suspension gently for 1 hour at 30°C the cells were pelleted at 2000*g* for 15 minutes at 4°C, resuspended in PBS with 5 mM $MgCl_2$, centrifuged again, and resuspended in 9 mL of 10 mM Tris-HCl (pH 8.8), 2 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethyl sulfonyl fluoride (PMSF). A volume of 1 mL of 10% protein grade Triton × 100 (Sigma Aldrich) was added and the suspension was agitated for 30 minutes at 4°C. The insoluble material was removed by centrifugation at 16,000*g* for 20 minutes and 1 mL of 2% deoxycholate, 50 μL of 20% SDS and 500 μL of Sepharose-SpA (Sigma Aldrich) were added to the supernatant. After agitating the mixture gently for 30 minutes at 4°C, the complex of Sepharose SpA-MAb-antigen was washed by centrifugation two times with 0.01% Triton × 100 in 10 mM Tris-HCl (pH 8.8) and resuspended in final sample buffer. The complex was submitted to SDS-PAGE and immunoblotting using MAb 1D9-POase as probe. As negative controls two additional immunoprecipitation experiments were carried out in parallel: in the first one MAb against LipL32 was omitted and in the second one the culture of *L. interrogans* L1-130 was replaced with *L. biflexa* serovar patoc. For the immunomagnetic separation (IMS) procedure, 1D9 MAb was absorbed onto protein A-coated microspheres (Bangs Laboratories Inc., Fishers, IN). Briefly, 100 μL of particles with 1% solids was suspended with 900 μL of 50 mM pH 8.2 borate buffer (BB), washed twice with BB, resuspended in 1 mL of BB containing 1.2 mg of MAb and incubated with gentle agitation at 4°C for 16 hours. The MAb-coated particles were washed twice with BB and resuspended in stock buffer (100 mM borate pH 8.5, 0.1% bovine serum albumin [BSA], 0.05 Tween 20, 10 mM EDTA, 0.1% NaN_3). An immunomagnetic separator (Invitrogen Corporation, Carlsbad, CA) was used in washing steps. Standard IMS was performed with 5 μL of MAb-coated particles per milliliter of different dilutions of a pool of sterile human sera artificially contaminated with pathogenic and saprophytic *Leptospira* species. The serum samples were gently agitated for 15 minutes at room temperature and then washed twice on a magnetic separator. The particles were then resuspended in 20 μL of lysis buffer (0.02 M Na_2HPO_4; 0.15 M NaCl; pH 7.2), boiled for 10 minutes and stored at −20°C until use in polymerase chain reaction (PCR) as described below.

PCR conditions

PCR primers *lipL32* F: 5′ CGC TTG TGG TGC TTT CGG TGG T 3′ and *lipL32* R: 5′ CTC ACC GAT TTC GCC TGT TGG G 3′ were used, resulting in a 264 bp amplicon of the *lipL32* coding region. Briefly, 2 μL of DNA-IMS template was added to a tube with 1 U *Taq* DNA polimerase (Invitrogen) 150 ng of primers, 2.5 μL of 10× reaction buffer containing $MgCl_2$ and 0.2 mM dNTP. For DNA template optimization, volumes of 10 μL, 5 μL, 3 μL, 2 μL, and 1 μL of DNA-IMS were tested in the same conditions. Amplification was carried out in a Perkin Elmer 2400 thermocycler (PE Biosystems, Foster City, CA) with 1 cycle at 94°C for 5 minutes, 35 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute, and an extension of 7 minutes at 72°C at the end of the final cycle. Aliquots were analyzed by electrophoresis in 2% agarose gel with ethidium bromide and visualized under UV transillumination.

RESULTS

Generation of MAbs

From a total of 54 hybridomas tested in the primary ELISA, 3, named 1D9, 36C4, and 412H4, have shown a specific reaction in the secondary ELISA screening and were selected for further characterization and testing in diagnostic applications.

TABLE 1. REACTIONS OF ANTI-LIPL32 MABS WITH DIFFERENT STRAINS OF *LEPTOSPIRA* IN IMMUNOBLOTTING

			MAbs		
Serogroup	*Serovar*	*Genomoespecies*	*1D9*	*412H*	*36C*
Australis	Australis	*L. Interrogans*	+	+	+
Australis	Bratislava	*L. Interrogans*	+	+	+
Autumnalis	Autumnalis	*L. Interrogans*	+	+	+
Autumnalis	Butembo	*L. Kirshneri*	+	+	+
Autumnalis	Rachmat	*L. Interrogans*	+	+	+
Bataviae	Bataviae	*L. Interrogans*	+	+	+
Canícola	Canícola	*L. Interrogans*	+	+	+
Hebdomadis	Hebdomadis	*L. Interrogans*	+	+	+
Icterohaemorrhagiae	Copenhageni	*L. Interrogans*	+	+	+
Icterohaemorrhagiae	Icterohaemorrhagiae	*L. Interrogans*	+	+	+
Pomona	Pomona	*L. Interrogans*	+	+	+
Pyrogenes	Pyrogenes	*L. Interrogans*	+	+	+
Sejroe	Wolffi	*L. Interrogans*	+	+	+
Sejroe	Hardjo	*L. Interrogans*	+	+	+
Djasiman	Sentot	*L. Interrogans*	+	+	+
Djasiman	Djasiman	*L. Interrogans*	+	+	+
Ballum	Castellonis	*L. Borgpetersneii*	+	+	+
Ballum	Ballum	*L. Borgpetersneii*	+	+	+
Celledoni	Withcombi	*L. Borgpetersneii*	+	+	+
Javanica	Javanica	*L. Borgpetersneii*	+	+	+
Tarassovi	Tarassovi	*L. Borgpetersneii*	+	+	+
Cynopteri	Cynopteri	*L. Kirshneri*	+	+	+
Grippotyphosa	Grippotyphosa	*L. Kirshneri*	+	+	+
Louisiana	Louisiana	*L. Noguchii*	+	+	+
Panamá	Panama	*L. Noguchii*	+	+	+
Shermani	Shermani	*L. Santarosai*	+	+	+
Celledoni	Celledoni	*L. Weilii*	+	+	+
Andamana	Andamana	*L. Biflexa*	−	−	−
Semaranga	Patoc	*L. Biflexa*	−	−	−
Semaranga	Semaranga	*L. Meyeri*	−	−	−

MAbs, monoclonal antibodies.

The immunoglobulin classes of MAbs 1D9, 36C4, and 412H4 were IgG2b, IgG2a, and IgG3, respectively. All MAbs reacted with a band of an estimated molecular mass of 32 kd and neither reacted with *E. coli* as seen by immunoblotting (Table 1).

ELISA with intact and heat-treated leptospira

To evaluate MAb reactivity against native LipL32 protein ELISA protocols with intact and heat-treated leptospiral cells were used. A decrease in the absorbance of ELISA with intact cells occurred as the concentration of antibodies decreased, demonstrating the specificity of the reaction (Fig. 1A). In addition, antisera against the cytoplasmatic protein GroEL did not react in this ELISA. A conjugate of MAb 1D9-POase reacted with native LipL32 in both intact and heat-treated cell ELISA, but higher absorbance values were observed with heat-treated bacteria (Fig. 1B). In these ELISA experiments MAbs did not react with saprophytic bacteria and sera against GroEL reacted with heat-treated cells (data not shown).

Direct and indirect immunofluorescence

Reaction of MAbs with native LipL32 on the outer membrane of pathogenic *Leptospira* was investigated by indirect immunofluorescence and by an in-house prepared conjugate of MAb 1D9 and FITC. Bacterial cells were applied to slides with and without methanol treatment to evaluate the effect of the fixing method on MAb reactivity. The three MAbs labeled leptospiral cells in both fixing methods as could be seen by the intense fluorescence (Fig. 2). Labeling of leptospiral cells with MAb anti-*Salmonella* or normal mouse serum was not observed (data not shown).

Immunoprecipitation of native LipL32 and use of a MAb on IMS-PCR

The MAbs were used in immunoprecipitation assays to investigate their ability in binding the surface of live and motile leptospiral cells in suspension. Two MAbs were able to precipitate LipL32 from the suspension of live pathogenic bacteria (Fig. 3). In addition, MAb 1D9 was adsorbed onto protein A magnetic beads to explore its potential for use in diagnostic assays, which associates IMS with PCR amplification. With the use of IMS for capturing bacterial cells prior to DNA extraction and PCR amplification, it was possible to detect as low as 10 leptospires per milliliter of artificially contaminated human sera (Fig. 4). Sera contaminated with saprophytic *Leptospira* submitted to IMS and PCR did not show any amplification product (data not shown).

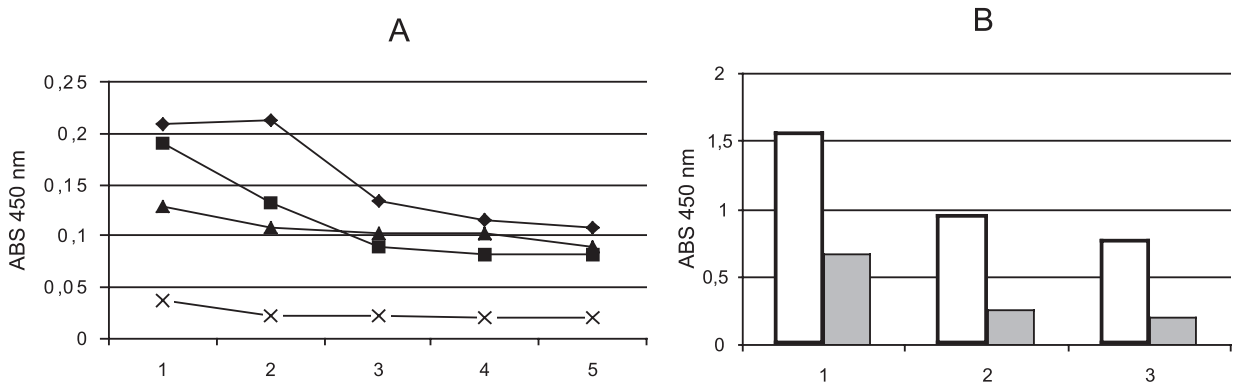

FIG. 1. Enzyme-linked immunosorbent assay (ELISA) reactions of monoclonal antibodies (MAbs) anti-LipL32 using intact and heat-treated *L. interrogans* L1-130 cells. **A**: ELISA using 10^9 intact leptospiral cells per milliliter (100 μl per well) and MAbs 1D9 (◆), 412H4 (■), 36C4 (▲), and GroEL antiserum (×) diluted 1:4 (1), 1:8 (2), 1:16 (3), 1:32 (4), and 1:64 (5). **B**: ELISA reactions with intact (□) and heat-treated cells (■) at 10^9 cell per milliliter (1), 4×10^8 cell per milliliter (2) and 2×10^8 cell per milliliter (3) and 1D9-POase conjugate diluted 1:1000.

DISCUSSION

Leptospiral outer membrane protein LipL32 is prominent on pathogenic strains and is the major detergent-phase protein extracted by Triton X 114.[4,16] Human sera from cases of leptospirosis reacted with native LipL32 and its recombinant form expressed in *E. coli*.[5] After these findings this antigen has been used for developing diagnostic tests that detect antibodies in human and animal leptospirosis.[6,7,17,18] However, diagnostic tests based on antibody detection suffer from lack of sensitivity in the first 10 days of infection. Antigen detection tests should be preferred when early diagnosis is important for initiating treatment, as is the case of leptospirosis.[1,19] MAbs are important tools for antigen detection tests because of their high specificity and permanent availability. The three MAbs reported in this study reacted with a 32-kd polypeptidic band from dif-

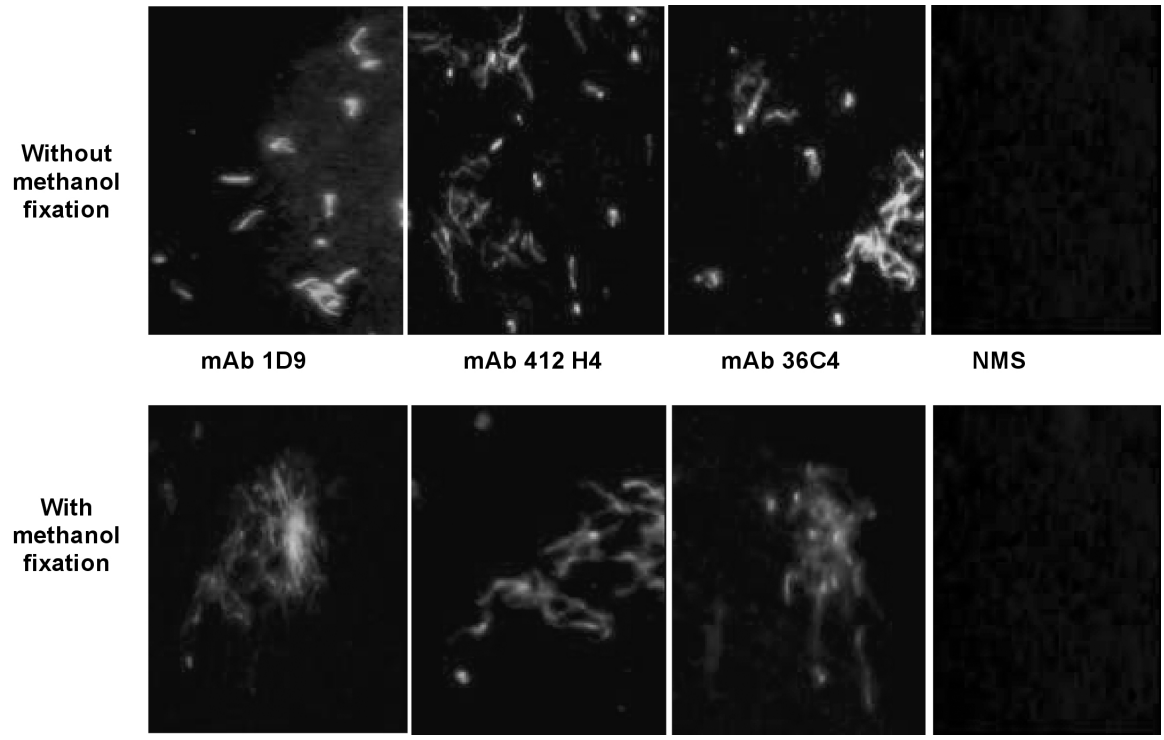

FIG. 2. Staining of LipL32 from *L. interrogans* L1-130 by indirect immunofluorescence. *L. interrogans* L1-130 was fixed to microscope slides with and without methanol and probed with monoclonal antibodies (MAbs). Test control slides were made by reacting fixed bacteria with normal mouse sera and rabbit anti-mouse fluorescein isothiocyanate (FITC).

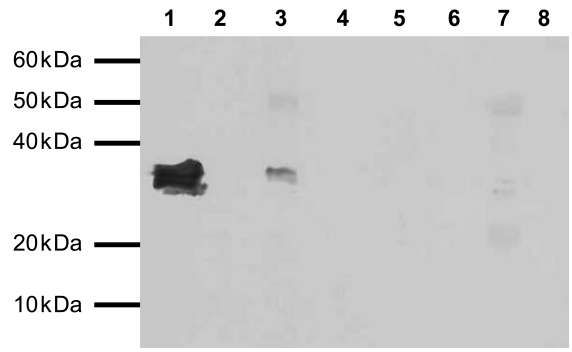

FIG. 3. Immunoprecipitation of native LipL32 on the surface of *L. interrogans* L1-130. Bacterial cells were reacted with monoclonal antibodies (MAbs) before Triton X-100 membrane fractionation and precipitated with protein A-Sepharose. Precipitated material was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blots were probed with MAb 1D9-POase. 1, rLipL32; 2, *L biflexa* Patoc, and MAb 1D9; 3, *L. interrogans* L1-130, and MAb 1D9; 4, *L. biflexa* Patoc and 412H; 5, *L. interrogans* L1-130 and 412H; 6, *L. biflexa* Patoc and 36C; 7, *L. interrogans* L1-130 and 36C; and 8, *L. interrogans* L1-130 treated with protein A-Sepharose only.

ferent leptospiral serovars on immunoblotting, a molecular mass similar to that of LipL32. Moreover, anti-LipL32 MAbs 1D9, 36C4 and 412H4 did not react with the other microorganisms tested. The MAbs were also able to detect native LipL32 antigen when used as primary antibody in indirect ELISA with intact and heat-treated leptospiral cells. Furthermore, MAb 1D9 maintained antibody activity after conjugation with peroxidase suggesting it is suitable for two-site immunoassays such as sandwich ELISA.

Indirect immunofluorescence was used to investigate MAb reaction after fixing leptospiral cells with or without methanol treatment. When anti-LipL32 MAb 1D9 was used conjugated to FITC, it was possible to visualize leptospiral cells by direct fluorescence. MAbs have been successfully used in immunofluorescence based diagnostic tests for Lyme and syphilis, two diseases also caused by spirochetes.[20,21] The antibodies reported here were able to attach to the bacterial outer membrane after the two fixing treatments, providing evidence of their usefulness for immunohistochemical tests.

Surface immunoprecipitation experiments were carried out to explore MAb potential for use in immunoseparation techniques or chromatographic assays. IMS has been used to increase PCR sensitivity and to improve bacterial isolation from clinical samples containing inhibitory substances or contaminating organisms.[22,24] Two of the MAbs obtained in this study were able to form complexes with live leptospiral cells suspended in a liquid phase suggesting that they coud be useful for IMS. One of these MAbs was adsorbed to magnetic particles and used to develop a method for *Leptospira* detection in association with PCR amplification of the *lipL32* gene. Using this novel method we were able to detect approximately 10 leptospiral cells per milliliter of human sera experimentally contaminated. An assay that uses MAb-based magnetic separation coupled to PCR amplification for detection of *L. borgpetersenii* serovar hardjo in urine from experimentally contaminated cattle has been reported.[25] Despite its good sensitivity, the assay consistently detected 10 organisms in urine samples spiked with leptospires, it may have an important limitation in specificity since the mAbs used for IMS are directed to LPS or a non-characterized outer envelope protein.

In conclusion, the results reported in this study demonstrated that MAbs generated against the recombinant form of LipL32 were able to recognize the native protein from pathogenic *Leptospira.* Because LipL32 is a surface protein conserved among pathogenic serovars and is not found on saprophytic strains, the MAbs against this target will be useful in the development of different test formats for diagnosis of human and animal leptospirosis.

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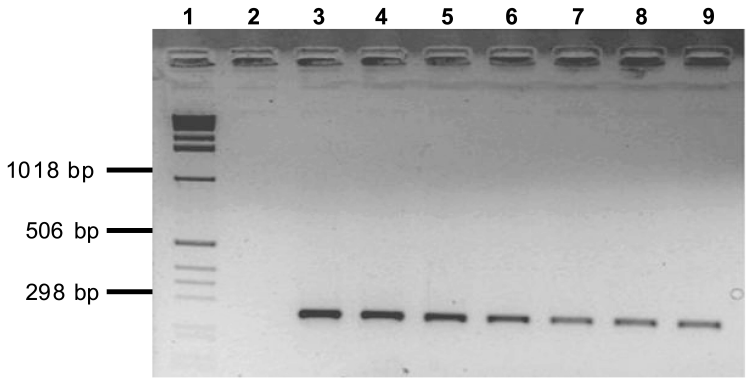

FIG. 4. Polymerase chain reaction (PCR) amplification of the *lipL32* coding region from *L. interrogans* L1-130 after immunomagnetic separation from experimentally contaminated human sera. 1, DNA ladder; 2, *L. biflexa* Patoc 10^7 cells per milliliter; 3, 10^7; 4, 10^6; 5, 10^5; 6, 10^4; 7, 10^3; 8, 10^2; 9, 10^1 cells per milliliter.

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