Exoproteome from Leptospira interrogans and Host Cells during Infection: Leptospiral Virulence Factors and Cellular Proteins Involved in Stress and Inflammation

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Abstract

**Background:** Leptospirosis, caused mainly by *Leptospira interrogans*, is a global zoonotic infectious disease. Macrophages and vascular endothelial cells are the main host cells for *L. interrogans* during infection, but the proteins released from the pathogen and the two host cells during infection remain mostly unknown.

**Results:** Cellular supernatant proteins (CSPs) from human THP-1 macrophages or umbilical vein endothelial cells (HUVECs) infected with *L. interrogans* strain Lai were extracted by TCA/FASP methods. The exoproteins in the CSPs were identified by LC-MS/MS. Viability of the leptospires and host cells during infection was confirmed by confocal microscopy and MTT. The results showed that higher co-culture temperature (from 28°C to 37°C) and different biochemical environments cause a large change in the exoproteome of the spirochete. *L. interrogans* increased levels of leptospiral exoproteins related to stress, signal transduction and virulence factors, while the lipoprotein antigens LipL41, LipL21 and/or Loa22 were not detected. During infection of macrophages and endothelial cells, there was a large increase in host-cell exoproteins involved in stress response, complement pathways (C4/5/7/8), inflammatory cytokines (IL-6, TNF-α, MIF, MCP-1 and GM-CSF), extracellular matrix proteins (FN, LN and COLs), and blood coagulation factors. One-third of the leptospires and infected THP-1 macrophages died during macrophage infection, but nearly all the leptospires and endothelial cells remained viable during endothelial cell infection.

**Conclusions:** Infection causes stress responses for both leptospires and human macrophages and vascular endothelial cells and release of virulence factors, alteration of surface leptospiral lipoprotein antigens and secretion of complement components and inflammatory cytokines from host cells.

**Background**

Leptospirosis, caused mainly by pathogenic *Leptospira* genospecies, is a global zoonotic infectious disease with approximately one million new patients and nearly sixty-thousand death cases per year [1-3]. The disease is endemic in Asia, Oceania and South America [4-6], but it is considered as an emerging or re-emerging infectious disease in Europe, North America and Africa, due to frequent case reports and several outbreaks in the recent years [7-9].

Many animals serve as the natural hosts of pathogenic *Leptospira* species, such as rodents, livestock and dogs [10]. Leptospirosis is transmitted from host animals to humans by contact with water or wet soil that had been contaminated with leptospire-containing animal urine. After invading the human body through the mucosa and impaired skin, the spirochetes enter the bloodstream to cause toxic septicemia with common clinical symptoms such as high fever, myalgia and superficial lymphadenectasis [11]. In many cases, the spirochetes can spread from the bloodstream into many internal organs and cerebrospinal fluid to generate different clinical types of leptospirosis with the severe cases often dying of pulmonary diffuse hemorrhage, acute renal failure and meningoencephalitis with a high fatality rate [10-
Therefore, the diffusion from the bloodstream into internal organs and tissues of pathogenic *Leptospira* species is a key step for the progression and aggravation of leptospirosis.

Infection results from the interaction between microbial pathogens and their hosts. During the process of invasion, surface and released proteins act as the crucial molecular determinants for the pathogens to respond to the microenvironment of infected tissues by change in their expression levels [13]. Previous studies showed that the transcription and expression levels of many genes of pathogenic *Leptospira interrogans* during infection of cells are significantly changed [14,15]. For example, the expression levels of virulence factors such as adherence factors, invasive enzymes and toxins of *L. interrogans* are significantly up-regulated during infection, but those of surface protein antigens are notably decreased [16-25]. However, it is believed that only released or surface proteins of microbes can play a direct role in the pathogenic process. Nonetheless, until now, the exoproteome from host cells and leptospires during *L. interrogans* infection remains poorly understood.

During the process of microbial invasion, the host cells can also respond by changing of their protein expression profiles. Macrophages play an important role in the innate immune response to infection by phagocytosis of the invaded pathogens and the adaptive immunity by presentation of microbial antigens [26]. In addition, macrophages from the bloodstream but not neutrophils have been shown to be the main infiltrating phagocytes in the infectious tissues of leptospirosis patients and *L. interrogans*-infected animals [27,28]. A previous study reported that the transcription levels of many genes in both human and murine macrophages were altered during infection with *L. interrogans* [29]. In many leptospirosis patients, leptospires can spread from the bloodstream to internal organs and tissues through small blood vessels by transcytosis [12,30]. Therefore, macrophages and vascular endothelial cells are the two types of host cells suitable for characterization of the exoproteome during leptospiral infection.

Pathogenic *Leptospira* consists a number of genospecies, in which *L. interrogans* is the most predominant causative agents of human leptospirosis in the world [31,32]. Although many serogroups and serovars of *L. interrogans* are prevalent in China, *L. interrogans* serogroup Icterohaemorrhagiae serovar Lai is responsible for disease in over 60% of leptospirosis patients [4,33]. In addition, the genomic sequence of *L. interrogans* serogroup Icterohaemorrhagiae serovar Lai strain Lai (No. 56601) is known [34]. Therefore, in this study, the proteins released from *L. interrogans* strain Lai, human macrophages and vascular endothelial cells during infection were characterized. The exoproteome profiles from the pathogen and two types of host cells during infection were examined and the functions of the leptospiral and cellular exoproteins were also analyzed.

## Results

**Overview of CSPs and exoproteins from *L. interrogans* and host cells before infection.**

The flow cytometric examination showed that 95.7% of THP-1 monocytes were differentiated into CD68+ macrophages after PMA treatment (Figure 1A). The SDS-PAGE examination showed the profiles of CSPs
from *L. interrogans* strain Lai cultured in EMJH medium at 28°C or in 2.5% FCS RPMI-1640 medium at 37°C for 24 h and from THP-1 macrophages and HUVECs cultured in 2.5% FCS RPMI-1640 medium at 37°C for 24 h (Figure 1B). The LC-MS/MS identified 57 or 61 leptospiral exoproteins from EMJH (28°C) or RPMI-1640 (37°C) medium but 62.3% (38/61) of the exoproteins were different (Table 1 and 2). In addition, the LC-MS/MS identified 27 or 28 exoproteins in the CSPs from THP-1 macrophages or HUVECs during incubation and 75.0% (21/28) of the exoproteins from different cells were identical (Table 3 and 4). The category analysis indicated that the main exoproteins of the spirochetes from EMJH-medium at 28°C were outer membrane/surface proteins and lipoproteins but the toxic proteins from the spirochetes were notably increased during incubation in RPMI-1640 medium at 37°C (Figure 1C). The data suggested that *L. interrogans* largely changed its released proteins in response to higher environmental temperature and different biochemical environments.

**Increased secretion of exoproteins from *L. interrogans* during infection of cells.**

The SDS-PAGE showed the profiles of total CSPs from co-cultures of *L. interrogans* strain Lai with THP-1 macrophages or HUVECs (Figure 2A). The LC-MS/MS identified 115 or 98 leptospiral exoproteins in the CSPs during infection of THP-1 macrophages or HUVECs, of which 45 or 40 exoproteins were also present in the leptospiral CSPs from EMJH and/or RPMI-1640 media and 25 exoproteins were identical during infection of THP-1 macrophages and HUVECs but 45 or 33 exoproteins were different during infection of THP-1 macrophages or HUVECs (Table 5 and 6). The heatmaps of leptospiral exoproteins during infection of cells were shown in Figure 2B. The outer membrane/surface proteins (33.87%) and toxic proteins (20.62%) were the main exoproteins of *L. interrogans* during infection (Figure 2C). Compared to the exoproteins from *L. interrogans* before infection, oxidoreductases, virulence factors and two-component signaling proteins were found as the main increased leptospiral exoproteins during infection (Table 5 and 6). In particular, outer membrane lipoprotein 32 (LipL32) was present in all the CSPs before and during infection of cells but OmpA family lipoprotein 22 (Loa22) and LipL41 disappeared during infection of THP-1 macrophages and LipL21 and LipL41 were absent during infection of HUVECs (Table 1, 2, 5 and 6). The data suggested that *L. interrogans* changes its metabolism during infection of human macrophages and vascular endothelial cells by increasing secretion of exoproteins involved in virulence, oxidative stress and signal transduction but decreasing secretion of some lipoprotein antigens.

**Increased secretion of exoproteins from host cells during infection with *L. interrogans*.**

The LC-MS/MS identified 95 or 163 cellular exoproteins in the total CSPs from co-cultures of *L. interrogans* strain Lai with THP-1 macrophages or HUVECs, of which 26 or 27 exoproteins were also present in the CSPs from THP-1 macrophages and/or HUVECs incubated in RPMI-1640 medium and 40 exoproteins were identical in the CSPs from THP-1 macrophages and HUVECs during infection but 24 or 90 exoproteins were different from THP-1 macrophages or HUVECs during infection (Table 7 and 8). The heatmaps of cellular exoproteins during infection were shown in Figure 3A and 3B. In the exoproteins, stress/immune response proteins (38.61% for THP-1 macrophages and 39.14% for HUVECs), such as
multiple heat shock proteins (HSPs), oxidoreductases and complement components, and adhesion proteins (21.67% for THP-1 macrophages and 20.81% for HUVECs), such as fibronectin (FN), laminin (LN) and many collagens (COLs) in extracellular matrix (ECM), were as the main exoproteins (Figure 3C, Table 7 and 8). In addition, the THP-1 macrophages released more antibacterial response proteins (17.97%) and fewer inhibitor activity proteins (1.92%) than HUVECs (6.14% and 13.16%) during infection (Figure 3C). In particular, THP-1 macrophages or HUVECs during infection released different inflammatory cytokines (IL-6 and TNF-α from THP-1 macrophages, MCP-1 and GM-CSF from HUVECs) (Table 7 and 8). Interestingly, both types of host cells released multiple blood coagulation factors during infection, such as coagulation factor X, thrombospondin-1/4 and prothrombin. The data suggested that *L. interrogans* also causes a significant metabolic change in human macrophages and vascular endothelial cells by increasing secretion of exoproteins involved in heat/oxidative stress, inflammation and immune response.

**Functional classes of leptospiral exoproteins before and during infection.**

The main exoproteins of *L. interrogans* strain Lai incubated in EMJH medium at 28°C play roles in two-component signaling system and flagellar assembly, but secretion of leptospiral ABC transporters was increased during incubation in RPMI-1640 medium at 37°C (Figure 4A and 4B). When the spirochetes were incubated with THP-1 macrophages and HUVECs, there was a significant increase in leptospiral exoproteins involved in oxidoreduction and metabolism for the stress response (Figure 4C and 4D). The data suggested that *L. interrogans* expresses more exoproteins to response to the infection of human macrophages and vascular endothelial cells.

**Functional classes of cellular exoproteins before and during infection.**

The main exoproteins of THP-1 macrophages and HUVECs incubated in RPMI-1640 medium play roles in protein binding, extracellular exosome and signal transduction (Figure 5A and 5B). When the cells were infected with *L. interrogans* strain Lai, the cellular exoproteins with functions in complement and coagulation cascades, ECM-receptor interaction and cytoskeleton rearrangement signal transduction were significantly increased (Figure 5C and 5D). The data suggested that human macrophages and vascular endothelial cells also express more exoproteins in response to the infection with *L. interrogans*.

**Viability of *L. interrogans* and host cells during infection**

The confocal microscopic examination showed that 19.2%-34.6% of the leptospires during infection of THP-1 macrophages had died, but nearly all of the leptospires during infection of HUVECs were still alive (Figure 6A and 6B). On the other hand, the MTT test showed that the THP-1 macrophages showed 65.6% viability percentage at 24 h during infection with *L. interrogans* strain Lai but nearly all of the HUVECs persistently maintained their viability during the whole infection process (Figure 6C). The data suggested that *L. interrogans* and human macrophages are mutually damaged during infection, but both leptospires and host cells remained viable during infection of vascular endothelial cells.

**Discussion**
Infection is an interactive process that takes place between pathogens and hosts [23,35]. During this interaction, hosts raise body temperature and mount an inflammatory reaction in order to eliminate the pathogens, but conversely, prokaryotic pathogens also change their metabolism in order to respond to the adverse environment for survival in the hosts. Host and pathogen proteins, including enzymes, play a major role in host defense against the pathogen and metabolic changes in the pathogen for environmental adaptation. Therefore, a characterization of exoproteins released from \textit{L. interrogans} and host cells during infection can enable us to further understand the interaction between the pathogen and host.

The \textit{Leptospira} genus includes a large group of helical, didermal prokaryotic microbes that can be classified into pathogenic and non-pathogenic saprophytic \textit{Leptospira} genospecies [32]. The optimal growth temperature of \textit{Leptospira} in medium in \textit{vitro} is 28°C. At this culture temperature, \textit{L. interrogans} strain Lai in EMJH medium released the OmpA family lipoprotein 22 (Loa22) and outer membrane lipoproteins 21, 32 and 41 (LipL21, LipL32 and LipL41). When the spirochetes were incubated in cellular RPMI-1640 medium, LipL21 and LipL41 were absent. The two lipoproteins have been described as the major surface antigens of pathogenic \textit{Leptospira} species [36-38]. In addition, most of Clp family proteins, which is composed of chaperones/protease complexes responsible for degradation of abnormal proteins, also disappeared in the CSPs from \textit{L. interrogans} strain Lai during incubation in cellular RPMI-1640 medium at 37°C while co-chaperonin GroEL and chaperone DnaK, also called heat shock protein 60 cofactor (Co-HSP60) and HSP70, and VagC, a toxic protein in the VagCB toxin-antitoxin module, were present in the supernatants [34,39,40]. The data indicated that higher cell-culture temperature and different chemical environments stimulate metabolic change of \textit{L. interrogans} by decreasing expression of surface lipoprotein antigens and increasing expression of heat stress and virulence proteins.

\textit{interrogans} can rapidly invade the bloodstream to cause septicaemia and diffuse from the bloodstream into internal organs and tissues, such as lungs, liver, kidneys and cerebrospinal fluid [12,30,32]. On the other hand, macrophages but not neutrophils have been confirmed as the main infiltrating phagocytes involved in the immune response during leptospirosis [28]. Therefore, PAM-differentiated human THP-1 macrophages and HUVECs were used as host cells in this study to generate cell infection models of \textit{L. interrogans}.

When \textit{L. interrogans} strain Lai was incubated with THP-1 macrophages or HUVECs, the spirochete quickly increased secretion of exoproteins related to outer membrane/surface protein antigens, virulence, oxidative stress and two-component signaling systems. Among the exoproteins, von Willebrand factor type A (vWA) domain-containing proteins (LB_054/055), ColA collagenase (LA_0872), Sph2 and TlyA hemolysins (LA_1029/0327) have been confirmed as pulmonary hemorrhage inducers, invasive enzyme and inflammatory stimulators of \textit{L. interrogans}, respectively [20,21,25]. Importantly, LipL21 and LipL41 disappeared from the supernatants during infection of THP-1 macrophages while Loa22 and LipL41 were absent during infection of HUVECs, but LipL32 was persistently present before or after infection of the two types of host cells. The data indicated that \textit{L. interrogans} can adapt to each specific host cell and
respond to the environments by increasing expression of virulence factors and decreasing surface lipoprotein antigens.

Macrophages, which are also the main leptosiral phagocytes in leptospirosis [28], play an important role in innate and adaptive anti-infection immunity due to its ability to phagocytose pathogens and present their antigen. Vascular endothelial cells also participate in anti-infection immunity through inflammatory reaction by release of multiple cytokines [41]. When THP-1 macrophages and HUVECs were infected with \textit{L. interrogans} \textit{in vitro}, the two types of host cells released many HSPs, oxidoreductases, complement components and inflammatory cytokines (IL-6 and TNF-α from THP-1 macrophages, MCP-1, MIF and GM-CSF from HUVECs). In particular, the mitochondrial HSP10, HSP60 and HSP70 imply a role for mitochondrial stress in the two types of host cells during infection [42]. TLR4 is often considered as an important pattern recognition receptor on phagocytes in response to bacterial infection [20,43]. In recent years, many components in the ECM of cells, such as FN, LN and COL1/3/4, have been shown to be receptors for many bacteria including \textit{L. interrogans} [44]. In the present study, THP-1 macrophages during infection with \textit{L. interrogans} expressed TLR4, FN and COL3, while the infected HUVECs presented FN, LN and COL3. The data suggested that human macrophages and vascular endothelial cells respond to infection of \textit{L. interrogans} through rapid generation of a heat/oxidative stress response an inflammatory reaction, and increased ability to recognize pathogens.

Pulmonary hemorrhage is a typical histopathological change observed in leptospirosis patients [10-12]. von Willebrand factor (vWF) plays a crucial role in blood coagulation by inducing platelet aggregation and activating blood coagulation factors [45]. Our previous study revealed that the proteins containing vWF A-region domains (vWA) from \textit{L. interrogans} cause pulmonary hemorrhage by competitive inhibition of vWF-mediated platelet aggregation [25]. In this study, THP-1 macrophages and HUVECs were shown to produce coagulation factor X during incubation in medium. To our surprise, during the infection process with \textit{L. interrogans}, both THP-1 macrophages and HUVECs are predicted to prevent hemorrhage by secretion of many blood coagulation factors, such as prothrombin, fibrinogen, coagulation factor V, and thrombospondin-1/4. The infected THP-1 macrophages or HUVECs also released coagulation factor IX or vWF. A recent study revealed that coagulation factors such as VII/IX/X factors act as effective antibacterial agents [46]. On the other hand, approximately one-third of the leptospires and infected THP-1 macrophages died during infection of macrophages, but almost all the leptospires and infected HUVECs survived during infection of the endothelial cells. These results are consistent with previous reports describing the interaction between the \textit{L. interrogans} and phagocytes (macrophages) or nonphagocytes (HUVECs) [30,36,37].

Taken together, our results show that infection of human macrophages or vascular endothelial cells by \textit{L. interrogans} can lead to heat and oxidative stress responses for both the pathogen and host cells, but release of virulence factors from \textit{L. interrogans} and inflammatory cytokines from both host cells. Moreover, the change in leptosiral surface lipoprotein antigens released during infection could be helpful in selection of suitable protein immunogens and development of specific vaccines against leptospirosis.
Conclusions

Leptospirosis is a zoonotic infectious disease of global importance. *Leptospira interrogans*, a major pathogenic genospecies of this disease, can invade the bloodstream to cause septicaemia and then diffuse from the bloodstream into many internal organs and tissues. Monocyte-differentiated macrophages but not neutrophils are the main infiltrating phagocytes in the immune response against the pathogen in *vitro* and in *vivo*. Therefore, the interaction between *L. interrogans* and macrophages and vascular endothelial cells, and the neighboring tissues via their secreted exoproteins, are expected to play a major role in the pathogenesis of leptospirosis. In this study, we found that both leptospires and host cells secreted many exoproteins during infection. The main exoproteins from the spirochete during infection were involved in stress responses, virulence and signal transduction while those from the infected cells were host stress proteins, complement proteins, inflammatory cytokines, extracellular matrix proteins and blood coagulation factors. Analysis of the secretome indicates that infection induces stress response in both pathogen and host cell, while leptospiral virulence factors and inflammatory cytokines from the host may alter the physiology of microenvironment and the host immune response.

Methods

**Leptospiral strain and culture**

*L. interrogans* serogroup Icterohaemorrhagiae serovar Lai strain Lai was provided by the Chinese National Institute for Control of Pharmaceutical and Biological Products. The strain was cultured in Ellinghausen-McCullough-Johnson-Harris (EMJH) medium at 28°C [47].

**Cell lines and culture**

Human THP-1 monocyte and umbilical vein endothelial cell (HUVEC) lines were provided by the Cell Bank of the Institute of Cytobiology in Shanghai, Chinese Academy of Sciences. The cells were cultured in RPMI-1640 medium (Gibco, USA), supplemented with 10% fetal calf serum (FCS, Gibco), 100 U/mL penicillin and 100 μg/mL streptomycin (Sigma, USA) at 37°C in an atmosphere of 5% CO₂. In particular, THP-1 monocytes were pretreated with 100 ng/mL phorbol-12-myristate-13-acetate (PMA, Sigma) at 37°C for 48 h to differentiate them into macrophages before use [24,48]. The PMA-differentiated CD68⁺ THP-1 macrophages were determined by flow cytometry using FITC-conjugated mouse anti-human macrophage surface marker CD68-IgG (BD Biosciences, USA) [24].

**Cell infection models**

Fresh cultured *L. interrogans* strain Lai was pelleted at 10,000×g centrifugation for 30 min (4°C). After washing twice with 0.01 M phosphate buffered saline (PBS, pH7.4) and centrifugation again, the leptospiral pellets were suspended in PBS for counting under a dark-field microscope using a Petroff-Hausser chamber (Fisher Scientific, USA) [24]. THP-1 macrophages or HUVECs (10⁶ per well) were seeded in 6-well culture plates with 2.5% FCS antibiotic-free RPMI-1640 medium for a 12-h incubation at 37°C to
form cell monolayers. The monolayers were infected with the spirochete at a multiplicity of infection of 50 (MOI\textsubscript{50}, 50 leptospires per cell) and then incubated at 37°C for 6, 12 or 24 h [15,29,47-49].

**Extraction of supernatant proteins from co-cultures**

Supernatants of the leptospire-cell co-cultures were centrifuged at 12,000×g for 15 min (4°C) and then passed through 0.45 and 0.22 µm filters (Millipore, USA) in turn. Trichloroacetic acid (TCA, Sigma) was added into the filtered supernatants at a final concentration 10% (W/V) TCA to precipitate the co-culture supernatant proteins (CSPs) overnight (4°C). After centrifugation at 17,200×g for 15 min (4°C), the CSP pellets were washed twice with ice-cold acetone and methanol to remove TCA [20,24]. In addition, the CSPs from *L. interrogans* strain Lai in EMJH medium at 28°C and THP-1 macrophages or HUVECs in 2.5% FCS antibiotic-free RPMI-1640 medium for a 6-, 12- or 24-h incubation at 37°C were also extracted as above. All the CSP extracts were dissolved in ultrapure double-distilled water (ddH\textsubscript{2}O, Millipore) for further analysis.

**Examination of CSP extracts by SDS-PAGE**

The CSP extracts were quantified using a BCA Protein Assay Kit (Thermo Scientific, USA) and then examined under a Gel Image Analyzer (Bio-Rad, USA) after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

**Pretreatment of CSP extracts**

The CSP extracts were purified using a ProteoPrep Blue Albumin and IgG Depletion Kit (Sigma) to remove FCS proteins and then treated by filter aided sample preparation (FASP) method as previously described [38-40]. Briefly, after determination of protein concentration as above, the CSP extracts were administrated with an ultrafiltration filter (10 kDa units, Millipore) at 12,000×g. The filter was rinsed with 200 µL urea buffer (6 M urea-10 mM Tris-HCl, pH 6.8) and then incubated with 10 mM 1,4-dithiothreitol (DTT, Sigma) for 1 h at 37°C. After the centrifuge, the samples were alkylated in 40 mM iodoacetamide (IAA, Sigma) for 30 min at room temperature in dark. After washing with urea buffer, then washed twice with 50 mM ammonium bicarbonate, the harvested CSPs were hydrolyzed with trypsin (Sigma) at a 1:50 (enzyme: protein) mass ratio at 37 °C for 12 h. After the centrifuge, the hydrolytic peptides were collected and dissolved with 0.1% trifluoroacetic acid (TFA). The samples were desalted with Ziptip according to the instruction before freeze-dried preservation.

**Label free LC-MS/MS detection**

The trypsin-digested proteins from the hydrolyzed CSPs were identified by high performance liquid chromatography plus tandem mass spectrometry (LC-MS/MS) as previously described [23,53]. Briefly, 1 ug of the trypsin-digested protein preparation was dissolved in 5 µL of 0.1% formic acid (FA, Sigma) solution. The peptide solution was loaded onto an in-house packed C18 trap column (100 µm ID × 2 cm, 5 µm, Reprosil-Pur C18 AQ, Dr. Maisch, Germany) for separation in an in-house packed C18 analytical
column (75 μm ID × 20 cm, 3 μm, Reprosil-Pur C18 AQ, Dr. Maisch) using mobile phase A solution (0.1% FA in ultrapure ddH₂O) and 5-95% gradient mobile phase B solutions (0.1% FA in acetonitrile) for a 78-min separation with a 280 NL/min flow rate in a LC system (EASY-nLC-1000, Thermo Scientific). The separated peptides were then identified in a MS/MS system (LTQ-Orbitrap-Elite, Thermo Scientific) using positive ion mode. The full-scan fragmentation MS/MS spectrum (300-1600 m/z) of each of the peptides carrying 2-5 positive charges were obtained from the Orbitrap analyzer at a high resolution of 70,000 (m/z 200) with an automatic gain control and a maximum fill time of 60 ms. All the obtained fragmentation MS/MS spectral data were further analyzed using Xcalibur v2.2 software.

**Determination of the leptospiral or cellular exoproteins**

The LC-MS/MS-identified peptides were searched for their corresponding exoproteins from *L. interrogans* strain Lai, THP-1 macrophages or HUVECs based on match of amino acid sequences. The exoproteins from the spirochete were determined using both NCBI and UniProt/Swiss Prot databases while the exoproteins from THP-1 macrophages and HUVECs were determined using UniProt/Swiss Prot database.

**Bioinformatic analysis**

The categories of exoproteins from *L. interrogans* strain Lai, THP-1 macrophages and HUVECs were classified into clusters of orthologous groups (COGs) using MicroScope and GenoScope softwares [54]. The heatmaps and clusters of the leptospiral and cellular exoproteins were generated using Complex-Heatmap package and R software [55]. GO and KEGG pathway analysis including cellular component, molecular function and biological process in gene ontology were analyzed as previously described [23,56-58].

**Detection of viabilities of *L. interrogans* and cells during infection**

THP-1 macrophages or HUVECs were infected with *L. interrogans* strain Lai as described above. After trypsinization, the co-cultures were centrifuged at 500×g for 10 min (4°C) to precipitate the extracellular leptospire-free cells and the supernatants were centrifuged at 10,000×g for 30 min (4°C) to precipitate the extracellular leptospires. The cells and leptospires were washed twice with PBS and then centrifuged as above. The viability of infected cells was detected by methyl thiazolyl tetrazolium (MTT) test using a Cell Proliferation Kit (Sigma) while the viability of collected leptospires was detected by confocal microscopy (Zeiss, Germany) (485/630 or 485/530 nm excitation/emission wavelengths for SYTO 9 or PI detection) using a LIVE/DEAD Bacterial Viability Kit (Invitrogen, USA) and the fold changes of green fluorescence intensity (FI) were analyzed for semi-quantification of the leptospiral viability [23,31]. In the tests, *L. interrogans* strain Lai, THP-1 macrophages and HUVECs without infection were used as the controls.

**Statistical analysis**

Data from a minimum of three independent experiments for detection of viability of *L. interrogans*, macrophages and HUVECs during infection were averaged and then presented as mean ± standard
deviation (SD). Significant differences were determined by $t$ and $\chi^2$ tests. Statistical significance was defined as $p < 0.05$.

Abbreviations

CSPs: Cellular supernatant proteins; HSPs: heat shock proteins; FN: fibronectin; LN: laminin; COLs: collagens; ECM: extracellular matrix; EMJH: Ellinghausen-McCullough-Johnson-Harris; PBS: phosphate buffered saline; MOI: multiplicity of infection; SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis; FASP: filter aided sample preparation; LC-MS/MS: liquid chromatography plus tandem mass spectrometry; FA: formic acid; COGs: clusters of orthologous groups; MTT: methyl thiazolyl tetrazolium; FI: fluorescence intensity; SD: standard deviation.

Declarations

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Author's Contributions

JY and WLH contributed to the design of the study. MI and KXL performed the experiments. MI, SAH, SJL, YMG and SLD analyzed the experimental data. MI, DMO, WLH and JY wrote the manuscript. All authors reviewed and approved the final version of the manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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Tables

Table 1. Same exoproteins of *L. interrogans* strain Lai in different culture conditions.
| No. | Exoprotein                                                                 | Gene   |
|-----|---------------------------------------------------------------------------|--------|
| 1   | S-adenosylmethionine dependent methyltransferase                           | LA_0082|
| 2   | OmpA family outer membrane lipoprotein 22 (Loa22)                          | LA_0222|
| 3   | ATP-dependent protease ClpA                                                | LA_0391|
| 4   | Elongation factor Tu                                                      | LA_0737|
| 5   | 50S ribosomal protein L6                                                  | LA_0754|
| 6   | Cytoplasmic membrane protein                                               | LA_0873|
| 7   | Cysteine synthase                                                          | LA_1719|
| 8   | Excinuclease ABC subunit A                                                | LA_2212|
| 9   | Endonuclease                                                              | LA_2250|
| 10  | Phosphoenolpyruvate-protein phosphotransferase                            | LA_2307|
| 11  | Endoflagellar filament core protein                                        | LA_2418|
| 12  | Outer membrane lipoprotein 32 (LipL32)                                     | LA_2637|
| 13  | Molecular chaperone GroEL (HSP60)                                          | LA_2655|
| 14  | ATP synthase subunit C                                                    | LA_2782|
| 15  | Peroxiredoxin                                                             | LA_2809|
| 16  | Prolipoprotein diacylglycerol transferase                                  | LA_3004|
| 17  | Acyl-CoA dehydrogenase                                                    | LA_3143|
| 18  | DNA-directed RNA polymerase subunit beta                                  | LA_3419|
| 19  | Precorrin-2 methyltransferase                                              | LB_159 |
| 20  | Hypothetical protein                                                       | LA_0505|
| 21  | Hypothetical protein                                                       | LA_0532|
| 22  | Hypothetical protein                                                       | LA_1202|
Table 2. Different exoproteins of *L. interrogans* strain Lai in different culture conditions.
| No. | Exoprotein / Gene                                      | Medium Conditions                          |
|-----|--------------------------------------------------------|-------------------------------------------|
| 1   | Outer membrane lipoprotein 21 (LipL21) / LA_0011       | EMJH medium at 28°C for 24 h              |
|     | RNA polymerase ECF-type sigma factor / LA_0165         | RPMI-1640 medium at 37°C for 24 h         |
| 2   | Outer membrane lipoprotein 41 (LipL41) / LA_0616       | RPMI-1640 medium at 37°C for 24 h         |
|     | Cell wall hydrolase / LA_0312                          |                                           |
| 3   | 30S ribosomal protein S1 / LA_0646                     |                                           |
|     | Methyltransferase / LA_0380                            |                                           |
| 4   | Acetolactate synthase large subunit / LA_0863          |                                           |
|     | Electron transfer flavoprotein subunit beta / LA_0412  |                                           |
| 5   | Transcription termination factor Rho / LA_1021         |                                           |
|     | 50S ribosomal protein L2 / LA_0742                      |                                           |
| 6   | Glutamine synthetase / LA_1313                         |                                           |
|     | ABC transporter permease / LA_0970                      |                                           |
| 7   | ATP-dependent protease ClpA / LA_1879                   |                                           |
|     | Virulence-associated protein VagC / LA1002             |                                           |
| 8   | ATP-dependent protease ClpP / LA_1953                   |                                           |
|     | Succinyl-CoA synthetase subunit alpha / LA_1101        |                                           |
| 9   | Endoflagellar filament core protein / LA_2017           |                                           |
|     | Histidine kinase sensor protein / LA_1348              |                                           |
| 10  | 16S rRNA methyltransferase RsmE / LA_2064              |                                           |
|     | Fructose-bisphosphatealdolase / LA_1532                |                                           |
| 11  | ABC transporter ATP-binding protein / LA_2114           |                                           |
|     | MCE-like protein / LA_2055                             |                                           |
| 12  | fructose-1,6-bisphosphatase / LA_2226                  |                                           |
|     | Excinuclease ABC subunit C / LA_2166                   |                                           |
| 13  | Transaldolase / LA_2286                                |                                           |
|     | Outer membrane lipoprotein 45 (LipL45) / LA_2295       |                                           |
| 14  | ATP-dependent protease ATP-binding ClpX / LA_2558       |                                           |
|     | Serine/threonine phosphatase / LA_2548                 |                                           |
| 15  | ATP-dependent Clp protease adaptor ClpS / LA_2631       |                                           |
|     | Co-chaperonin GroES (Co-HSP60) / LA_2654                |                                           |
| 16  | Iron-sulphuroxidoreductase / LA_2712                    |                                           |
|     | P-loop domain-containing protein / LA_2685              |                                           |
| 17  | ATP synthase F0F1 subunit beta / LA_2776                |                                           |
|     | Transpeptidase / penicillin binding protein / LA_2755   |                                           |
| 18  | Alpha/beta hydrolase / LA_3147                         |                                           |
|     | TolB protein precursor / LA_3067                        |                                           |
|   | Protein Name                                      | Protein ID   | Protein Name                                      | Protein ID   |
|---|-------------------------------------------------|--------------|-------------------------------------------------|--------------|
| 19| Flagellar filament sheath protein / LA_3380     | cAMP-binding protein / LA_3372 |
| 20| Leucylaminopeptidase / LA_3441                  | Flagellar filament sheath protein / LA_3379 |
| 21| Cytoplasmic membrane protein / LA_3725          | Mrr restriction system protein / LA_3528 |
| 22| Sulfite reductase subunit beta / LA_4216        | DNA-binding ferritin-like protein / LA_3598 |
| 23| Ketol-acid reductoisomerase / LA_4242           | Molecular chaperone DnaK (HSP70) / LA_3705 |
| 24| Acetyl-CoA synthetase / LA_4254                 | Valyl-tRNA synthetase / LA_3763 |
| 25| Delta-aminolevulinic acid dehydratase / LB_012  | Ribonuclease D / LA_4091 |
| 26| HD-GYP domain-containing protein / LB_124       | Acyl carrier protein / LB_083 |
| 27| Aconitate hydratase / LB_327                    | Hypothetical protein / LA_0709 |
| 28| Hypothetical protein / LA_0471                  | Hypothetical protein / LA_0927 |
| 29| Hypothetical protein / LA_1204                  | Hypothetical protein / LA_0954 |
| 30| Hypothetical protein / LA_1939                  | Hypothetical protein / LA_1060 |
| 31| Hypothetical protein / LA_3097                  | Hypothetical protein / LA_1312 |
| 32| Hypothetical protein / LA_3696                  | Hypothetical protein / LA_1412 |
| 33| Hypothetical protein / LA_3762                  | Hypothetical protein / LA_1443 |
| 34| Hypothetical protein / LB_334                   | Hypothetical protein / LA_1698 |
| 35| Hypothetical protein / LA_2123                  | Hypothetical protein / LA_2123 |
| 36| Hypothetical protein / LA_2443                  | Hypothetical protein / LA_2443 |
| 37| Hypothetical protein / LA_2859                  | Hypothetical protein / LA_2859 |
| 38| Hypothetical protein / LA_3271                  | Hypothetical protein / LA_3271 |

**Table 3.** Same exoproteins of THP-1 macrophages and HUVECs in culture.
| No. | Exoprotein                                                                 | Protein ID |
|-----|----------------------------------------------------------------------------|------------|
| 1   | Ceruloplasmin (RPMI-1640 medium at 37°C for 24 h)                          | E9PFZ2     |
| 2   | Coagulation factor X                                                        | P00742     |
| 3   | Complement C3                                                              | P01024     |
| 4   | Apolipoprotein C-III                                                       | P02656     |
| 5   | Fibronectin                                                                | P02751     |
| 6   | Retinol binding protein 4                                                  | Q5VY30     |
| 7   | Lactotransferrin                                                           | P02788     |
| 8   | Keratin, type II cytoskeletal 6B                                            | P04259     |
| 9   | Heat shock protein HSP 90-alpha                                            | P07900     |
| 10  | Heat shock protein HSP 90-beta                                             | P08238     |
| 11  | Isoform 2 of cartilage oligomeric matrix protein                           | P49747     |
| 12  | Bax inhibitor 1                                                            | F8W1V3     |
| 13  | Actin, cytoplasmic 2                                                       | P63261     |
| 14  | Histone H4                                                                 | P62805     |
| 15  | Actin, alpha skeletal muscle                                               | P68133     |
| 16  | Desmoglein-1                                                               | Q02413     |
| 17  | Peroxiredoxin-1                                                            | A0A0A0MSI0 |
| 18  | NAD(P)(+)-arginine ADP-ribosyltransferase                                   | H7C2G2     |
| 19  | Testis-specific Y-encoded-like protein 2                                   | Q9H2G4     |
| 20  | HCG1745306, isoform CRA_a                                                  | G3V1N2     |
| 21  | Isoform 2 of Rho guanine nucleotide exchange factor 5                       | Q12774     |

Table 4. Different exoproteins of THP-1 macrophages and HUVECs in culture.
| No. | Exoprotein / Protien ID (in RPMI-1640 medium at 37˚C for 24 h) | THP-1 macrophages | HUVECs |
|-----|-------------------------------------------------------------|-------------------|-------|
| 1   | L-lactate dehydrogenase A chain / P00338                     |                   | Fructose-bisphosphatealdolase A / P04075 |
| 2   | Transthyretin / A0A087WV45                                  |                   | Collagen alpha-1(COL-VI) chain / A0A087X0S5 |
| 3   | Thioredoxin / P10599                                        |                   | Dermcidin / P81605 |
| 4   | Elongation factor 2 / P13639                                |                   | Calmodulin-like protein 5 / Q9NZT1 |
| 5   | 14-3-3 protein zeta/delta / P63104                           |                   | Protein disulfide-isomerase / I3NI03 |
| 6   | Protein S100 / R4GN98                                       |                   | Annexin / H0YMM1 |
| 7   | Isoform 2 of tubulin alpha-1B chain / P68363                |                   |       |

Table 5. Exoproteins of *L. interrogans* strain Lai during infection of THP-1 macrophages.
| No. | Exoprotein / Gene | Before infection | Time of infection (h) |
|-----|------------------|------------------|----------------------|
|     |                  |                  | 6        | 12 | 24 |
| 1   | Elongation factor Tu / LA_0737Δ | √                | √         | √  | √  |
| 2   | Outer membrane lyoprotein 32 (LipL32) / LA_2637Δ | √                | √         | √  | √  |
| 3   | Molecular chaperone GroEL (HSP60) / LA_2655Δ | √                | √         | √  | √  |
| 4   | S-adenosylmethionine-dependent methytransferase / LA_0082Δ | √                | √         | √  | 0  |
| 5   | Cysteine synthase / LA_1719Δ | √                | √         | √  | 0  |
| 6   | Endonuclease / LA_2250Δ | √                | √         | √  | 0  |
| 7   | ATP synthase subunit C / LA_2782Δ | √                | √         | √  | 0  |
| 8   | Peroxiredoxin / LA_2809Δ | √                | 0         | √  | √  |
| 9   | Endoflagellar filament core protein / LA_2418Δ | √                | 0         | 0  | √  |
| 10  | Hypothetical protein / LA_3961Δ | √                | √         | √  | √  |
| 11  | Hypothetical protein / LA_0505Δ | √                | 0         | 0  | √  |
| 12  | Endoflagellar | √                | √         | √  | √  |
|   | Protein Name                                                                 |   |   |   |   |
|---|------------------------------------------------------------------------------|---|---|---|---|
| 13| Translaldolase / LA_2286                                                   | ✓ | ✓ | ✓ | ✓ |
| 14| Cytoplasmic membrane protein / LA_3725                                      | ✓ | ✓ | 0 | 0 |
| 15| Outer membrane lipoprotein LipL21 / LA_0011                                 | ✓ | 0 | 0 | ✓ |
| 16| ATP-dependent protease ATP-binding subunit ClpX / LA_2558                   | ✓ | 0 | 0 | ✓ |
| 17| PBS lyase HEAT-like protein / LA_0471                                      | ✓ | 0 | 0 | ✓ |
| 18| Iron-sulphuroxidor eductase / LA_2712                                      | ✓ | 0 | 0 | ✓ |
| 19| Endoflagellar filament sheath protein / LA_3380                             | ✓ | 0 | 0 | ✓ |
| 20| Hypothetical protein / LA_3097                                              | ✓ | 0 | 0 | ✓ |
| 21| Methyltransferase / LA_0380*                                                | ✓ | ✓ | ✓ | 0 |
| 22| Cell wall hydrolase / LA_0312*                                             | ✓ | ✓ | 0 | 0 |
| 23| Virulence-associated protein VagC / LA_1002*                                | ✓ | ✓ | 0 | 0 |
|   | Protein Name                                                                 | Gene ID  | $\sqrt{\text{Character}}$ | $\sqrt{\text{Character}}$ | 0 | 0 |
|---|------------------------------------------------------------------------------|----------|-----------------------------|-----------------------------|---|---|
|24 | Excinuclease ABC subunit C / LA_2166*                                        |          | $\sqrt{\text{Character}}$   | $\sqrt{\text{Character}}$   | 0 | 0 |
|25 | Serine/threonine phosphatase / LA_2548*                                     |          | $\sqrt{\text{Character}}$   | $\sqrt{\text{Character}}$   | 0 | 0 |
|26 | TolB protein precursor / LA_3067*                                            |          | $\sqrt{\text{Character}}$   | $\sqrt{\text{Character}}$   | 0 | 0 |
|27 | cAMP-binding protein / LA_3372*                                             |          | $\sqrt{\text{Character}}$   | $\sqrt{\text{Character}}$   | 0 | 0 |
|28 | valyl-tRNAsynthetase / LA_3763*                                             |          | $\sqrt{\text{Character}}$   | $\sqrt{\text{Character}}$   | 0 | 0 |
|29 | Acyl carrier protein / LB_083*                                              |          | $\sqrt{\text{Character}}$   | $\sqrt{\text{Character}}$   | 0 | 0 |
|30 | RNA polymerase ECF-type sigma factor / LA_0165*                             |          | $\sqrt{\text{Character}}$   | 0                           | $\sqrt{\text{Character}}$ | 0 |
|31 | ABC transporter / LA_2055*                                                  |          | $\sqrt{\text{Character}}$   | 0                           | $\sqrt{\text{Character}}$ | 0 |
|32 | P-loop domain-containing protein / LA_2685*                                 |          | $\sqrt{\text{Character}}$   | 0                           | $\sqrt{\text{Character}}$ | 0 |
|33 | Flagellar filament sheath protein / LA_3379*                                |          | $\sqrt{\text{Character}}$   | 0                           | 0 | $\sqrt{\text{Character}}$ |
|34 | DNA-binding ferritin-like protein / LA_3598*                                 |          | $\sqrt{\text{Character}}$   | 0                           | 0 | $\sqrt{\text{Character}}$ |
|35 | Molecular chaperone DnaK (HSP70) / LA_3705*                                 |          | $\sqrt{\text{Character}}$   | 0                           | 0 | $\sqrt{\text{Character}}$ |
|   | Hypothetical protein / LA_1312* | √ | √ | √ | 0 |
|---|---------------------------------|---|---|---|---|
| 37 | Hypothetical protein / LA_0709* | √ | 0 | √ | √ |
| 38 | Hypothetical protein / LA_1443* | √ | √ | 0 | 0 |
| 39 | Hypothetical protein / LA_2123* | √ | 0 | √ | 0 |
| 40 | Hypothetical protein / LA_2443* | √ | 0 | √ | 0 |
| 41 | Hypothetical protein / LA_0954* | √ | 0 | 0 | √ |
| 42 | Hypothetical protein / LA_1060* | √ | 0 | 0 | √ |
| 43 | Hypothetical protein / LA_2295* | √ | 0 | 0 | √ |
| 44 | Hypothetical protein / LA_2859* | √ | 0 | 0 | √ |
| 45 | Hypothetical protein / LA_3271* | √ | 0 | 0 | √ |
| 46 | von Willebrand factor type A domain-containing protein / LB_054# | 0 | √ | √ | √ |
| 47 | Sulfatase / LA_0726# | 0 | √ | √ | 0 |
| 48 | ABC transporter, ATP-binding protein / LA_0969# | 0 | √ | √ | 0 |
| 49 | FAD | 0 | √ | √ | 0 |
|   | Protein Name                                                                 | Value 1 | Value 2 | Value 3 | Value 4 |
|---|------------------------------------------------------------------------------|---------|---------|---------|---------|
|50 | Gamma-glutamyltranspeptidase / LA_2105#                                     | 0       | √       | √       | 0       |
|51 | Two component system sensor histidine kinase / LA_2715#                     | 0       | √       | √       | 0       |
|52 | Exonuclease / LA_3974#                                                      | 0       | √       | √       | 0       |
|53 | Oxidoreductase FAD-binding family protein / LB_163#                         | 0       | √       | √       | 0       |
|54 | Microbial collagenase ColA / LA_0872#                                       | 0       | 0       | √       | √       |
|55 | Zn-dependent peptidase (metalloproteinase) / LA_0948 #                      | 0       | 0       | √       | √       |
|56 | von Willebrand factor type A domain-containing protein / LB_055#            | 0       | 0       | √       | √       |
|57 | Hemolysin A (TlyA) / LA_0327#                                              | 0       | √       | 0       | 0       |
|58 | HD family protein / LA_0866#                                                | 0       | √       | 0       | 0       |
|59 | Sphingomyelinase C                                                          | 0       | √       | 0       | 0       |
|   | Gene Name                        | Gene ID | Predicted Activity | Known Activity | Experiment Type | Item of Interest |
|---|---------------------------------|---------|--------------------|---------------|----------------|------------------|
| 60| Citrate lyase / LA_2841         | 0       | √                  | 0             | 0              | 0                |
| 61| DNA helicase UvrD / LA_2317     | 0       | 0                 | √             | 0              | 0                |
| 62| Oxidoreductase / LA_4224        | 0       | 0                 | √             | 0              | 0                |
| 63| Two-component hybrid sensor and regulator / LB_014 | 0 | 0 | 0 | √ |
| 64| Hypothetical protein / LA_4154  | 0       | √                  | √             | 0              | 0                |
| 65| Hypothetical protein / LA_1553  | 0       | √                  | 0             | 0              | 0                |
| 66| Hypothetical protein / LA_1774  | 0       | √                  | 0             | 0              | 0                |
| 67| Hypothetical protein / LA_0089  | 0       | 0                 | √             | 0              | 0                |
| 68| Hypothetical protein / LA_1063  | 0       | 0                 | √             | 0              | 0                |
| 69| Hypothetical protein / LA_3612  | 0       | 0                 | √             | 0              | 0                |
| 70| Hypothetical protein / LA_3677  | 0       | 0                 | √             | 0              | 0                |
| 71| Oxidoreductase-like protein / LA_0630 & | 0 | √ | √ | 0 |
| 72| Flagellar                      | 0       | √                  | 0             | 0              | 0                |
| Line | Description                                                                 | Gene ID(s) |
|------|-----------------------------------------------------------------------------|------------|
| 73   | Protein G / La_0025                                                        |            |
| 74   | HSP20/alpha crystallin molecular chaperone / La_1563                        |            |
| 75   | Leucyl-tRNA synthetase / La_3714                                           |            |
| 76   | Acriflavine resistance protein / La_3739                                    |            |
| 77   | Adenosylcobalamin biosynthesis bifunctional protein / CobDQ / Cobyric acid synthase / LB_151 |            |
| 78   | NADH dehydrogenase (ubiquinone) chain G / La_0160                          |            |
| 79   | Acyl-CoA dehydrogenase / La_1130                                           |            |
| 80   | Cell division protein ZapA / La_1246                                       |            |
| 81   | Esterase / La_1702                                                        |            |
| 82   | Penicillin binding protein / La_2187                                       |            |
|      | Thioesterase                                                              |            |
|   | Description                                                                 | Score | Activity | Note | RAF     |
|---|------------------------------------------------------------------------------|-------|----------|------|---------|
| 83| GTP-binding protein / LA_3634 &                                               | 0     | 0        | ✓    | 0       |
| 84| Macro domain-containing protein / LA_4133 &                                  | 0     | 0        | ✓    | 0       |
| 85| Chloride channel protein EriC / LA_4210 &                                    | 0     | 0        | ✓    | 0       |
| 86| ATPase / LA_0038 &                                                          | 0     | 0        |      | ✓       |
| 87| Cell shape determination protein / LA_0799 &                                 | 0     | 0        |      | ✓       |
| 88| Preprotein translocase subunit YajC / LA_1141 &                             | 0     | 0        |      | ✓       |
| 89| Transcriptional regulator / LA_1282 &                                       | 0     | 0        |      | ✓       |
| 90| Catalase / LA_1859 &                                                        | 0     | 0        |      | ✓       |
| 91| Succinate dehydrogenase flavoprotein subunit / LA_1897 &                    | 0     | 0        |      | ✓       |
| 92| RNA polymerase sigma-70 factor / LA_2232 &                                  | 0     | 0        |      | ✓       |
| 93| Type II secretory pathway component                                         | 0     | 0        |      | ✓       |
|   | Enzyme/Protein                             | Column 1 | Column 2 | Column 3 | Column 4 | Column 5 |
|---|-------------------------------------------|----------|----------|----------|----------|----------|
| 94 | Esterase/lipase / LA_2505                 | 0        | 0        | 0        |          | √        |
| 95 | Cytoplasmic membrane lipoprotein LipL31 / LA_2512 | 0        | 0        | 0        |          | √        |
| 96 | ATP synthase F0F1 subunit alpha / LA_2779 | 0        | 0        | 0        |          | √        |
| 97 | Carbon-nitrogen hydrolase / LA_2860      | 0        | 0        | 0        |          | √        |
| 98 | Oxidoreductase / LA_3709                 | 0        | 0        | 0        |          | √        |
| 99 | Adenosine/AMP deaminase / LA_3969       | 0        | 0        | 0        |          | √        |
| 100| Choline dehydrogenase / LA_3999         | 0        | 0        | 0        |          | √        |
| 101| Hypothetical protein / LB_268            | 0        |          |          |          | √        |
| 102| Hypothetical protein / LA_0012           | 0        |          |          |          | √        |
| 103| Hypothetical protein / LA_0905           | 0        |          |          |          | √        |
| 104| Hypothetical protein / LA_1124a          | 0        |          |          |          | √        |
| 105| Hypothetical protein /                  | 0        |          |          |          | √        |
| No. | Hypothetical protein / LA_1141a & | 0 | √ | 0 | 0 |
|-----|----------------------------------|---|----|---|---|
| 107 | Hypothetical protein / LA_1737 & | 0 | √ | 0 | 0 |
| 108 | Hypothetical protein / LA_3390 & | 0 | √ | 0 | 0 |
| 109 | Hypothetical protein / LA_3827 & | 0 | √ | 0 | 0 |
| 110 | Hypothetical protein / LA_4319 & | 0 | 0 | √ | 0 |
| 111 | Hypothetical protein / LA_0172 & | 0 | 0 | √ | 0 |
| 112 | Hypothetical protein / LA_1177 & | 0 | 0 | √ | 0 |
| 113 | Hypothetical protein / LA_3026 & | 0 | 0 | √ | 0 |
| 114 | Hypothetical protein / LA_3697 & | 0 | 0 | 0 | √ |
| 115 | Hypothetical protein / LA_0793 & | 0 | 0 | 0 | √ |

Δ: The 11 exoproteins (No.1-11) also presented in the CSPs of *L. interrogans* incubated in both EMJH medium at 28°C and RPMI-1640 medium at 37°C. ▲: The 9 exoproteins (No.12-20) also presented in the CSPs of *L. interrogans* incubated only in EMJH medium. *: The 25 exoproteins (No.21-45) also presented in the CSPs of *L. interrogans* incubated only in RPMI-1640 medium. #: The 25 exoproteins (No.46-70) also presented in the CSPs of *L. interrogans* during infection of HUVECs. &: The 45 exoproteins (No.71-115) only presented in the CSPs of *L. interrogans* during infection of THP-1 macrophages.
Table 6. Exoproteins of *L. interrogans* strain Lai during infection of HUVECs.
| No. | Exoproteins / Genes | Before infection | Time of infection (h) |
|-----|---------------------|-----------------|----------------------|
|     |                     |                 | 6        | 12        | 24        |
| 1   | Elongation factor Tu / LA_0737Δ | √               | √        | √        | √        |
| 2   | Molecular chaperone GroEL (HSP60) / LA_2655Δ | √               | √        | √        | √        |
| 3   | Outer membrane lypoprotein 32 (LipL32) / LA_2637Δ | √               | √        | √        | √        |
| 4   | Peroxiredoxin / LA_2809Δ | √               | √        | √        | √        |
| 5   | Cysteine synthase / LA_1719Δ | √               | √        | √        | √        |
| 6   | Endonuclease / LA_2250Δ | √               | √        | √        | √        |
| 7   | OmpA family lipoprotein 22 (Loa22) / LA_0222Δ | √               | 0        | √        | √        |
| 8   | Endoflagellar filament core protein / LA_2418Δ | √               | 0        | √        | √        |
| 9   | S-adenosylmethionine-dependentmethyltransferase / LA_0082Δ | √               | √        | 0        | 0        |
| 10  | ATP synthase subunit C / LA_2782Δ | √               | 0        | √        | 0        |
| 11  | DNA-directed RNA polymerase subunit beta / LA_3419Δ | √               | 0        | 0        | √        |
| 12  | Hypothetical protein / LA_3961Δ | √               | √        | √        | √        |
| 13  | Endoflagellar filament core protein / LA_2017▲ | √               | √        | √        | √        |
| 14  | Translaldolase / LA_2286▲ | √               | √        | √        | 0        |
| 15  | Alpha/beta hydrolase / LA_3147▲ | √               | √        | 0        | 0        |
| 16  | Glutamine synthetase / LA_1313▲ | √               | 0        | 0        | √        |
|   | Protein Name                                                                 |   |   |   |   |
|---|-----------------------------------------------------------------------------|---|---|---|---|
| 17| ATP synthase F0F1 subunit beta / LA_2776*                                  | ✓ | 0 | 0 | ✓ |
| 18| Sulfite reductase subunit beta / LA_4216*                                  | ✓ | 0 | 0 | ✓ |
| 19| Hypothetical protein / LA_1939*                                           | ✓ | 0 | 0 | ✓ |
| 20| Hypothetical protein / LA_3097*                                           | ✓ | 0 | 0 | ✓ |
| 21| Acetyl carrier protein / LB_083*                                          | ✓ | ✓ | ✓ | ✓ |
| 22| Methyltransferase / LA_0380*                                              | ✓ | ✓ | ✓ | 0 |
| 23| Co-chaperonin GroES / LA_2654*                                            | ✓ | 0 | ✓ | ✓ |
| 24| ABC transporter permease / LA_0970*                                       | ✓ | ✓ | 0 | 0 |
| 25| P-loop domain-containing protein / LA_2685*                                | ✓ | ✓ | 0 | 0 |
| 26| Cell wall hydrolase / LA_0312*                                            | ✓ | 0 | ✓ | 0 |
| 27| Transpeptidase/penicillin binding protein / LA_2755*                      | ✓ | 0 | ✓ | 0 |
| 28| Electron transfer flavoprotein subunit beta / LA_0412*                    | ✓ | 0 | 0 | ✓ |
| 29| 50S ribosomal protein L2 / LA_0742*                                      | ✓ | 0 | 0 | ✓ |
| 30| Succinyl-CoA synthetase subunit alpha / LA_1101*                          | ✓ | 0 | 0 | ✓ |
| 31| Histidine kinase sensor protein / LA_1348*                                | ✓ | 0 | 0 | ✓ |
| 32| Fructose-bisphosphatasealdolase / LA_1532*                                | ✓ | 0 | 0 | ✓ |
| 33| cAMP-binding protein / LA_3372*                                          | ✓ | 0 | 0 | ✓ |
| 34| Hypothetical protein / LA_2123*                                          | ✓ | ✓ | ✓ | 0 |
| 35| Hypothetical protein /                                                   | ✓ | 0 | ✓ | 0 |
|    | protein                        | La_1412* | La_1698* | La_3271* | La_1060* | La_2295* | LB_054#   | LB_055#   | La_0726# | La_2317# | La_0872# | LA_0948# | La_0327# | LA_1029# | LA_2105# | LA_2841# | Oxidoreductase / |
|----|--------------------------------|----------|----------|----------|----------|----------|-----------|-----------|----------|----------|----------|----------|----------|----------|----------|-----------|-----------------|
| 36 | Hypothetical protein           | √        | 0        | √        | 0        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 37 | Hypothetical protein           | √        | 0        | √        | 0        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 38 | Hypothetical protein           | √        | 0        | √        | 0        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 39 | Hypothetical protein           | √        | 0        | 0        | √        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 40 | Hypothetical protein           | √        | 0        | 0        | √        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 41 | von Willebrand factor type     | 0        | √        | √        | √        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 42 | von Willebrand factor type     | 0        | √        | √        | √        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 43 | Sulfatase                      | 0        | √        | √        | 0        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 44 | DNA helicase UvrD              | 0        | √        | √        | 0        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 45 | Microbial collagenase          | 0        | 0        | √        | √        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 46 | Zn-dependent peptidase         | 0        | 0        | √        | √        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 47 | Hemolysin A (TlyA)             | 0        | √        | 0        | 0        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 48 | Sphingomyelinase C precursor   | 0        | √        | 0        | 0        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 49 | Gamma-glutamyltranspeptidase   | 0        | √        | 0        | 0        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 50 | Two component system sensor    | 0        | √        | 0        | 0        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 51 | Citrate lyase                  | 0        | √        | 0        | 0        |         |           |           |          |          |          |          |          |          |          |          |                  |
| 52 | Oxidoreductase                 | 0        | √        | 0        | 0        |         |           |           |          |          |          |          |          |          |          |          |                  |
|   | Protein Description                                                                 | Line 1 | Line 2 | Line 3 | Line 4 | Line 5 |
|---|-------------------------------------------------------------------------------------|--------|--------|--------|--------|--------|
| 53 | HD family protein / LA_0866#                                                        | 0      | 0      | √      | 0      |
| 54 | ABC transporter ATP-binding protein / LA_0969#                                       | 0      | 0      | √      | 0      |
| 55 | FAD dependent oxidoreductase / LA_1578#                                             | 0      | 0      | √      | 0      |
| 56 | Exonuclease / LA_3974#                                                               | 0      | 0      | √      | 0      |
| 57 | Two-component hybrid sensor and regulator / LB_014#                                  | 0      | 0      | √      | 0      |
| 58 | Oxidoreductase FAD-binding family protein / LB_163#                                 | 0      | 0      | √      | 0      |
| 59 | Hypothetical protein / LA_1063#                                                      | 0      | √      | √      | 0      |
| 60 | Hypothetical protein / LA_4154#                                                      | 0      | √      | √      | 0      |
| 61 | Hypothetical protein / LA_1553#                                                      | 0      | √      | 0      | 0      |
| 62 | Hypothetical protein / LA_3612#                                                      | 0      | √      | 0      | 0      |
| 63 | Hypothetical protein / LA_3677#                                                      | 0      | √      | 0      | 0      |
| 64 | Hypothetical protein / LA_0089#                                                      | 0      | 0      | √      | 0      |
| 65 | Hypothetical protein / LA_1774#                                                      | 0      | 0      | √      | 0      |
| 66 | Zn-dependent peptidase (metalloprotease) / LA_3401#                                 | 0      | √      | √      | 0      |
| 67 | Alcohol dehydrogenase / LA_0477#                                                    | 0      | √      | 0      | 0      |
|   | Description                                                                                       | FC | Log2FC | p-value | q-value |
|---|-------------------------------------------------------------------------------------------------|----|--------|---------|---------|
| 68| Pyridoxal phosphate-dependent aminotransferase / LA_1595&                                      | 0  | √      | 0       | 0       |
| 69| Transcriptional regulator / LA_2253&                                                              | 0  | √      | 0       | 0       |
| 70| ISlin1 transposase / LA_3281&                                                                     | 0  | √      | 0       | 0       |
| 71| DNA repair protein Rad50 / LA_0293&                                                                | 0  | 0      | √       | 0       |
| 72| Short chain dehydrogenase / LA_0971&                                                               | 0  | 0      | √       | 0       |
| 73| Glucose-1-phosphate thymidylyltransferase / LA_1662&                                              | 0  | 0      | √       | 0       |
| 74| O-antigen ligase/O-antigen polymerase / LA_2089&                                                 | 0  | 0      | √       | 0       |
| 75| Endonuclease III / LA_2163&                                                                       | 0  | 0      | √       | 0       |
| 76| Zn-dependent protease / LA_2184&                                                                   | 0  | 0      | √       | 0       |
| 77| Aspartyl/glutamyl-tRNA amidotransferase subunit A / LA_2507&                                      | 0  | 0      | √       | 0       |
| 78| 50S ribosomal protein L7/L12 / LA_3421&                                                           | 0  | 0      | √       | 0       |
| 79| SAM-dependent methyltransferase / LA_3546&                                                         | 0  | 0      | √       | 0       |
| 80| Alpha/beta hydrolase / LA_3770&                                                                    | 0  | 0      | √       | 0       |
| 81| Galactokinase / LA_3885&                                                                          | 0  | 0      | √       | 0       |
| 82| Arginine/ornithine transport system ATPase / LB_275&                                              | 0  | 0      | √       | 0       |
| 83| Thiamine biosynthesis protein ThiC / LA_0980&                                                      | 0  | 0      | 0       | √       |
|   | Description                                                                 | M3 | M4 | M5 | M6 | Status |
|---|------------------------------------------------------------------------------|----|----|----|----|--------|
| 84| Glyceraldehyde 3-phosphate dehydrogenase / LA_1704&                           | 0  | 0  | 0  | 0  | √      |
| 85| S-adenosylmethionine synthetase / LA_2633&                                   | 0  | 0  | 0  | 0  | √      |
| 86| Transmembrane outer membrane protein L1 (OmpL1) / LA_3138&                   | 0  | 0  | 0  | 0  | √      |
| 87| Molybdopterinoxidoreductase iron-sulfur-binding subunit / LA_3267&            | 0  | 0  | 0  | 0  | √      |
| 88| Short-chain dehydrogenase / LA_4052&                                        | 0  | 0  | 0  | 0  | √      |
| 89| Fatty acid synthase subunit beta / LA_4159&                                 | 0  | 0  | 0  | 0  | √      |
| 90| Hypothetical protein / LA_2024&                                             | 0  | √  | √  | √  |        |
| 91| Hypothetical protein / LA_0135&                                             | 0  | √  | 0  | 0  |        |
| 92| Hypothetical protein / LA_2900&                                             | 0  | √  | 0  | 0  |        |
| 93| Hypothetical protein / LA_1402&                                             | 0  | 0  | √  | 0  |        |
| 94| Hypothetical protein / LA_1467&                                             | 0  | 0  | √  | 0  |        |
| 95| Hypothetical protein / LA_4237&                                             | 0  | 0  | √  | 0  |        |
| 96| Hypothetical protein / LA_1005&                                             | 0  | 0  | 0  | √  |        |
| 97| Hypothetical protein / LA_1137&                                             | 0  | 0  | 0  | √  |        |
| 98| Hypothetical protein / LA_1584&                                             | 0  | 0  | 0  | √  |        |
Δ: The 12 exoproteins (No.1-12) also presented in the CSPs of *L. interrogans* incubated in both EMJH medium at 28°C and RPMI-1640 medium at 37°C. ▲: The 8 exoproteins (No.13-20) also presented in the CSPs of *L. interrogans* incubated only in EMJH medium. *: The 20 exoproteins (No.21-40) also presented in the CSPs of *L. interrogans* incubated only in RPMI-1640 medium. #: The 25 exoproteins (No.41-65) also presented in the CSPs of *L. interrogans* during infection of THP-1 macrophages. &: The 33 exoproteins (No.65-98) only presented in the CSPs of *L. interrogans* during infection of HUVECs.

Table 7. Exoproteins of THP-1 macrophages during infection with *L. interrogans*.
| No. | Exoprotein / Protien ID                                      | Before infection | Time of infection (h) |
|-----|--------------------------------------------------------------|------------------|-----------------------|
|     |                                                              |                  | 6         | 12         | 24         |
| 1   | Coagulation factor X / P00742Δ                               | √                | √         | √          | √          |
| 2   | Complement C3 / P01024Δ                                     | √                | √         | √          | √          |
| 3   | Apolipoprotein C-III / P02656Δ                               | √                | √         | √          | √          |
| 4   | Fibronectin (FN) / P02751Δ                                  | √                | √         | √          | √          |
| 5   | Retinol binding protein 4 / Q5VY30Δ                          | √                | √         | √          | √          |
| 6   | Lactotransferrin / P02788Δ                                   | √                | √         | √          | √          |
| 7   | Heat shock protein HSP 90-alpha / P07900Δ                    | √                | √         | √          | √          |
| 8   | Heat shock protein HSP 90-beta / P08238Δ                     | √                | √         | √          | √          |
| 9   | Actin, cytoplasmic 2 / P63261Δ                               | √                | √         | √          | √          |
| 10  | Histone H4 / P62805Δ                                        | √                | √         | √          | √          |
| 11  | Ceruloplasmin / E9PFZ2Δ                                     | √                | √         | √          | 0          |
| 12  | Keratin, type II cytoskeletal 6B / P04259Δ                   | √                | √         | √          | 0          |
| 13  | Isoform 2 of cartilage oligomeric                           | √                | √         | √          | 0          |
| No. | Protein Name                                      | PDB ID   | Licenced | Future | DeepMind | Total |
|-----|--------------------------------------------------|----------|----------|--------|----------|-------|
| 14  | Bax inhibitor 1 / F8W1V3Δ                         | √        | √        | √      | 0        | 0     |
| 15  | Actin, alpha skeletal muscle / P68133Δ            | √        | √        | √      | 0        | 0     |
| 16  | Desmoglein-1 / Q02413Δ                           | √        | √        | √      | 0        | 0     |
| 17  | Peroxiredoxin-1 / A0A0A0MSI0 Δ                   | √        | √        | √      | 0        | 0     |
| 18  | NAD(P)(+)-arginine ADP-ribosyltransferase / H7C2G2Δ | √        | √        | √      | 0        | 0     |
| 19  | Testis-specific Y-encoded-like protein 2 / Q9H2G4Δ | √        | √        | √      | 0        | 0     |
| 20  | HCG1745306, isoform CRA_a / G3V1N2Δ               | √        | √        | √      | 0        | 0     |
| 21  | L-lactate dehydrogenase A chain / P00338▲        | √        | √        | √      | √        |       |
| 22  | Thioredoxin / P10599▲                            | √        | √        | √      | √        |       |
| 23  | Elongation factor 2 / P13639▲                    | √        | √        | √      | √        |       |
| 24  | Transthyretin / A0A087WV45 ▲                      | √        | √        |        | 0        | 0     |
| 25  | 14-3-3 protein zeta/delta /                       | √        | √        |        | 0        | 0     |
|   | Protein Name                                      | ID          | 26   | 27   | 28   | 29   | 30   | 31   | 32   | 33   | 34   | 35   | 36   | 37   | 38   | 39   |
|---|--------------------------------------------------|-------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 26| Protein S100                                     | R4GN98      | √    |      |      |      |      |      |      |      |      |      |      |      |      |      |
| 27| Dermcidin / P81605*                              |             |      | √    |      |      |      |      |      |      |      |      |      |      |      |      |
| 28| Calmodulin-like protein 5 / Q9NZT1*              |             |      |      | √    |      |      |      |      |      |      |      |      |      |      |      |
| 29| Collagen alpha-1(COL-VI) chain / A0A087X0S5*     |             |      |      |      | √    |      |      |      |      |      |      |      |      |      |      |
| 30| Fructose-bisphosphate aldolase A/ P04075*        |             |      |      |      |      | √    |      |      |      |      |      |      |      |      |      |
| 31| Isoform 2 of tubulin alpha-1B chain / P68363*    |             |      |      |      |      |      | √    |      |      |      |      |      |      |      |      |
| 32| Calmodulin-1 / G3V226#                          |             |      |      |      |      |      |      | √    |      |      |      |      |      |      |      |
| 33| Cartilage oligomeric matrix protein / P49747#    |             |      |      |      |      |      |      |      | √    |      |      |      |      |      |      |
| 34| Alpha-2-HS-glycoprotein / P02765#                |             |      |      |      |      |      |      |      |      | √    |      |      |      |      |      |
| 35| Tетranectin / E9PHK0#                           |             |      |      |      |      |      |      |      |      |      | √    |      |      |      |      |
| 36| Gelsolin / A0A0A0MS51 #                          |             |      |      |      |      |      |      |      |      |      |      | √    |      |      |      |
| 37| Thrombospondin-1 / P07996#                       |             |      |      |      |      |      |      |      |      |      |      |      | √    |      |      |
| 38| Prothrombin / E9PIT3#                            |             |      |      |      |      |      |      |      |      |      |      |      |      | √    |      |
| 39| Hemoglobin subunit alpha                          |             |      |      |      |      |      |      |      |      |      |      |      |      |      | √    |      |
|   | Protein Name                                                                 | Score 1 | Score 2 | Score 3 | Score 4 | Match |
|---|-----------------------------------------------------------------------------|---------|---------|---------|---------|-------|
| 40| Alpha-2-macroglobulin / P01023#                                             | 0       | 0       | 0       | 0       | √     |
| 41| Inter-alpha-trypsin inhibitor heavy chain H3 / A0A087WW43#                  | 0       | 0       | 0       | 0       | √     |
| 42| Fibulin-1 / P23142#                                                         | 0       | 0       | 0       | 0       | √     |
| 43| Beta-2-glycoprotein 1 / J3KS17#                                             | 0       | 0       | 0       | 0       | √     |
| 44| Coagulation factor V / P12259#                                              | 0       | 0       | 0       | 0       | √     |
| 45| L-lactate dehydrogenase B chain / P07195#                                   | 0       | 0       | 0       | 0       | √     |
| 46| Thrombospondin-4 / E7ES19#                                                  | 0       | 0       | 0       | 0       | √     |
| 47| Vitamin D-binding protein / P02774#                                         | 0       | 0       | 0       | 0       | √     |
| 48| Inter-alpha-trypsin inhibitor heavy chain H1 / H7C0N0#                      | 0       | 0       | 0       | 0       | √     |
| 49| Histone H2B type 1-K / O60814#                                              | 0       | 0       | 0       | 0       | √     |
| 50| Hemoglobin subunit delta / E9PFT6#                                          | 0       | 0       | 0       | 0       | √     |
| 51| Plasminogen                                                                | 0       | 0       | 0       | 0       | √     |
| 52 | Hepatocyte growth factor activator / Q04756# | 0 | 0 | 0 | √ |
| 53 | Alpha-amylase 1 / P04745# | 0 | 0 | 0 | √ |
| 54 | Pigment epithelium-derived factor / P36955# | 0 | 0 | 0 | √ |
| 55 | Complement C4-B / A0A0G2JL54 # | 0 | 0 | 0 | √ |
| 56 | Complement component C8 beta chain / F5GY80# | 0 | 0 | 0 | √ |
| 57 | Retina-specific copper amine oxidase / Q75106# | 0 | 0 | 0 | √ |
| 58 | HPX protein / Q9BS19# | 0 | 0 | 0 | √ |
| 59 | Vitronectin / P04004# | 0 | 0 | 0 | √ |
| 60 | Antithrombin-III / Q8TCE1# | 0 | 0 | 0 | √ |
| 61 | Apolipoprotein B-100 / P04114# | 0 | 0 | 0 | √ |
| 62 | Complement C5 / P01031# | 0 | 0 | 0 | √ |
| 63 | Inter-alpha-trypsin inhibitor heavy chain H4 / Q14624# | 0 | 0 | 0 | √ |
| 64 | Elongation | 0 | 0 | 0 | √ |
|   | Description | Accession | Annotation | Dates | Protein names |
|---|-------------|-----------|------------|-------|---------------|
| 65 | Collagen alpha-1 (COL-III) chain / P02461# | 0 0 0 0 | √ | | |
| 66 | Superoxide dismutase [Cu-Zn] / H7BYH4# | 0 0 0 0 | √ | | |
| 67 | Inter-alpha-trypsin inhibitor heavy chain H2 / Q5T985# | 0 0 0 0 | √ | | |
| 68 | Complement component C7 / P10643# | 0 0 0 0 | √ | | |
| 69 | 10 kDa heat shock protein, mitochondrial / B8ZZ54# | 0 0 0 0 | √ | | |
| 70 | Interleukin-1 receptor accessory protein / C9J1D9# | 0 0 0 0 | √ | | |
| 71 | Pregnancy zone protein / P20742# | 0 0 0 0 | √ | | |
| 72 | Tumor necrosis factor-alpha (TNF-α) / P01375& | 0 0 | √ | √ | |
| 73 | Immunoglobulin kappa constant / P01834& | 0 | √ | 0 0 | |
| 74 | Immunoglobulin heavy constant alpha 1 / P01876& | 0 | √ | 0 0 | |
|   | Protein Name                          | Score | Present | Rank |
|---|--------------------------------------|-------|---------|------|
| 75| Cleavage stimulation factor subunit 3 / Q12996& | 0     | √       | 0    | 0    |
| 76| Mucin-5B / Q9HC84&                    | 0     | √       | 0    | 0    |
| 77| Heparin cofactor 2 / P05546&          | 0     | 0       | 0    | √    |
| 78| Apolipoprotein E / P02649&            | 0     | 0       | 0    | √    |
| 79| Nucleophosmin / P06748&               | 0     | 0       | 0    | √    |
| 80| Heat shock 70 kDa protein 1A / V9GZ37&| 0     | 0       | 0    | √    |
| 81| Stress-70 protein, mitochondrial / P38646& | 0     | 0       | 0    | √    |
| 82| Pyruvate kinase PKM / H3BN34&         | 0     | 0       | 0    | √    |
| 83| Extracellular matrix protein 1 (ECM-P1) / Q16610& | 0     | 0       | 0    | √    |
| 84| Hepatocyte growth factor-like protein / P26927& | 0     | 0       | 0    | √    |
| 85| Haptoglobin / H3BS21&                 | 0     | 0       | 0    | √    |
| 86| Interleukin-6 (IL-6) / P05231&         | 0     | 0       | 0    | √    |
| 87| Neural cell adhesion molecule 1 / H7BYX6& | 0     | 0       | 0    | √    |
| 88| Coagulation                          | 0     | 0       | 0    | √    |
| No. | Exoprotein Description | Gene ID | THP-1 | HUVECs | Infection |
|-----|-----------------------|---------|-------|--------|-----------|
| 89  | CD44 antigen (Fragment) | H0YDX6 & | 0     | 0      | 0         | √         |
| 90  | Plastin-2 OS           | P13796 & | 0     | 0      | 0         | √         |
| 91  | Apolipoprotein A-I     | F8W696 & | 0     | 0      | 0         | √         |
| 92  | Fibrinogen beta chain  | D6REL8 & | 0     | 0      | 0         | √         |
| 93  | Moesin                 | P26038 & | 0     | 0      | 0         | √         |
| 94  | Toll-like receptor 4 (TLR4) | O00206 & | 0     | 0      | 0         | √         |
| 95  | Kininogen-1            | P01042 & | 0     | 0      | 0         | √         |

Δ: The 20 exoproteins (No.1-20) also presented in the CSPs of both THP-1 macrophages and HUVECs incubated in RPMI-1640 medium. ▲: The 6 exoproteins (No.21-26) also presented only in the CSPs of THP-1 macrophages incubated in RPMI-1640 medium. *: The 5 exoproteins (No.27-31) also presented only in the CSPs of HUVECs incubated in RPMI-1640 medium. #: The 40 exoproteins (No.32-71) also presented in the CSPs of HUVECs during infection. &: The 24 exoproteins (No.72-95) only presented in the CSPs of THP-1 macrophages during infection.

Table 8. Exoproteins of HUVECs during infection with *L. interrogans*. 

| No. | Exoprotein / Protein ID | Before infection | Time of infection (h) |
|-----|------------------------|------------------|----------------------|
|     |                        |                  | 6   | 12  | 24  | 24 |
| 1   | Coagulation factor X / P00742$^\Delta$ | √    | √   | √   | √   | √   |
| 2   | Complement C3 / P01024$^\Delta$ | √    | √   | √   | √   | √   |
| 3   | Apolipoprotein C-III / P02656$^\Delta$ | √    | √   | √   | √   | √   |
| 4   | Fibronectin / P02751$^\Delta$ | √    | √   | √   | √   | √   |
| 5   | Heat shock protein HSP 90-alpha / P07900$^\Delta$ | √    | √   | √   | √   | √   |
| 6   | Heat shock protein HSP 90-beta / P08238$^\Delta$ | √    | √   | √   | √   | √   |
| 7   | Actin, cytoplasmic 2 / P63261$^\Delta$ | √    | √   | √   | √   | √   |
| 8   | Histone H4 / P62805$^\Delta$ | √    | √   | √   | √   | √   |
| 9   | Peroxiredoxin -1 / A0A0A0MS10$^\Delta$ | √    | √   | √   | √   | √   |
| 10  | Testis-specific Y-encoded-like protein 2 / Q9H2G4$^\Delta$ | √    | √   | √   | √   | √   |
| 11  | Lactotransferrin / E7EQB2$^\Delta$ | √    | √   | √   | √   | √   |
| 12  | Keratin, type II cytoskeletal 6B / P04259$^\Delta$ | √    | √   | √   | 0   | 0   |
| 13  | Isoform 2 of cartilage | √    | √   | √   | 0   | 0   |
| Oligomeric matrix protein / P49747Δ |
|-----------------------------------|
| Bax inhibitor 1 / F8W1V3Δ         |
| √   | √   | √   | 0   |
| Actin, alpha skeletal muscle / P68133Δ |
| √   | √   | √   | 0   |
| NAD(P)(+)-arginine ADP-ribosyltransferase / H7C2G2Δ |
| √   | √   | √   | 0   |
| HCG1745306, isoform CRA_a / G3V1N2Δ |
| √   | √   | √   | 0   |
| Isoform 2 of Rho guanine nucleotide exchange factor 5 / Q12774Δ |
| √   | √   | √   | 0   |
| Ceruloplasmin / E9PFZ2Δ          |
| √   | 0   | √   | 0   |
| Desmoglein-1 / Q02413Δ           |
| √   | 0   | √   | 0   |
| Retinol binding protein 4 / Q5VY30Δ |
| √   | 0   | 0   | √   |
| Dermcidin / P81605Δ              |
| √   | √   | √   | √   |
| Calmodulin-like protein 5 / Q9NZT1Δ |
| √   | √   | √   | 0   |
| Fructose-bisphosphate aldolase A / P04075Δ |
| √   | √   | 0   | 0   |
| Collagen alpha-1(COL-VI) chain / |
| √   | √   | 0   | 0   |
|  | A0A087X0S5 ▲ | 26 | Protein disulfide-isomerase / I3NI03 ▲ | √ | 0 | √ | 0 |
|---|---|---|---|---|---|---|---|
| 27 | Isoform 2 of tubulin alpha-1B chain / P68363 ▲ | √ | 0 | 0 | √ |
| 28 | L-lactate dehydrogenase A chain / P00338* | 0 | √ | √ | √ |
| 29 | Thioredoxin / P10599* | 0 | √ | √ | √ |
| 30 | Elongation factor 2 / P13639* | 0 | √ | √ | √ |
| 31 | 14-3-3 protein zeta/delta / P63104* | 0 | √ | √ | √ |
| 32 | Transthyretin / A0A087WV45 * | 0 | √ | √ | 0 |
| 33 | Protein S100 / R4GN98* | 0 | √ | √ | 0 |
| 34 | Calmodulin-1 / G3V226# | 0 | √ | √ | 0 |
| 35 | Cartilage oligomeric matrix protein / P49747# | 0 | 0 | 0 | √ |
| 36 | Alpha-2-HS-glycoprotein / P02765# | 0 | 0 | 0 | √ |
| 37 | Tetranectin / E9PHK0# | 0 | 0 | 0 | √ |
| 38 | Gelsolin / A0A0A0MS51 # | 0 | 0 | 0 | √ |
| 39 | Thrombospo | 0 | 0 | 0 | √ |
| # | Protein Name / Gene ID | Score | Mismatch | Coverage | Valid |
|---|-----------------------|-------|----------|----------|-------|
| 40 | Prothrombin / E9PIT3# | 0     | 0        | 0        | √     |
| 41 | Hemoglobin subunit alpha / P69905# | 0 | 0        | 0        | √     |
| 42 | Alpha-2-macroglobulin / P01023# | 0 | 0        | 0        | √     |
| 43 | Inter-alpha-trypsin inhibitor heavy chain H3 / A0A087WW43# | 0 | 0        | 0        | √     |
| 44 | Fibulin-1 / P23142# | 0     | 0        | 0        | √     |
| 45 | Beta-2-glycoprotein 1 / J3KS17# | 0 | 0        | 0        | √     |
| 46 | Coagulation factor V / P12259# | 0 | 0        | 0        | √     |
| 47 | L-lactate dehydrogenase B chain / P07195# | 0 | 0        | 0        | √     |
| 48 | Thrombospondin-4 / E7ES19# | 0 | 0        | 0        | √     |
| 49 | Vitamin D-binding protein / P02774# | 0 | 0        | 0        | √     |
| 50 | Inter-alpha-trypsin inhibitor heavy chain H1 / P19827# | 0 | 0        | 0        | √     |
| 51 | Histone H2B type 1-K / | 0 | 0        | 0        | √     |
| #  | Protein Name                                                                 | Accession | Score | False Discovery Rate | Significant?  |
|----|------------------------------------------------------------------------------|-----------|-------|----------------------|-------------|
| 52 | Hemoglobin subunit delta / E9PFT6#                                            | 0         | 0     | 0                    | ✓           |
| 53 | Plasminogen / P00747#                                                        | 0         | 0     | 0                    | ✓           |
| 54 | Hepatocyte growth factor activator / Q04756#                                 | 0         | 0     | 0                    | ✓           |
| 55 | Alpha-amylase 1 / P04745#                                                    | 0         | 0     | 0                    | ✓           |
| 56 | Pigment epithelium-derived factor / I3L4F9#                                  | 0         | 0     | 0                    | ✓           |
| 57 | Complement C4-B / A0A0G2JL54 #                                               | 0         | 0     | 0                    | ✓           |
| 58 | Complement component C8 beta chain / F5GY80#                                 | 0         | 0     | 0                    | ✓           |
| 59 | Retina-specific copper amine oxidase / O75106#                               | 0         | 0     | 0                    | ✓           |
| 10 | HPX protein / Q9BS19#                                                        | 0         | 0     | 0                    | ✓           |
| 61 | Vitronectin / P04004#                                                        | 0         | 0     | 0                    | ✓           |
| 62 | Antithrombin-III / P01008#                                                   | 0         | 0     | 0                    | ✓           |
| 63 | Apolipoprotein B-100 / P04114#                                               | 0         | 0     | 0                    | ✓           |
| 64 | Complement C5 / P01031*                                                      | 0         | 0     | 0                    | ✓           |
|   | Protein Name                                                                 | Score 1 | Score 2 | Score 3 | Score 4 | Score 5 |
|---|------------------------------------------------------------------------------|---------|---------|---------|---------|---------|
| 65| Inter-alpha-trypsin inhibitor heavy chain H4 / Q14624#                      | 0       | 0       | 0       |         | ✓       |
| 66| Elongation factor Tu, mitochondrial / H3BNU3#                               | 0       | 0       | 0       |         | ✓       |
| 67| Collagen alpha-1 (COL-III) chain / P02461#                                 | 0       | 0       | 0       |         | ✓       |
| 68| Superoxide dismutase [Cu-Zn] / H7BYH4#                                     | 0       | 0       | 0       |         | ✓       |
| 69| Inter-alpha-trypsin inhibitor heavy chain H2 / Q5T985#                      | 0       | 0       | 0       |         | ✓       |
| 70| Complement component C7 / P10643#                                          | 0       | 0       | 0       |         | ✓       |
| 71| 10 kDa heat shock protein, mitochondrial / B8ZZ54#                          | 0       | 0       | 0       |         | ✓       |
| 72| Interleukin-1 receptor accessory protein / C9J1D9#                          | 0       | 0       | 0       |         | ✓       |
| 73| Pregnancy zone protein / P20742#                                           | 0       | 0       | 0       |         | ✓       |
| 74| Metalloprotease inhibitor 1 / Q5H9B4#                                      | 0       | ✓       | ✓       | ✓       |         |
| 75| Triosephosphate isomerase / U3KQF3#                                        | 0       | ✓       | ✓       | ✓       |         |
| 76| 78 kDa glucose-                                                            | 0       | 0       | ✓       | ✓       |         |
|    | Protein Name | P11021 & | P18206 & | H0YJ11 & | B4DLR8 & | P60709 & | P06733 & | B4DNK4 & | E9PKE3 & | P31947 & | P62258 & |
|----|--------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|
| 77 | Vinculin /   | 0        | 0        | √        | √        |          |          |          |          |          |          |
|    | P11021       |          |          |          |          |          |          |          |          |          |          |
| 78 | Alpha-actinin-1 / H0YJ11 & | 0        | 0        | √        |          |          |          |          |          |          |          |
| 79 | NAD(P)H dehydrogenase [quinone] / B4DLR8 & | 0        | 0        | √        |          |          |          |          |          |          |          |
| 80 | Cytoplasmic 1 / P60709 & | 0        | 0        |          |          | 0        |          |          |          |          |          |
| 81 | Alpha-enolase / P06733 & | 0        | 0        | 0        |          |          |          |          |          |          |          |
| 82 | Pyruvate kinase / B4DNK4 & | 0        | 0        | 0        |          |          |          |          |          |          |          |
| 83 | Heat shock cognate 71 kDa protein / E9PKE3 & | 0        | 0        | 0        |          |          |          |          |          |          |          |
| 84 | Vimentin / B0YJC5 & | 0        | 0        | 0        |          |          |          |          |          |          |          |
| 85 | GTP-binding nuclear protein Ran / P62826 & | 0        | 0        | 0        |          |          |          |          |          |          |          |
| 86 | Galectin-1 / P09382 & | 0        | 0        | 0        |          |          |          |          |          |          |          |
| 87 | Urokinase-type plasminogen activator / E7ET40 & | 0        | 0        | 0        |          |          |          |          |          |          |          |
| 88 | 14-3-3 protein sigma / P31947 & | 0        | 0        | 0        |          |          |          |          |          |          |          |
| 89 | 14-3-3 protein epsilon / P62258 & | 0        | 0        | 0        |          |          |          |          |          |          |          |
|   | Protein Name | PDB ID | Ref1 | Ref2 | Ref3 | Ref4 |
|---|-------------|--------|------|------|------|------|
| 90 | Prelamin-A/C | Q3BDU5 & | 0    | 0    | 0    | ✓    |
| 91 | Peroxiredoxin-2 | P32119 & | 0    | 0    | 0    | ✓    |
| 92 | Profilin-1 | P07737 & | 0    | 0    | 0    | ✓    |
| 93 | Tubulin beta chain | Q5JP53 & | 0    | 0    | 0    | ✓    |
| 94 | Stanniocalcin-2 | H0YB13 & | 0    | 0    | 0    | ✓    |
| 95 | Peptidyl-prolyl cis-trans isomerase A | P62937 & | 0    | 0    | 0    | ✓    |
| 96 | Insulin-like growth factor-binding protein 7 | Q16270 & | 0    | 0    | 0    | ✓    |
| 97 | Pentraxin-related protein PTX3 | P26022 & | 0    | 0    | 0    | ✓    |
| 98 | Phosphoglycerate mutase 2 | P15259 & | 0    | 0    | 0    | ✓    |
| 99 | 14-3-3 protein beta/alpha | P31946 & | 0    | 0    | 0    | ✓    |
| 100 | Alpha-2-antiplasmin | A0A0J9YY65 & | 0    | 0    | 0    | ✓    |
| 101 | Alpha-fetoprotein | P02771 & | 0    | 0    | 0    | ✓    |
| 102 | Nucleolin | P19338 & | 0    | 0    | 0    | ✓    |
| 103 | Hemoglobin subunit | | 0    | 0    | 0    | ✓    |
| No. | Protein Name                                                                 | Value | TS  | GC  | FDKT |
|-----|------------------------------------------------------------------------------|-------|-----|-----|------|
| 104 | Haptoglobin-related protein / J3KTC3&                                        | 0     | 0   | 0   | √    |
| 105 | Serotransferrin / C9JVG0&                                                     | 0     | 0   | 0   | √    |
| 106 | Activated RNA polymerase II transcriptional coactivator p15 / P53999&         | 0     | 0   | 0   | √    |
| 107 | Hemoglobin subunit delta / E9PFT6&                                           | 0     | 0   | 0   | √    |
| 108 | SH3 domain-binding glutamic acid-rich-like protein 3 / Q5T123&                | 0     | 0   | 0   | √    |
| 109 | Complement C1s subcomponent / B5MCV4&                                        | 0     | 0   | 0   | √    |
| 110 | Filamin-A / F8WE98&                                                          | 0     | 0   | 0   | √    |
| 111 | Fibrinogen gamma chain / C9JEU5&                                             | 0     | 0   | 0   | √    |
| 112 | Metalloproteinase inhibitor 2 / B4DFW2&                                      | 0     | 0   | 0   | √    |
| 113 | Heterogeneous nuclear ribonucleoproteins A2/B1 / A0A087WUI2 &                | 0     | 0   | 0   | √    |
| 114 | Endoplasmin / P14625&                                                        | 0     | 0   | 0   | √    |
| 115 | 40S ribosomal protein SA / F8WD59 & | 0 | 0 | 0 | √ |
| 116 | 60 kDa heat shock protein, mitochondrial / P10809 & | 0 | 0 | 0 | √ |
| 117 | Protein S100-A11 / P31949 & | 0 | 0 | 0 | √ |
| 118 | Tubulin alpha-1C chain / A0A1W2PQM 2 & | 0 | 0 | 0 | √ |
| 119 | Elongation factor 1-alpha 1 / A6PW80 & | 0 | 0 | 0 | √ |
| 120 | Adenylyl cyclase-associated protein 1 / Q5T0R7 & | 0 | 0 | 0 | √ |
| 121 | Metallothionein-2 / P02795 & | 0 | 0 | 0 | √ |
| 122 | Eukaryotic initiation factor 4A-I / J3KS93 & | 0 | 0 | 0 | √ |
| 123 | Pantetheinase / O95497 & | 0 | 0 | 0 | √ |
| 124 | Cathepsin Z / Q9UBR2 & | 0 | 0 | 0 | √ |
| 125 | Complement component C9 / P02748 & | 0 | 0 | 0 | √ |
| 126 | Chloride intracellular channel protein 1 / O00299 & | 0 | 0 | 0 | √ |
| 127 | Phosphoglyc | 0 | 0 | 0 | √ |
| No. | Protein Name                                                                 | Accession No. | Score 1 | Score 2 | Score 3 | Score 4 | Validity |
|-----|------------------------------------------------------------------------------|---------------|---------|---------|---------|---------|----------|
| 128 | Adenosylhomocysteinase / P23526                                            |               | 0       | 0       | 0       | √       |
| 129 | EGF-containing fibulin-like extracellular matrix protein 1 / Q12805          |               | 0       | 0       | 0       | √       |
| 130 | Epididymal secretory protein E1 / G3V2V8                                    |               | 0       | 0       | 0       | √       |
| 131 | Protein-glutamine gamma-glutamyltransferase 2 / P21980                       |               | 0       | 0       | 0       | √       |
| 132 | Lumican / P51884                                                            |               | 0       | 0       | 0       | √       |
| 133 | Plasma protease C1 inhibitor / H9KV48                                       |               | 0       | 0       | 0       | √       |
| 134 | Tropomyosin alpha-1 chain / H0YL80                                         |               | 0       | 0       | 0       | √       |
| 135 | Cofilin-1 / E9PLJ3                                                          |               | 0       | 0       | 0       | √       |
| 136 | Putative heat shock 70 kDa protein 7 / P48741                               |               | 0       | 0       | 0       | √       |
| 137 | Protein disulfideisomerase A4 / P13667                                      |               | 0       | 0       | 0       | √       |
| 138 | Amyloid-beta A4 protein / H7C0V9                                            |               | 0       | 0       | 0       | √       |
| 139 | Macrophage                                                                  |               | 0       | 0       | 0       | √       |
| ID  | Description                                                                 | Eukaryotic translation initiation factor 5A | MIF / P14174 | von Willebrand factor / P04275 | Protein CYR61 / O00622 | ADP-ribosylation factor 3 / P61204 | Calreticulin / P27797 | Peptidylprolyl isomerase / Q1JUQ5 | 14-3-3 protein gamma / P61981 | Aspartate aminotransferase, mitochondrial / P00505 | Acyl-CoA-binding protein / P07108 | Alpha-actinin-4 / O43707 | Chromobox protein homolog 2 / Q14781 | Insulin-like |
|-----|----------------------------------------------------------------------------|-------------------------------------------|-------------|--------------------------------|------------------------|--------------------------------|-----------------------|--------------------------------|-----------------------|--------------------------------|------------------------|-------------------|------------------------|-------------|
| 140 | Eukaryotic translation initiation factor 5A / F8WCJ1                        | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 141 | von Willebrand factor / P04275                                              | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 142 | Protein CYR61 / O00622                                                      | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 143 | ADP-ribosylation factor 3 / P61204                                           | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 144 | Calreticulin / P27797                                                       | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 145 | Peptidylprolyl isomerase / Q1JUQ5                                          | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 146 | 14-3-3 protein gamma / P61981                                               | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 147 | Aspartate aminotransferase, mitochondrial / P00505                           | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 148 | Acyl-CoA-binding protein / P07108                                            | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 149 | Alpha-actinin-4 / O43707                                                    | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 150 | Chromobox protein homolog 2 / Q14781                                         | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 151 | Insulin-like                                                                | 0                                         | 0           | 0                               | 0                      | 0                               | 0                     | 0                               | 0                      | 0                               | 0                      | 0                               | 0                       | ✓                  |
| 152 | Laminin (LN) subunit alpha-5 / O15230\(^a\) | 0 | 0 | 0 | √ |
| 153 | Myosin regulatory light chain 12A / P19105\(^a\) | 0 | 0 | 0 | √ |
| 154 | Myosin-9 / P35579\(^a\) | 0 | 0 | 0 | √ |
| 155 | Nucleoside diphosphate kinase A / P15531\(^a\) | 0 | 0 | 0 | √ |
| 156 | Monocyte chemoattractant protein-1 (MCP-1) / Q6UZ82\(^a\) | 0 | 0 | 0 | √ |
| 157 | Protein-L-isoaspartate(D-aspartate) O-methyltransferase / P22061\(^a\) | 0 | 0 | 0 | √ |
| 158 | Thyroxine-binding globulin / P05543\(^a\) | 0 | 0 | 0 | √ |
| 159 | Ankyrin repeat domain-containing protein 53 / C9JZ61\(^a\) | 0 | 0 | 0 | √ |
| 160 | Solute carrier organic anion transporter family member / F5H8K0\(^a\) | 0 | 0 | 0 | √ |
|   |   |   |   |   |
|---|---|---|---|---|
| 161 | Sorting nexin-33 / H3BPR3 & | 0 | 0 | 0 | √ |
| 162 | Complement factor I / A0A087X0I2 & | 0 | 0 | 0 | √ |
| 163 | Granulocyte-macrophage colony stimulating factor (GM-CSF) / P15509 & | 0 | 0 | 0 | √ |

Δ: The 21 exoproteins (No.1-21) also presented in the CSPs of both THP-1 macrophages and HUVECs incubated in RPMI-1640 medium. ▲: The 6 exoproteins (No.22-27) also presented only in the CSPs of HUVECs incubated in RPMI-1640 medium. *: The 6 exoproteins (No.28-33) also presented only in the CSPs of THP-1 macrophages incubated in RPMI-1640 medium. #: The 40 exoproteins (No.34-73) also presented in the CSPs of THP-1 macrophages during infection. &: The 90 exoproteins (No.74-163) only presented in the CSPs of HUVECs during infection.

**Figures**
CSPs and exoproteins of *L. interrogans* and different host cells before infection. (A). Differentiation of human THP-1 monocytes into CD68+ macrophages after PMA treatment, determined by flow cytometry. (B). CSPs from cultures of *L. interrogans* and cells incubated in different media for 24 h, examined by SDS-PAGE. Lane M: protein marker. Lane 1 or 2: CSPs from *L. interrogans* strain Lai in EMJH medium at 28°C for 7 d or in RPMI-1640 medium at 37°C for 24 h. Lane 3 or 4: CSPs from THP-1 macrophages or HUVECs in RPMI-1640 medium at 37°C for 24 h. (C). Classification of the exoproteins released from *L. interrogans* strain Lai incubated in EMJH medium at 28°C or RPMI-1640 medium at 37°C and from THP-1 macrophages and HUVECs in RPMI-1640 medium for 24 h, determined by MicroScope software.
Increased exoproteins from L. interrogans during infection of host cells. (A). CSPs from co-cultures of L. interrogans and different host cells for the indicated infection times, examined by SDS-PAGE. Lane M: protein marker. Lanes 1-3: total CSPs from co-cultures of L. interrogans strain Lai and THP-1 macrophages for 6, 12 or 24 h. Lanes 4-6: total CSPs from co-cultures of L. interrogans strain Lai and HUVECs for 6, 12 or 24 h. (B). Heatmaps and clusters of exoproteins from L. interrogans strain Lai during infection of THP-1 macrophages and HUVECs for the indicated times, determined by Complex-Heatmap package and R software. The T6, T12 and T24 or H6, H12 and H24 indicate the exoproteins from the spirochetes during infection of THP-1 macrophages (T) or HUVECs (H) for 6, 12 and 24 h, respectively. The control indicates the exoproteins from the spirochetes incubated in EMJH medium at 28°C for 24 h.
(before infection). (C). Categories of exoproteins from L. interrogans strain Lai during infection of THP-1 macrophages and HUVECs, determined by MicroScope software.
Secretion of exoproteins from host cells during infection with L. interrogans. (A). Heatmaps and clusters of exoproteins from THP-1 macrophages and HUVECs during infection with L. interrogans strain Lai for the indicated times, determined by Complex-Heatmap package and R software. The T6, T12 or T24 indicate the cellular exoproteins during infection for 6, 12 or 24 h. The control indicates the cellular exoproteins for a 24-h incubation in RPMI-1640 medium (before infection). (B) Heatmaps and clusters of exoproteins from HUVECs during infection with L. interrogans strain Lai for the indicated times, determined by Complex-Heatmap package and R software. The legends are similar to that shown in Figure 3A. (C). Categories of exoproteins from THP-1 macrophages and HUVECs during infection with L. interrogans strain Lai, determined by GenoScope software.
Figure 4

Functional classes of leptospiral exoproteins released before and during infection. (A). Function of the exoproteins from L. interrogans strain Lai incubated in EMJH medium at 28°C, determined by GO and KEGG pathway analysis. (B). Function of the exoproteins from L. interrogans strain Lai incubated in RPMI-1640 medium at 37°C, determined by GO and KEGG pathway analysis. (C). Function of the exoproteins from L. interrogans strain Lai during infection of THP-1 macrophages, determined by GO and KEGG pathway analysis. (D). Function of the exoproteins from L. interrogans strain Lai during infection of HUVECs, determined by GO and KEGG pathway analysis.
Figure 5

Functional classes of cellular exoproteins before and during infection. (A). Function of exoproteins from THP-1 macrophages incubated in RPMI-1640 medium, determined by GO and KEGG pathway analysis. (B). Function of exoproteins from HUVECs incubated in RPMI-1640 medium, determined by GO and KEGG pathway analysis. (C). Function of exoproteins from THP-1 macrophages during infection with L. interrogans strain Lai, determined by GO and KEGG pathway analysis. (D). Function of exoproteins from HUVECs during infection with L. interrogans strain Lai, determined by GO and KEGG pathway analysis.
Viability of L. interrogans and host cells during infection. (A). Viability of L. interrogans strain Lai during infection of cells for the indicated times, detected by confocal microscopy. The green or red leptospires indicate living or dead leptospires. (B). Statistical summary of green fluorescence intensity values reflecting living L. interrogans during infection of cells. Statistical data from experiments such as shown in A. Bars show the means ± SD of three independent experiments. The green fluorescence intensity (FI) values of L. interrogans before infection were set as 1.0. *: p<0.05 vs the green FI values of the spirochetes before infection. (C). Viability of THP-1 macrophages or HUVECs during infection with L. interrogans for the indicated times, examined by MTT test. Bars show the means ± SD of three independent experiments. *: p<0.05 vs the viability of the cells before infection.