Proteomic identification of endothelial cell surface proteins isolated from the hepatic portal vein of mice infected with *Schistosoma bovis*.

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Abstract

Schistosomes are blood parasites adapted to their intravascular habitat that have evolved mechanisms to evade the immune and haemostatic responses of their hosts. It has been observed that the schistosome can regulate the endothelium function along the infection, which contributes to modulation of host defensive responses and parasite survival. The purpose of this work was the analysis of the changes induced by *S. bovis* adult worms in the proteome expressed by infected mice on the endothelial surface of their portal vein. With this aim, we have utilized a methodology that allows the purification, identification and relative quantification of endothelial cell surface proteins after their selective *in vivo* labeling with biotin. Trypsin digestion of the biotinylated proteins and subsequent liquid chromatography and tandem mass spectrometry analysis (LC-MS/MS) resulted in the identification of a total 127 non redundant proteins. All these proteins have been classified according to their function and cellular location, and the differences between *S. bovis*-infected and non-infected mice in their endothelial surface proteomes have been analyzed. The present work provides the first data on the proteome of the endothelial surface of the portal vein, and identifies some of the changes induced in it after an infection by *S. bovis*.

Keywords: Schistosoma bovis; vascular endothelium; biotinylation; mass spectrometry; proteome.

1. Introduction.

Animal schistosomiasis, caused by *Schistosoma bovis*, is a serious veterinary problem, affecting large numbers of animals and causing significant economic losses to farmers in many areas of the world. *S. bovis*, a close relative to human schistosome *S. haematobium* [1], lives in the mesenteric veins of its host and is distributed across almost the whole of Africa and south western Asia, where it has been estimated that there are 165 million of infected animals. In Europe it is present on the Mediterranean islands and in Spain [2].

Schistosome cercariae infect their vertebrate hosts through the skin. Then, they enter the blood circulation, performing an intra-organic migration along which they mature and reach their final localization (portal vein and mesenteric vessels for *S. bovis*), where they transform into adults and live for years. During its migration through the host, larvae of the parasite migrate through the blood vessels of several organs and tissues, including the subcutaneous tissue, the lungs, heart, the portal hepatic system and mesenteric veins, where they interact with vascular endothelial cells [3, 4]. This interaction occurs through the tegument surface and through the suckers of the worm, which are used to adhere to the vessel wall. Additionally, the vascular endothelium interacts with the products released by the parasites, namely, components shed from the tegument and excretion-secretion products.

The vascular endothelium is a smooth surface formed by a monolayer of cells that acts as a semipermeable barrier between the lumen of blood vessels (arteries, veins and capillaries) and the surrounding tissues. The endothelium regulates vascular tone and permeability, coagulation and thrombosis, and governs the passage of leukocytes towards areas of inflammation through the expression of surface proteins and the

secretion of soluble mediators [5, 6]. Additionally, endothelial cells are the targets of cytokines that alter cell function during inflammation [7, 8].

Schistosomes are perfectly adapted to their intravascular habitat and have evolved mechanisms to evade the immune and haemostatic responses of their hosts [9-12]. In this context, it has been observed that parasite molecules can regulate the function of the endothelium along the infection, which contributes to the modulation of host defensive responses and parasite survival. Some *in vitro* studies have shown that the products secreted by the schistosomula of *S. mansoni* limit inflammation through the induction of IL-6 and down regulation of the adhesion molecules E-selectin and VCAM-1 in endothelial cells, thereby avoiding the immune/inflammatory response during their transit through the lungs [13-15]. It has also been found that the adult worms of the same species exert a regulatory effect on the release of nitric oxide [16]. More recently, studies performed with endothelial cell cultures from mesenteric microvasculature have demonstrated that schistosomes increase vascular permeability and endothelial cell-leukocyte interactions, and that these effects are partially caused by a decrease in the expression of endothelial NO synthase (eNOS) [17]. In addition, *S. mansoni* infections are known to produce an increase in the plasma concentration of the soluble form of ICAM-1, a classic marker of endothelial activation in inflammation, which correlates with the severity of the disease [18].

In spite of the above, and in light of the importance of the regulation of endothelial function in schistosome infections, studies dealing with schistosome-endothelium interactions are very scant, and their results are sometimes contradictory [17]. Additionally, little information is available concerning the changes that they produce on the surface of the endothelium along infection, and there is also a lack of information regarding the parasite molecules involved in such a regulation.

Accordingly, in the present work we were prompted to investigate the proteome of the endothelial surface of the portal vein expressed as a result of the presence of adult worms. To accomplish this, we used a methodology consisting of the vascular perfusion of mice (infected and not with *S. bovis*) with a biotin ester reactive. In this way the endothelial surface proteins showing free amino groups would become biotinylated and efficiently purified by streptavidin affinity chromatography. Trypsin digestion of biotinylated proteins and subsequent liquid chromatography and tandem mass spectrometry analysis (LC-MS/MS) resulted in the identification of a total of 127 proteins in hepatic portal vein endothelium from *S. bovis*-infected and non-infected mice. The proteins were classified according to their biological function and cellular location, and the differences between the infected and non-infected mice in their endothelial surface proteomes were analyzed.

2. Material and Methods

2.1. Experimental animals and infection protocol.

Forty NMRI mice weighing 30-35 g (Harlan Laboratories Models, S.L.) were used. The mice were distributed in the following experimental groups: R0 (control group, n= 10), non-infected mice, perfused with buffer; R1 (n= 15), non-infected mice, perfused with biotin; R2 (n= 15), mice infected with *S. bovis* and perfused with biotin. All mice were subjected to the same surgical and perfusion procedure, as described below.

Mouse infection with *S. bovis* (R2 group) was done by tail immersion with 200 *S. bovis* cercariae for 60 min. Four months later, when the infections were fully established, the animals were perfused with sulfo-NHS-LC-biotin.

All animal manipulations were done according to the rules from the ethical and animal welfare Committee from the Institution where the experiments were conducted (IRNASA, CSIC), following the corresponding EU rules and regulations.

2.2. Perfusion procedure for *in vivo* biotinylation.

We followed the procedure for *in vivo* perfusion described by Roesli et al. [19].

The mice were anesthetized with a combined subcutaneous injection of 200 mg/kg ketamine (Imalgene 1000, Merial) and 20 mg/kg xylazine (Xilagesic 2%, Laboratories Calier, S.A.). The chests of the anesthetized mice were opened through a median sternotomy. Perfusion was performed from the aorta through the hepatic portal system. To do this, a perfusion needle (24-gauge catheter, BD Insyte) was inserted into the aortic artery at the exit from the left ventricle, through which the solutions were administered and another catheter was inserted at the posterior end of the portal vein to allow outflow of the perfusion solutions. Blood components were washed away with pre-warmed PBS (38°C) supplemented with 10% dextran 40 (Sigma) as a plasma expander for 10 min. Immediately afterwards, the mice were perfused with 10 ml of biotinylation solution at a flow rate of 1 ml/min. The perfusion solution contained 1 mg/ml of sulfo-NHS-LC-biotin (Thermo Scientific) in PBS (pH 7.4, 38 °C) and 10% of dextran 40. To prevent hydrolysis of the sulfo-NHS-LC-biotin, the reagent was dissolved immediately before the beginning of the *in vivo* perfusion. Subsequently, to neutralize unreacted biotinylation reagent the mice were then perfused for 10 min with 50 mM Tris-HCl in PBS and 10% dextran 40. Mice in the R0 control group were perfused as above, except without the addition of sulfo-NHS-LC-biotin to the perfusion solution. During perfusion with the biotinylation solution the abdomens of the animals were wetted with a quenching solution (50 mM Tris-HCl in PBS, pH 7.4, 38°C) to avoid

the biotinylation of organ surfaces. All the solutions were perfused using a peristaltic pump and the flow rate was tuned so as not to exceed a counter pressure of 100 mmHg.

Throughout the operation, the mice remained fixed to a solid surface, which was slightly inclined to help the drainage of excess liquid, and were kept warm (38°C) with the aid of a lamp.

After perfusion, a sample of the portal vein through which the perfused solutions had passed was excised and the specimen was either freshly snap-frozen for the preparation of organ homogenates or fixed during 24 hours in 4% saline formaldehyde for histochemical analysis.

2.3. Protein extraction and purification.

In all groups, the samples of portal vein were processed in batches of 4 mice. Hepatic portal vein samples were resuspended in lysis buffer (2% SDS, 50 mM Tris, 10 mM EDTA, complete EDTA-free proteinase inhibitor cocktail (Roche Diagnostics) in PBS, pH 6.8.) at a ratio of 40 µl of buffer per mg of sample. The samples were homogenized six times using an Ultra-Turrax T10 disperser (IKA-Werke), 2 min each at full power. The homogenates were sonicated for 3 min, incubated at 95 °C for 20 min and, after cooling to room temperature, centrifuged at 17,000 g for 20 min. Then, to eliminate as much SDS as possible and to avoid interference in biotin-streptavidin binding, the supernatant was dialyzed with PBS for 24 hours. The concentration of the total protein extract of the supernatant was determined using the BCA Protein Assay Reagent kit (Pierce).

Biotinylated proteins from this extract were purified by affinity chromatography using a streptavidin-sepharose resin (GE Healthcare). The slurry resin was washed three times in a buffer containing 1% Nonidet P-40 and 0.1% SDS in PBS (pH 7.4). Five mg

of total protein extract was added to 215 μ l of resin and biotinylated proteins were captured for 2 h at room temperature in a revolving mixer at low rotation speed. The unbound proteins were eliminated by washing 3 times with the above-mentioned buffer and centrifugation at 470 g.

2.4. On-resin tryptic digestion

The resin with the bound biotinylated protein was washed three times with 400 μ l of 50 mM NH_4HCO_3 . Following this, proteins were digested overnight at 37 °C with 2 μ g of sequencing grade trypsin (Promega) in a total volume of 400 μ l of 50 mM NH_4HCO_3 . The reaction was stopped with 20 μ l of 10% trifluoroacetic acid (TFA), after which the digestion mixture was collected by centrifugation and the resin was washed once with 200 μ l of 5% acetonitrile and 0.1% TFA in water. All the fractions combined were dried in a vacuum centrifuge and resuspended in 200 μ l of 5% acetonitrile and 0.1% TFA. Tryptic peptide samples were analysed by liquid chromatography-tandem mass spectrometry.

The concentration of the peptides was estimated by UV spectrometry, assuming that a 1 mg/ml solution of proteins has an extinction of 1.1 absorbance units at 280 nm.

2.5. Liquid chromatography and tandem mass spectrometry (LC-MS/MS).

For the R1 and R2 groups, two independent batches of four portal vein samples each were subjected to LC-MS/MS. For the control group (R0), one batch of four portal vein samples was subjected to LC-MS/MS. For all the samples, two technical replicas of the chromatogram were performed.

Peptide separation by LC-MS/MS was performed using an Ultimate nano-LC system (LC Packings) and a QSTAR XL Q-TOF hybrid mass spectrometer (ABSciex).

LC-MS/MS was performed as described before by De la Torre-Escudero et al. [20] with some modifications. Briefly, an aliquot corresponding to 5 μ l containing 3.9 μ g of digested peptides was loaded onto a trap column (PepMap C18, 300 μ m $\times$ 5 mm, LC Packings) and desalted with 0.1% TFA at 30 μ l/min for 3 min. The peptides were then loaded onto an analytical column (PepMap C18 3 μ 100 Å, 75 μ m $\times$ 15 mm, LC Packings) equilibrated in 5% acetonitrile and 0.1% formic acid. Elution was carried out with a linear 2-30% gradient of 95% acetonitrile and 0.1% formic acid over 110 min at a flow rate of 300 μ l/min. The peptides eluted were analyzed with a nanoESI-Q-TOF mass spectrometer (QSTAR-XL, AB Sciex) in information-dependent acquisition mode (IDA), in which a 1-s TOF MS scan from 400–2000 m/z was performed, followed by 3-s product ion scans from 65–2000 m/z on the three most intense doubly or triply charged ions.

2.6. Database search and protein identification.

Database searches were performed in the NCBI *Mus musculus* database (17005982 sequences; 5838490176 residues) using MASCOT v2.2 (Matrix Science) and ProteinPilot v4.0 (AB Sciex) search engines. For samples from the R2 group, an additional search was performed using MASCOT v2.2 in a custom made *Schistosoma mansoni* + *S. haematobium* EST database (341544sequences; 86460946 residues) from the Sanger Institute (<ftp://ftp.sanger.ac.uk/pub/pathogens/Schistosoma/>). This searching was done to evaluate the potential presence of proteins of parasite origin.

For Mascot searches, the peak lists were generated directly from QSTAR wiff files by Mascot Daemon v 2.2.2 (Matrix Science) with Sciex Analyst import filter options using the default parameters. The NCBI *Mus musculus* database was searched using the following parameters: tryptic specificity, allowing two missed cleavages and a

tolerance on the mass measurement of 100 ppm in MS mode and 0.8 Da for MS/MS ions. The carbamidomethylation of Cys was set as a fixed modification, and Met oxidation and Asn/Gln deamidation were set as variable modifications. The significance threshold was set at 0.05. Only proteins for which there were two or more unique peptides matches were selected and shown in the results.

For ProteinPilot searches, default parameters were used to generate the peak list directly from QSTAR wiff files of portal vein samples. The Paragon algorithm of ProteinPilot was used to search the NCBI *Mus musculus* protein database with the following parameters: trypsin specificity and cys-alkylation. To avoid using the same spectral evidence in more than one protein, the proteins identified were grouped based on MS/MS spectra, using the Protein-Pilot Progroup algorithm. Thus, proteins sharing MS/MS spectra were grouped, regardless of the peptide sequence assigned. The protein within each group able to explain more spectral data with confidence is shown as the primary protein of the group. Only proteins with a Protein Pilot unused scored above 1.3, which is equivalent to a protein confidence threshold greater than 95%, and for which there were at least one unique peptides matches with a confidence > 95% were selected and shown in the results.

The relative abundance of a protein in the sample was quantified using the protein abundance index (PAI), which is defined as the number of observed peptides in the experiment divided by the number of observable tryptic peptides for each protein within a given mass range of the mass spectrometer employed [21]. PAI was modified exponentially to give emPAI, the exponential form of PAI minus one, which is directly proportional to the protein content in a sample [22]. The protein content in a particular sample was calculated as a percentage by dividing the emPAI value of a protein by the sum of all emPAI values in that sample multiplied by 100.

In the Results section, redundant identifications are eliminated from the lists of identified proteins, in each case choosing the protein hit with the highest score. When different, the emPAI values for a common hit in the two replicas were used to calculate the mean.

2.7. Bioinformatic analysis of the proteins identified

For the proteins identified, their biological function and cellular location were assigned according to the Gene Ontology database (<http://amigo.geneontology.org>), the SwissProt/UniProt databases (<http://amigo.geneontology.org>), the National Center for Biotechnology Information database (NCBI: <http://www.ncbi.nlm.nih.gov/sites/entrez>) and the literature.

The presence of transmembrane helices was predicted using the TMHMM Server v. 2.0 at <http://www.cbs.dtu.dk/services/TMHMM-2.0>; the prediction of signal peptides with SignalP 3.0 [23] at <http://www.cbs.dtu.dk/services/SignalP> and the search for glycosyl-phosphatidyl anchors with the big-PI Predictor [24] at http://mendel.imp.ac.at/sat/gpi/gpi_server.html were also performed.

2.8. Histochemical staining in portal vein sections.

Biotin tagging of the vascular endothelium surface was verified by immunohistochemistry. Samples of perfused portal vein were fixed with 4% formaldehyde in PBS for 24 h and then dehydrated and embedded in paraffin following standard protocols. Microtome-cut 5 μ m sections were placed on microscope slides, deparaffinised in xylene, and rehydrated. The biotin tag was detected in these sections by fluorescence, using a streptavidin-FITC probe, and by chromogenic staining using

streptavidin-horseradish peroxidase (HRP), 3,3'-diaminobenzidine (DAB) substrate and haematoxylin as counter stain.

For immunofluorescence, hepatic portal vein sections were blocked with 1% BSA in PBS containing 0.05% Tween 20 (PBST) for 1 h at 37 °C, and incubated with the streptavidin-Fluorescein isothiocyanate (FITC) (Sigma) diluted 1/500 in blocking buffer for 1 h at 37 °C. Samples were washed five times with PBST and mounted in an antifade reagent (Prolong Gold, Invitrogen).

For DAB-haematoxylin staining, sections were treated with 3% H₂O₂ for 10 min to block the activity of the tissue peroxidase. After washing, samples were blocked with 1% BSA in PBST and incubated with streptavidin-HRP (Sigma) diluted 1/1,000 in blocking buffer for 1 h at 37 °C. Samples were washed three times, incubated with DAB for 3 min and counterstained with haematoxylin for 1.5 min. All incubations were performed in a humid chamber.

2.9. SDS-PAGE and western blotting

The composition of the total protein extract, the biotinylated protein and the efficiency of purification were checked by SDS-PAGE and western blot with streptavidin-HRP. Samples of 5 µg of protein extract, the supernatant after capture, and resin-bound proteins were subjected to electrophoresis in 10% acrylamide gels. After running at 60 V, the gels were either stained with Coomassie brilliant blue or electrotransferred onto nitrocellulose membranes at 400 mA for 90 min. The membranes were blocked in 2% BSA, washed with PBST, and then incubated with streptavidin-HRP (Sigma) diluted 1/5,000 for 1h at room temperature. After washing, the reactive bands were developed using 4-chloro-1-naphthol as chromogen.

3. Results

3.1. Verification of successful *in vivo* biotinylation

The efficiency of vascular endothelium biotinylation after *in vivo* perfusion was assessed in portal vein samples taken from the mice from groups R1 and R2. This verification was performed by immunohistochemistry of paraffin sections of portal vein and by western blotting with streptavidin-HRP.

The images of the portal vein sections stained with DAB and with FITC clearly showed that after perfusion the biotin was bound to components of the wall of the portal vein (Fig. 1). In the portal vein sections from the R0 mice, perfused only with PBS and dextran, no fluorescence or colorimetric staining was observed (not shown).

Biotin labelling was also confirmed by western blotting, as can be observed in Fig. 2, which is described in the following section.

3.2. Analysis of protein extracts and purification of biotinylated proteins.

Total proteins were extracted from two independent batches from the R1 and R2 groups and from one batch of the R0 group.

After staining with Coomassie, the total protein extracts of the portal vein samples from the R0, R1 and R2 mice showed a similar band pattern, displaying many components, most of them with a molecular weight above 35 kDa (Fig. 2A). In the western blot of the protein extract from the R0 group (not perfused with biotin) two bands around 130 and 80 kDa were revealed (Fig. 2B). These molecular weights coincided with those of the enzymes pyruvate carboxylase (129,684 Da), propionyl CoA carboxylase (79,940 Da) and methylcrotonyl CoA carboxylase (79,369 Da). These proteins, which contain several biotin-binding sites, were abundantly identified in the

analysis by LC-MS/MS of this sample (next section). The pattern of biotinylated bands from the R1 and R2 groups was apparently similar to each other; up to 10 bands appeared with a molecular weight above 45 kDa.

As can be observed in Fig. 2B, the biotinylated proteins were efficiently captured on the streptavidin-sepharose resin and the bulk of non-biotinylated proteins was washed away during the washing step. We performed digestion with trypsin while the proteins were still bound to the streptavidin-sepharose resin and subjected the resulting peptides to LC-MS/MS.

3.3. Identification of biotinylated proteins

A comparative proteomic analysis was performed between the samples of hepatic portal vein taken from the two groups of mice perfused with biotin: the group not infected with *S. bovis* (R1) and the group infected with this parasite four months before perfusion (R2). We also included the R0 control group (not infected, not perfused with biotin) in order to identify the proteins that might have been retained non-specifically in the resin.

We classified the proteins identified in the portal vein according to currently available data concerning cellular location and their biological function and, based on their primary amino acid sequences, we also assessed the presence of a signal peptide, transmembrane domains and glycosyl-phosphatidyl anchors.

Seventeen proteins were identified in portal vein samples from the control mice (R0). Together with the above-mentioned proteins which contain several biotin-binding sites, we also identified several keratins (or unknown protein similar to keratin), and one uncharacterized protein (gi|12842691), which was not identified in the samples from the R1 and R2 mice (Supplementary Table 1).

Table 1, 2 and 3 show the non-redundant proteins identified in the samples from the R1 and R2 groups. These results are the sum of the identifications performed with the Mascot and ProteinPilot searches in the two portal vein samples analyzed in each group of mice (4 mice/sample). The proteins which were also identified in the R0 sample and the contaminating proteins such as trypsin, keratins, and proteins similar to keratins were not included in these lists.

As a result, we identified a total of 127 non-redundant proteins: 73 in the portal vein of non-infected mice (R1) and 100 in those infected with *S. bovis* (R2). Of these proteins, 27 were identified only in the portal vein of the R1 group; 54 only in the R2 mice (Table 2) and 46 in both groups of mice (Table 3).

Considering the cellular location of the proteins identified in each group of mice, we observed that most of them -54.8% and 47.0% in the R1 and R2 groups, respectively- were annotated as externally-bound proteins or extracellular matrix proteins (Fig. 3A). As well as being the most numerous, these proteins proved to be the most abundant ones in both groups of mice, with an overall emPAI percentage of 59.2% in the R1 group and 75.0% in the R2 group (Fig. 3A). The proteins grouped in other categories (plasma membrane, cytoplasm, cytoskeleton, nucleus, other) were comparatively much less numerous and abundant, being the most pronounced difference the higher proportion of cytoskeletal proteins in the portal vein of the infected mice (16% in R2 *versus* 5.4% in R1). Proteins in other locations (ribosomal, mitochondrial) or unknown ontology were labelled “Other”.

Analysis of the sequences of the identified proteins detected signal peptide in all the proteins classified as extracellular matrix and externally-bound secreted proteins. Likewise, all the proteins classified as being present in the plasma membrane contained one or several transmembrane domains, except the protein disulfide-isomerase

(P09103), which did not show any transmembrane domain. The assignment of a plasma membrane location to this last protein was done according to the Gene Ontology and the SwissProt/UniProt databases. No GPI anchors were identified in the sequence of any of the proteins (Tables 1, 2 and 3).

Among the different biological functions found, cell adhesion molecules accounted for the largest category (Fig. 3B). In the samples from the infected mice (R2), as compared to the non-infected (R1), we identified a greater number of structural proteins (15.0 % protein number and 5.22 % emPAI for R2 *versus* 6.8 % protein number and 1.46% emPAI for R1), and of proteins involved in the immune response (8.0% protein number and 9.48 % emPAI for R2 *versus* 4.1% protein number and 3.1% emPAI for R1).

Proteins with oxido-reductase activity were only identified in the portal vein from the R2 mice (glutathione peroxidase 3, protein disulfide-isomerase and peroxiredoxin-1). As may be seen in Fig. 3B, the group of proteins that showed a clearly higher emPAI than the rest of the proteins were those involved in transport. This higher abundance was mainly due to haemoglobin, which showed 20.75% of emPAI in R1 and 25.11% in R2. Among the remaining categories, the most abundant proteins were those involved in cell adhesion, most of which are part of the extracellular matrix. Proteins having functions related to blood coagulation (fibrinogen alpha, beta and gamma), and proteins of unknown ontology were all included in the “Other” category (Fig. 3B). (Supplementary Tables 2 and 3 show the proteins classified according to their biological function)

Fig. 4 summarizes the proteins identified only in the portal vein of non-infected (R1) or infected (R2) mice and the proteins identified in both groups of mice (R1+R2), classified according to their function and cellular location.

Among the 27 proteins identified only in the R1 mice, the most numerous were those included in the “Other” category, i.e., proteins with ribosomal or mitochondrial location or of unknown ontology; among the others, the most numerous were externally-bound proteins and nuclear proteins and, regarding function, the proteins with regulatory or catalytic activities (Table 1, Fig. 4).

Among the 54 proteins identified only in the R2 group, the most numerous were cytoskeletal (26%) and externally-bound (17%) proteins, which were indeed the most abundant (9.47% emPAI). A large difference found in the R2 mice as compared to the R1 mice was the presence in the R2 group of immune response and oxido-reductase proteins and a notably higher amount of structural proteins (Table 2, Fig. 4).

Regarding the proteins present in both samples, the highest proportion (39%) corresponded to proteins of the extracellular matrix, many of them involved in cell adhesion, and to blood components such as haemoglobin, fibrinogen, transferrin, etc, which proved to be the most abundant (Table 3, Fig. 4).

The searching of the custom-made *Schistosoma* EST database with the samples from group R2 (infected with *S. bovis*) identified a total of 100 non-redundant hits. The subsequent BLAST analysis of these sequences allowed us to identify 64 proteins and 28 hypothetical proteins (Supplementary Table 4). To determine which of those proteins could be from *S. bovis* we applied the following requirements: (i) to be present in the excretion-secretion products and/or in the tegument of *Schistosoma* spp., and (ii) not to be identified in the *Mus musculus* NCBI database. Bearing in mind these requirements, we found three tegumental proteins: glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Smp_056970), also present in the excretion/secretion products, Venom allergen-like (VAL) 7 protein (Smp_070240) and CD9-like protein Sm-TSP-1 (Smp_095630).

Discussion

In schistosomiasis, recent research efforts have addressed the identification of the components involved in parasite-host relationships and have attempted to elucidate the mechanisms used by the parasite to ensure its survival in the host. Currently we have a fairly detailed knowledge of the parasite molecules that form part of the parasite-host interface [20, 25-30], and of the mechanisms of modulation and evasion of the immune response [9]. Although scarcer, there is also information regarding the interaction of the parasite with the haemostatic system and about the anticoagulant and fibrinolytic properties of certain parasite proteins [10-12].

Regarding the interaction between schistosomes and the vascular endothelium, several authors have reported that along the course of infection the parasite can regulate the endothelial cell signalling and function to its own benefit, thereby ensuring its survival [13-18]. However, the data in this particular field of schistosome research are scant, and information about the proteins expressed on the endothelial surface along infection is lacking, as is information about the parasite molecules that might be involved in the regulation of the functioning of the endothelium.

In order to improve our knowledge of the mechanisms involved in the regulation of the vascular endothelium by schistosomes, here we were prompted to identify the changes induced by adult worms of *S. bovis* in the proteome of the endothelial surface of the host portal vein.

To conduct this study, we used a methodology that would allow the purification, identification, and relative quantification of the cell surface proteins after selective labelling *in vivo*. This method has been used in several disease models, such as tumours, Alzheimer's disease and diabetic nephropathy, in order to establish an atlas of vascular

proteins in health and disease [31-36]. However, this is the first time that this method has been used in a study aimed at establishing the differential molecular profile between a healthy and pathologic status in an infection due to an intravascular pathogen.

The application of this methodology in an *in vivo* model has important advantages as compared to similar studies carried out *in vitro*. Studies involving endothelial cell cultures have important limitations, mainly due to the phenotypic heterogeneity of endothelial cells related to their anatomical location and to the regulation exerted by the tissue microenvironment in the protein expression of endothelial cells, a regulation that cannot be duplicated in standard cell cultures [6]. For example, in a comparative proteomic analysis of endothelial cell surface membranes isolated from rat lung *versus* cultured cells, Durr et al. [37] found that only 51% of the integral membrane and plasma membrane-associated proteins identified were expressed in common.

The isolation of biotinylated proteins from mouse hepatic portal vein by streptavidin-sepharose resin followed by LC-MS/MS analysis led to the identification of 127 non-redundant proteins, of which 100 were identified in the vascular endothelium of *S. bovis*-infected mice and 73 in non-infected mice. As in other studies addressing the aortic artery and the lung microvascular endothelial cell surface, most of the proteins identified were involved in cell adhesion including externally-bound proteins, components of the extracellular matrix and proteins associated with the plasma membrane [33, 37]. However, here we also found that one fraction of the proteins identified were categorized as intracellular (20.0% in the R1 group and 34.0% in the R2 group). This was somewhat unexpected since, at least theoretically, labelling with biotin must be limited to the proteins of the cell surface. Notwithstanding, it has been observed that the sulfo-NHS-LC-biotin label could pass through the cell membrane and

could label intracellular proteins as well [38]. In addition, Durr et al [37] suggested the possibility that some of these proteins simply reside in more than one subcellular location. It is not unusual for proteins initially categorized as organelle-specific to be discovered later elsewhere in the cell. In fact, three of the proteins identified here are examples of this. Protein disulfide-isomerase, commonly classified as an endoplasmic reticulum (ER) protein, has been detected in sinusoidal rat liver plasma membranes [39]. The 78 kDa glucose-regulated protein is a stress protein belonging to the Hsp70 multigene family that is located inside the ER, but also expressed in the cell membrane and present in the peripheral circulation of humans [40]. Elongation factor-1 alpha is primarily known as a component of the protein synthesis machinery but also has a less well-known function in cytoskeletal reorganization [41]. Finally, it cannot be discarded that the worms would have caused some injury to the endothelial cells resulting in intracellular protein biotinylation.

Some well known surface receptors including CD146, CD91, vascular adhesion protein and integrin were identified, but we were unable to detect other known markers of endothelial surfaces such as eNOS, VE-cadherin, E-selectin, ICAM-1 or PECAM-1. As remarked by Arjunan et al. [42], it is possible that the level of expression of these molecules in the cell membrane might be too low to be detected, being masked by other much more abundant proteins, such as those found in blood or the components of the extracellular matrix. It should be also brought in mind that since the biotinylation reagent interacts with primary amine groups (lysine side-chains or protein N-termini), the lack of primary amines in the extracellular domain of membrane spanning proteins, as well as inaccessibility due to the three-dimensional structure of the protein, may prevent efficient biotinylation [31].

Comparative proteomic analysis of endothelial cell surface proteins isolated from the portal vein of mice infected and non-infected with *S. bovis* revealed obvious differences. A total of 27 proteins were uniquely expressed in the portal vein of mice from the R1 group (non-infected) and 54 exclusively expressed in that of the R2 (infected) animals. However, given the low score and the reduced number of unique peptides identified (PN) for some of these proteins (Table 1 and 2), it is very possible that some of the differences observed in the protein composition between R1 and R2 groups could in fact represent quantitative differences in the expression level of the proteins, such that the least abundant would be below the threshold for detection by MS.

Nevertheless clear differences in the protein composition between both groups of mice were as follows: (i) a greater number of cytoskeletal proteins in the samples from the infected mice (15 in R2 *versus* 4 in R1); (ii) a greater number of proteins related to the immune response in the infected mice, mainly immunoglobulins (8 in R2 *versus* 3 in R1), and (iii) the identification only in the infected mice of three proteins with oxidoreductase activity (peroxiredoxin, glutathione peroxidase and protein disulfide-isomerase).

The cytoskeleton not only serves for the maintenance of cellular shape but also plays roles in other cellular functions, including cellular and organelles movement, cell division, endocytosis and as a mediator of inflammation [43]. Regarding infections by schistosomes, *in vitro* studies have shown that pulmonary schistosomula attach to the lung endothelium through at least one endothelial cytoskeleton component [13]. It is therefore possible that adult worms could use the same mechanism to become attached to the wall of the portal vein and mesenteric vessels, which would account for the higher expression of cytoskeletal proteins in the infected animals. Further studies would be necessary to confirm this.

The higher number of immunoglobulins identified on the surface of the vascular tissue of the infected animals is a clear sign of the immune response induced by the infection.

Peroxioredoxin and glutathione peroxidase are two anti-oxidant enzymes that may be involved in protection against the reactive oxygen species released by host immune effector cells. Glutathione peroxidase is an enzyme that can be secreted, and peroxiredoxin, although this is mainly found in the cytosol and lacks a signal peptide, recent studies have demonstrated that it can be possibly secreted via a non-classic secretory pathway [44]. These molecules exposed on the endothelial surface could play a crucial role in the prevention of host responses to pathogen products and protect host tissues from the reactive oxygen species produced during the anti-schistosome immune response [45, 46].

Protein disulfide-isomerase is a dithiol-disulfide oxidoreductase chaperone of the thioredoxin superfamily that plays multiple roles in redox signalling and homeostasis. This protein is expressed in the ER and also on the surface of endothelial cells, where it exerts an inhibitory effect on coagulation through the regulation of tissue factor [47, 48]. Regarding these functions, another protein expressed in the vascular endothelium of infected mice that also inhibits the procoagulant activity of the tissue factor is the 78-kDa Glucose-Regulated Protein (GRP78) [49]. Taking into account the biology of the parasite, it is not surprising that schistosomes are able to regulate the endothelium promoting anticoagulant conditions. It has been shown that the parasite itself has molecules with anticoagulant, profibrinolytic and vasodilator activity [11, 12 50]. Evidently, the ability to elude coagulation or promote vasodilatation would permit the worm free mobility in the vasculature without eliciting harmful ischemia and hypoxia, which could damage them [50].

Regarding the three proteins of possible *S. bovis* origin identified in the infected mice samples, namely, GAPDH, Sm_VAL-7 and Sm_TSP-1, there is no information about their possible interaction with the vascular endothelium. However, since all of them are part of the host-schistosome interface [25, 27, 51, 52], it cannot be ruled out that they might play some kind of regulation on the endothelium. GAPDH is a multifunctional protein with functions other than glycolysis including regulation of cellular response to oxidative stress and fibrinolysis [53, 54]. The SmVAL is a member of the SCP/TAPS (Sperm-Coating Protein/Tpx-1/Ag5/PR-1/Sc7) protein superfamily, with a signal peptide allowing it to be secreted to interact with their immediate environment, which may be important in host-pathogen interactions [52]. Tetraspanins have been identified on the surface of *S. mansoni* and their functions there are as yet unknown [51]. Nevertheless, bearing in mind that endothelial cells can express many tetraspanins of great importance in endothelial cell biology it is possible that we could be dealing with a protein from the host [55]. Obviously, further studies are needed to confirm if these three parasite proteins play some kind of regulation on the vascular endothelium of the host.

In sum, the present work provides the first data on the proteome of the endothelial surface of the mouse portal vein, and identifies some of the changes induced in it after infection by *S. bovis*.

Further studies should shed further light on these and other *S. bovis*-induced endothelial changes, and could pinpoint the parasite molecules involved in the regulation of the vascular endothelium. Since these molecules could be essential for parasite survival, they could constitute potential targets for the development of new drugs and anti-schistosome vaccines.

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Figure legend.

Figure 1. Biotinylation of the hepatic portal vein endothelium in the mice perfused with biotin. Paraffin sections of the portal vein. (A) Fluorescence staining with streptavidin-FITC. (B) Chromogenic staining using streptavidin-horseradish peroxidase (HRP), 3,3'-diaminobenzidine (DAB) substrate and haematoxylin as counter stain. The right images show the zones depicted in the left panels in greater detail. The biotinylation signals in infected and non-infected mice were similar. These images are representative of both groups of mice.

Figure 2. Analysis of the extracted proteins from mouse portal vein. The vascular portal vein of infected (R2) and non-infected (R1) mice were biotinylated with Sulfo-NHS-LC-biotin via systemic perfusion. The group of non-infected mice (R0) were perfused only with PBS and dextran. (A) Coomassie-stained SDS-PAGE of the protein extracts from portal vein samples from the mice in the groups R0, R1 and R2. (B) Western blot analysis of the biotin-labelled proteins in the total protein extracts, in the supernatants after capture (unbound proteins) and in the fraction of proteins bound to the streptavidin-sepharose resin.

Figure 3. Proteins identified in the portal vein of the infected (R2, white bars) and non-infected (R1 black bars) mice. The proteins are classified in categories according to their cellular location (A) and biological function (B). % Protein number is the ratio between the number of proteins identified in each category with respect to the total number of proteins identified. The emPAI percentage of each category is the sum of the % emPAI of each protein.

Figure 4. Classification of the proteins differentially identified in each group of mice by cellular location and biological function. (R1) Proteins identified in the portal vein from

non-infected mice only. (R2) Proteins identified in portal vein from *Schistosoma bovis*-infected mice only. (R1 and R2) Proteins identified simultaneously in both groups of mice. Pie charts represent the percentages of proteins in each category. Numbers in brackets indicate the % emPAI for each category calculated as the sum of the % emPAI of all the proteins in that category. In R1 and R2 charts, both values of % emPAI are given (R1- R2, respectively).

Table 1. Proteins identified only in the portal vein endothelium of uninfected mice (R1).

SwissProt locus	NCBI locus	Protein	% emPAI ¹	Score (Mascot/Pilot) ²	PN (Mascot/Pilot) ₃	Predicted TMs ⁴	Signal peptide
Externally bound (Secreted, blood)							
Q19LI2	124486702	Alpha-1B-glycoprotein precursor	0.36	79/3.07	2/2	0	yes
Q64285	6753406	Bile salt-activated lipase	0.6	64	2	0	yes
P23953	192854	Liver Carboxylesterase	0.55	50/2.09	1/2	0	yes
Q6P8U6	37674236	Pancreatic triacylglycerol lipase	0.36	66/4.47	1/3	0	yes
Extracellular matrix							
Q64739	841122	Collagen alpha-2(XI)	—	3.25	2	1	yes
Q62000	6679166	Mimecan precursor	1.2	140/5.77	4/3	0	yes
P82198	74215223	Transforming growth factor	—	1.54	1	0	yes
Plasma membrane							
Q8R420	19918916	ATP-binding cassette transporter	0.18	59	3	9	no
Q60849	973182	CD98 heavy chain	0.66	67/2.25	2/1	1	no
Q9EPB2	9954443	Natural killer cell receptor	—	44	2	1	no
Cytoplasm							
Q9QZ05	12847465	Eukaryotic translation initiation factor 2-alpha kinase 4	0.48	78/8.53	3/5	0	no
D3Z6P0	109731005	Protein disulfide-isomerase A2	0.72	123/2.91	3/2	0	yes
Q6PFX9	87239974	Tankyrase-1	—	54	2	0	no
Cytoskeleton							
A2ARZ3	123232783	Fibrous sheath-interacting protein	—	65	2	0	no
D3Z667	124486773	Dynein heavy chain 2	—	52	3	0	no
Nucleus							
Q69ZA1	124486698	Cyclin-dependent kinase	—	37	2	0	no
O08550	115495457	Histone MLL4	—	41	2	0	no
Q8VCQ3	12833067	Nuclear receptor-binding factor	—	47	2	0	no
O35134	2330007	RNA polymerase I	—	44	2	0	no
Miscellaneous or unknown ontology							
P47911	38571759	60S ribosomal protein L6	0.6	44	2	0	no
Q6GQX6	67906181	Ankyrin repeat and SAM domain-containing protein 6	0.24	76	3	0	no
—	14277826	Chain B, Crystal Structure of G3-519	1.47	51	2	0	no
Q924L1	19527322	LETM1 domain-containing protein 1	—	42	2	1	yes
—	148708988	mCG20427	—	50	3	0	no
—	148709576	mCG66490	—	48	2	6	no
Q52KE6	62871717	Pgbd5 protein	—	2.00	1	0	no
Q3V0M1	85701949	Uncharacterized protein	0.28	53	2	0	no

¹% emPAI (percentage of exponentially modified protein abundance index) was calculated by dividing the emPAI value of a protein by the sum of all emPAI values multiplied by 100.

²Scores with decimals belong to Protein Pilot-derived identification; scores without decimal identification correspond to MASCOT-derived identifications.

³PN. Number of unique peptides identified. Protein Pilot-derived identifications show the number of peptides identified with a confidence > 50%.

⁴TMs, N° of transmembrane domains

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Table 2. Proteins identified only in the portal vein endothelium of mice infected with *Schistosoma bovis* (R2).

SwissProt locus	NCBI locus	Protein	% emPAI ¹	Score (Mascot/Pi lot) ²	PN (Mascot/Pi lot) ³	Predicted TMs ⁴	Signal peptide
Externally bound (Secreted, blood)							
Q3V2G1	74203337	Apolipoprotein A-I	0.89	117/2.27	3/1	0	yes
P46412	15011841	Glutathione peroxidase 3	1.09	92/2.06	2/2	0	yes
Q61646	8850219	Haptoglobin precursor	0.7	80/2.34	2/2	0	yes
P28666	112914	Murinoglobulin	0.15	68/2.97	2/2	0	yes
—	148589114	Anti-human c-myc monoclonal antibody 9E10	0.23	104	4	0	yes
—	1421599	Chain L, Idiotype-Anti-Idiotype Fab Complex	2.64	134	5	0	no
—	110077	Ig gamma-1 chain C region	0.47	105	3	0	no
—	110434	Ig kappa ligh chain	2.6	139/5.01	5/4	0	no
—	34785917	Igh protein	0.7	121/12.6	4/7	0	yes
Extracellular matrix							
P28481	30410850	Collagen alpha-1(II)	—	41/2.82	3/2	0	yes
B1AWB9	223461042	Collagen alpha-1(V)	—	2.00	1	1	yes
Q61554	726324	Fibrillin-1	0.23	154	5	0	yes
F8VQ43	117647249	Laminin alpha 2	—	2.00	1	0	yes
Q62009	7657429	Periostin	0.43	99/3.32	3/3	0	yes
Plasma membrane							
P20029	1304157	78 kDa glucose-regulated protein	0.35	68	2	1	yes
Q91ZX7	49942	Alpha-2-macroglobulin receptor (CD91)	—	39	2	1	yes
Q8R2Y2	10566953	Cell surface glycoprotein MUC18	0.35	85	2	1	yes
P09103	129729	Protein disulfide-isomerase	0.23	122	4	0	yes
Q8C310	27229187	Roundabout homolog 4 precursor	—	46	2	2	yes
Cytoplasm							
O54774	6671565	AP-3 complex subunit delta-1	—	45	2	0	no
Q8VDR9	122065169	Dedicator of cytokinesis protein 6	—	42	2	0	no
A2APV2	29126933	Formin-like protein 2	—	42	2	0	no
Q6P5H2	50363232	Nestin	—	52	2	0	no
P35700	6754976	Peroxisome oxidin-1	—	47	2	0	no
Q99K08	13529638	Phosphofructokinase	—	53	3	0	no
Q6P9N8	40254203	Trafficking protein, kinesin binding 2	—	56	2	0	no
Q8K466	22023780	TSP50	—	38	2	0	yes
A2ALK8	218505829	Tyrosine-protein phosphatase non-receptor type 3	—	45	2	0	no
Cytoskeleton							
P59242	27503680	Cingulin	0.94	62	2	0	no
Q3UJV1	75677484	Coiled-coil domain-containing protein 61	—	54	3	0	no
Q45VK7	72534792	Dynein 2 heavy chain 1	—	42	2	0	no
Q91XQ0	153792273	Dynein heavy chain 8	0.35	50	3	0	no

B1AR51	153791933	Dynein heavy chain 9	—	66	3		no
Q91ZU6	15077863	Dystonin	—	70	4	0	no
—	90508	Gelsolin	0.31	50	2	0	no
Q60575	407339	Kinesin-like protein	—	39	2	0	no
Q8VDD5	17978023	Myosin II-A	0.12	103/2.28	4/1	0	no
Q9QXS1	40849908	Plectin 1	—	67	4	0	no
Q8BUJ6	77812	Titin isoform N2-A	—	106	7	0	no
Q9JHJ0	8394460	Tropomodulin-3	—	46	2	0	no
P05213	202210	Tubulin alpha-1B chain	0.23	54	2	0	no
P20152	55291	Vimentin	0.35	196	5	0	no
Nucleus							
E9Q616	61743961	AHNAK nucleoprotein isoform 1	0.04	86	3	0	no
Q6ZPV2	26333853	DNA helicase INO80	—	42	2	0	no
P84228	51301	Histone H3.2	1.9	113/2	3/1	0	no
P50540	6754766	Max-interacting protein 1 isoform a	—	47	2	0	no
Q61312	1154873	Transcription factor AP-2 gamma	0.82	49	2	0	no
Miscellaneous or unknown ontology							
Q61315	12643510	Adenomatous polyposis coli protein	—	48	3	0	no
—	148696491	mCG11496	—	46	3	1	no
—	148667581	mCG115458, isoform CRA_a	—	53	3	0	no
—	148704071	mCG141014	—	50	2	0	no
—	148672709	Phospholipase A2, group VI, isoform CRA_a	—	45	2	0	no
Q8BFU3	26324710	RING finger protein 214	—	53	2	0	no
—	148687286	Zinc finger, CW type with PWWP domain 1	—	49	2	0	no

¹% emPAI (percentage of exponentially modified protein abundance index) was calculated by dividing the emPAI value of a protein by the sum of all emPAI values multiplied by 100.

²Scores with decimals belong to Protein Pilot-derived identification; scores without decimal identification correspond to MASCOT-derived identifications.

³PN, Number of peptides identified. Protein Pilot-derived identifications show the number of peptides identified with a confidence > 50%.

⁴TMs, N° of transmembrane domains.

Table 3. Proteins identified in the portal vein endothelium of both groups of mice, the uninfected (R1) and the infected with *Schistosoma bovis* (R2).

SwissPro t locus	NCBI locus	Protein	% emPAI ¹		Score (Mascot/Pilot) ²		PN (Mascot/Pilot) ³		Predicted d TMs ⁴	Signal peptid e
			R1	R2	R1	R2	R1	R2		
Externally bound (Secreted, blood)										
P07724	26340966	Albumin	4.81	4.50	770/25.65	1000/32.19	15/19	20/23	0	no
P07758	6678079	Alpha-1-antitrypsin 1-1 precursor	1.92	0.27	204/8.39	82/6.77	5/6	3/4	0	yes
Q61838	199086	Alpha-2-macroglobulin	0.24	0.31	98/1.59	285/4.44	2/3	8/4	0	yes
P01027	1352102	Complement C3	0.12	0.35	73/7.7	291/10.9	3/5	9/9	1	yes
P06909	116132	Complement factor H	–	0.08	2.00	49/1.70	1	1/1	0	yes
P07759	50442	Contrapsin	0.9	0.89	149	150/3.00	4	3/3	0	yes
Q8K0E8	33859809	Fibrinogen beta chain precursor	0.72	1.28	162/3.75	342/8.87	4/2	8/7	0	yes
Q8VCM7	19527078	Fibrinogen gamma chain precursor	2.29	1.13	248/5.4	252/6.18	6/5	7/6	0	yes
Q99K47	33563252	Fibrinogen, alpha polypeptide isoform 2	1.8	1.4	259/9.79	402/12.79	8/6	11/9	0	yes
P01942	122441	Hemoglobin subunit alpha	7.52	6.83	127/12.79	189/12.46	4/9	6/9	0	no
A8DUP3	12846616	Hemoglobin subunit beta-1	13.23	18.28	284/11.21	442/16.98	7/9	9/10	0	no
Q91X72	1881768	Hemopexin	0.36	1.09	50	280/8.31	2	9/5	0	yes
–	3688704	Immunoglobulin heavy chain variable region	2.95	2.41	61	96/2.00	2	3/1	0	no
P00688	111607467	Pancreatic alpha amylase	0.72	1.24	125/6.38	275/9.73	3/4	6/7	0	yes
Q921I1	74178118	Serotransferrin	1.68	2.72	254/13.52	682/22.57	8/8	15/12	0	yes
Extracellular matrix										
Q99MQ4	27754118	Asporin preproprotein	0.96	0.62	201	140	3	3	0	yes
P28653	348962	Biglycan	2.1	1.78	204/6.55	218/8.00	5/4	6/4	0	yes
O88493	3236370	Collagen alpha-3 (VI)	0.42	0.66	393/16.9	867/16.5	10/11	23/12	1	yes
Q04857	6753484	Collagen alpha-1(VI)	0.18	1.05	87/6	205/15.83	2/5	7/13	0	yes
P08122	556299	Collagen alpha-2 (IV)	0.12	0.07	75/4.05	63/2.06	2/2	1/2	1	yes
Q02788	49809	Collagen alpha-2(VI)	–	2.23	95	332/4.28	3	9/2	0	yes
P11087	470674	Collagen pro-alpha-1(I)	1.2	2.69	411/111.4	451/90.19	9/60	11/53	0	yes
Q01149	50489	Collagen pro-	1.5	2.58	461/111.4	386/72.4	9/60	9/40	0	yes

		alpha-2(I)			6					
Q80X19	30420885	Collagen alpha-1(XIV)	0.3	0.23	193/6.6	44/2.00	5/4	1/1	1	yes
Q7TT32	30851550	Collagen alpha-1(III)	—	0.59	57/59.17	79/56.1	2/31	3/31	0	yes
P28654	6681143	Decorin preproprotein	3.67	3.45	358/14.81	423/19.72	11/10	11/11	0	yes
P11276	74218046	Fibronectin 1	—	0.08	4.52	42	2	3	0	yes
Q61292	2497599	Laminin subunit beta-2	0.18	0.08	87	71	3	3	0	yes
P02468	126370	Laminin subunit gamma-1	0.3	0.16	172	2.73	6	3	0	yes
P51885	1168179	Lumican	3.07	2.48	196/10.07	260/8.51	5/6	6/7	0	yes
P10493	53384	Nidogen-1	0.12	0.19	53/4.45	80/2.04	2/2	2/1	0	yes
B1B0C7	122891114	Perlecan	0.12	0.12	135/6.55	269/14.77	3/4	7/12	0	yes
Q9JK53	229608920	Prolargin	2.64	3.07	180/6.42	358/5.82	5/5	8/5	0	yes
Plasma membrane										
Q64314	19526792	Hematopoietic progenitor cell antigen CD34 isoform 2 precursor	0.55	—	40	50	3	3	1	yes
P09055	762977	Integrin beta-1	0.42	0.16	90/1.49	93	3/1	2	1	yes
—	6754382	Leukocyte surface antigen CD47	—	0.35	1.52	104/2	2	1/1	5	yes
A2AGL3	123210264	Ryanodine receptor 3	—	—	39	9	2	3	6	no
O70423	6753066	Vascular adhesion protein 1	—	0.31	5.81	144/6.66	4	3	1	no
Cytoplasm										
P10126	50797	Eukaryotic elongation factor 1	0.84	1.43	225	292/9.38	5	6/7	0	no
E9Q624	51988896	Zinc finger protein 644	—	—	58	58	3	2	0	no
Cytoskeleton										
P68033	387090	Actin	0.5	1.79	134/5.79	221/11.32	4/5	5/8	0	no
Q8R384	20070691	Myh11 protein	0.36	0.12	109	118	5	5	0	no
Nucleus										
Q8CGP0	76826886	Histone H2B	—	0.89	1.53	55/3.52	1	1/2	0	no
P62806	51311	Histone H4	4.15	2.68	112/2.15	124/3.22	2/1	3/2	0	no
Miscellaneous or unknown ontology										
Q6A074	50510473	mKIAA0400 protein	—	—	85	73	3	3	0	no
Q99MV3	118722338	Testis expressed gene 15	—	—	41	39	2	2	0	no

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ACCEPTED MANUSCRIPT

Figure 1

Figure 2

Figure 3

Figure 4.

Graphical Abstract

Highlights

- We have isolated mouse endothelial cell surface proteins by *in vivo* biotinylation.
- We provide the first data on the proteome of the endothelial surface of mouse portal vein.
- We identify changes in protein expression in portal vein endothelium induced by *S. bovis*.