An in vivo proteomic study of the interaction between Salmonella Typhimurium and porcine ileum mucosa

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ABSTRACT

The enteropathogen Salmonella Typhimurium is one of the main causes of porcine and human enterocolitis. We have used a 2-DE, MALDI-TOF/TOF-based approach to characterize in vivo proteome changes in porcine ileum mucosa after pathogen interaction. Ileum samples from non-infected and orally infected animals were collected at 2 days post infection and S. Typhimurium presence was confirmed by immunohistochemistry. Fifty one proteins, involved in immune response (acute phase response, inflammation and immune response regulation), apoptosis and pathogen-mediated cell invasion, were identified as being differentially expressed after pathogen challenge. Overall, anti-inflammatory signals and a possible down-regulation of dendritic cell maturation were observed. According to this, we identified the up-regulation of FK506-binding protein 4 (FKBP4), a negative regulator of the transcription factor IRF4 (interferon regulatory factor 4), implicated in Th2 and Th17 response. Transcriptional analysis using RT-qPCR indicated a general trend toward down-regulation of Th2 and Th17 cytokines genes, which would be in agreement with an IRF4 reduced transactivation activity. On the other hand, proteins that could be involved in maturation of Salmonella-containing vacuole and intracellular pathogen survival were up-regulated. Results derived from this study would be valuable to better characterize a possible pathogen led modulation of host responses in vivo.

1. Introduction

Salmonella enterica subsp. enterica serovar Typhimurium (S. Typhimurium) is a broad-host range serotype able to colonize the lower intestinal tract of a wide range of animals, including humans [1].

In swine, S. Typhimurium infection causes enterocolitis, frequently with sub-clinical symptoms, and can be maintained in a carrier-state by infected animals [2]. S. Typhimurium has become the most commonly non-typhoidal serotype isolated in pigs and recent data point pigs/pork as the main source of human salmonellosis in USA and European countries [2]. Although, in general, human salmonellosis is not a life-threatening disease, annually 3 million deaths due to non-typhoidal Salmonella are estimated occur worldwide [3]. Moreover, the prevalence of multidrug-resistant S. Typhimurium...
phagetypes in pigs, such DT104 used in this study, makes swine salmonellosis a major public-health concern [4,5]. Currently, the implementation of animal breeding projects and the design of effective vaccines are the bases for effective and sustainable disease control; therefore, a better understanding of host responses against Salmonella infection at the mucosal barrier is crucial.

Proteomics studies, including two-dimensional electrophoresis (2-DE), have been carried out in order to achieve a better understanding of S. Typhimurium physiology. Those studies include the proteomic characterization of different Salmonella spp. and strains [6], identification of translational changes occurring in the pathogen under in vivo mimicking conditions [7,8], and the characterization of the intracellular pathogen proteome [9,10]. However, little proteomic information is available from the host’s point of view, and the few reported studies were carried out using in vitro infections of isolated cell lines [11]. In a recent work, we identified important differences in the inflammatory response at transcriptional level throughout the porcine gut upon S. Typhimurium infection [12]. Our results showed ileum mucosa as unable to up-regulate some pro-inflammatory cytokines, which could help to a more successful colonization of this site by the infecting bacteria. In view of these results, the aim of this present study was to identify changes in the porcine ileum proteome as a consequence of S. Typhimurium infection in vivo. Moreover, we sought to identify changes that could be directed by the pathogen in order to achieve a successful entrance and survival as intracellular pathogen in the intestine, i.e., we focused on the early response of colonized ileum mucosa to S. Typhimurium.

2. Materials and methods

2.1. Bacterial strain

The Salmonella enterica subs. enterica serovar Typhimurium phagetype DT104 was an isolate from a carrier pig [5]. Bacteria growing at 37°C in LB broth to log stationary phase (OD600nm of 0.8) was harvested by centrifugation at 8000 g, and adjusted to a final concentration of 10⁸ CFU/ml in PBS.

2.2. Experimental infection

Eight 4-week old crossbreed weaned piglets were used. Before infection, all the animal’s fecal samples were confirmed as being free of Salmonella. Pigs were housed in an environmentally controlled isolation facility at 25°C and under constant light with ad libitum access to feed and water. After an acclimation period of 5 days, four pigs (one female and three males) were infected orally with 10⁸ CFU of S. Typhimurium, whereas the control group (two females and two males) received sterile medium orally. The four non-infected control pigs were necropsied 2 h prior to experimental infection. The four infected pigs were necropsied at 2 dpi. The ileum was carefully emptied of its content and sectioned in pieces that were immediately snap frozen in liquid nitrogen and stored at −80°C for protein and RNA isolation, or, alternatively, fixed in 10% neutral-buffered formalin for 24 h for subsequent immunostaining assays. All the infected animals were fecal-culture positive for Salmonella and developed similar clinical signs of gastrointestinal disease, including increased rectal temperature, diarrhea and lethargy. Piglets were housed in the experimental isolation facilities of the University of León (Spain). Animal care and procedures were in accordance with the guidelines of the Good Experimental Practices (GEP), under the supervision of the Ethical and Animal Welfare Committee of the University of León.

2.3. Immunohistochemistry using a S. Typhimurium specific antibody

Formalin-fixed tissues were embedded in paraffin wax following standard procedures. Sections of 5 μm were placed on slides coated with Poly-l-Lysine (Sigma-Aldrich) and kept at 55°C for 45 min. Slides were dewaxed in xylene and rehydrated through graded alcohols to distilled water. Slides were then subjected to heat-mediated antigen retrieval in 0.01 M citric acid, incubated against S. Typhimurium specific antibody, and developed similar clinical signs of gastrointestinal disease.

2.4. Mucosa isolation, protein extraction and protein concentration determination

Ileum sections of around 2 cm were thawed onto an ice-cold plate and opened by means of a longitudinal cut. The luminal surface was thoroughly cleaned using sterile gauze and PBS to eliminate mucus, and blotted dry onto dried gauze. Mucosa scrapings were obtained using a razor, weighed and homogenized using a glass tissue-lyser and lysis buffer in a proportion of 1 ml of buffer per 500 mg of mucosa. Lysis buffer composition was: 7 M urea, 2 M thiourea, CHAPS 4% (Sigma-Aldrich), DTT 1% (Sigma-Aldrich), 50 U DNAsase I (Roche) per ml of lysis buffer, Proteinase Inhibitor Cocktail P8340 (Sigma-Aldrich), Bio-Lyte ampholytes 0.8% (Bio-Rad) and Milli-Q water. The whole homogenization process was carried out in ice. Samples were incubated by 20 min in an orbital shaker and finally centrifuged at 16,000 g for 15 min at 4°C. Supernatants were individually recovered and used for subsequent analysis. Protein concentration was determined using Bradford Protein Assay (Bio-Rad) according to the manufacturer’s instructions.

2.5. Two-dimensional gel electrophoresis (2-DE) and image analysis

IPG strips (17 cm, 4–7 linear pH gradient) (Bio-Rad) were rehydrated with 600 μg of the protein solution in a total volume of 300 μl. Control and infected samples were focused simultaneously in a Protean IEF Cell (Bio-Rad), using the following parameters: 1) active rehydration at 50 V for 12 h, 2) at 250 V for 15 min without pause after rehydration; 3) rapid ramp until reaching 10,000 V h and 4) until 60,000 V h with slow ramped voltage. After IEF, the IPG strips were equilibrated by soaking first for 10 min in 50 mM Tris–HCl, pH 8.8, 6 M Urea, 2% SDS, 30% glycerol and 2% (w/v) DTT (Sigma-Aldrich) and then for 10 min in the solution containing 2.5% (w/v) iodoacetamide (Sigma-Aldrich). Second dimension was performed on 12% SDS-polyacrylamide gels using Protean Plus Dodeca Cell (Bio-Rad). Gels were stained with SYPRO Ruby protein gel.
stain (Bio-Rad), according to the manufacturer’s instructions. Gel images (three replicates per sample) were digitized with the FX Pro Plus Multiimager system (Bio-Rad) and analyzed with the PD Quest version 7.2 software (Bio-Rad). Spots detected by the software were matched between each gel in each group. Intensity levels of the spots were normalized by expressing the intensity of each protein spot in a 2-DE gel as a proportion of the total protein intensity detected for the entire gel. Normalized protein spot volume (area multiplied by stain intensity) was calculated for each gel. Intensity data were used to calculate differences in protein expression between groups (controls vs. infected samples). All data were then carefully reviewed by the operator to account for any discrepancies. A preliminary list of spots with a significance of 95% among infected and control samples was provided by the software. Finally, significant differences between groups in spot intensity were confirmed using the Mann–Whitney U test and a significance of P < 0.05 with the SPSS 15.0 software (SPSS Inc.). Fold-change values larger than 1.5 or smaller than −1.5 were set as a threshold indicating significant changes. Differential expressed spots were selected for protein identification by MALDI-TOF/TOF mass spectrometry.

2.6. Protein identification by mass spectrometry

Spots were automatically excised in a ProPic station (Genomic Solutions) and digested with modified porcine trypsin (sequencing grade; Promega), by using a ProGest digestion station (Genomic Solutions). Gel pieces were destained twice over 30 min at 37 °C with 200 mM ammonium bicarbonate/40% ACN, and subsequently subjected to three consecutive dehydration/rehydration cycles with pure ACN and 25 mM ammonium bicarbonate in 50% ACN, respectively. Finally, gel pieces were dehydrated for 5 min with pure ACN and dried out over 4 h at room temperature. Then, 20 μl trypsin (12.5 ng/μl in 25 mM ammonium bicarbonate) was added to dried gel pieces and digestion proceeded at 37 °C for 12 h. Peptides were extracted from gel plugs by adding 1 μl of 10% (v/v) trichloroacetic acid (TCA) and incubating for 15 min. Then, they were desalted and concentrated by using μC-18 ZipTip columns (Millipore) in a ProMS station (Genomic Solutions) and directly loaded onto the MALDI plate using α-cyano hydroxycinnamic acid as the matrix.

Mass analysis of peptides of each sample was performed with a 4800 MALDI-TOF/TOF Analyzer (Applied Biosystems) in automatic mode with the following setting for the MS data, m/z range 800 to 4000 with an accelerating voltage of 20 kV and delayed extraction, peak density of maximum 50 peaks per 200 Da, minimal S/N ratio of 10 and maximum peak at 65. Spectra were internally calibrated with peptides from trypsin autolysis (M+H+=842.509, M+H+=2211.104).

For the MS/MS data, fragment selection criteria were a m/z range 800 to 4000 with an accelerating voltage of 20 kV and delayed extraction, peak density of maximum 50 peaks per 200 Da, minimal S/N ratio of 5 and maximum peak at 65. Spectra were internally calibrated with peptides from trypsin autolysis (M+H+=842.509, M+H+=2211.104).

Western blot assays were carried out to validate up-regulation of protein FKBP4. Rabbit polyclonal antibody to human FKBP4 (Abcam) (1:1000 v/v dilution in 5% skimmed milk in TBS-T) was used for porcine FKBP4 detection after overnight incubations at 4 °C. Protein lysate from human intestinal epithelial cells INT-407 (ATCC Number: CCL-6) were used as positive control. One dimension SDS-PAGE in 10% acrylamide gels and Western blot were carried out following standard procedures. Thirty micrograms of protein from each animal (four non-infected control pigs and four infected pigs at 2 dpi) was used for FKBP4 detection. Protein load homogeneity and transference efficiency to PVDF membranes (Millipore) were checked using Ponceau S Red staining (0.2% Ponceau in 1% acetic acid) previous to blocking with 5% skimmed milk in TBS-T. A horseradish peroxidase (HRP)-conjugated goat anti-rabbit antibody (Sigma) was used as a secondary antibody at a 1:10,000 (v/v) dilution. Immobilon Western chemiluminescent HRP substrate (Millipore) was used for protein detection.

2.8. RNA isolation and real-time quantitative PCR assays

In order to check IRF4 and IRF5 transactivation activity upon S. Typhimurium infection, we quantified, using reverse transcription quantitative real-time PCR (RT-qPCR), the level of mRNA expression of a set of cytokine genes representatives of Th1, Th2 and Th17 response (Table 2). RNA was isolated from ileum mucosa samples following the same procedure previously described [12]. From each animal, 1.5 μg of RNA was independently reverse transcribed to cDNA using the qScript cDNA Synthesis kit (Quanta). Cycle of quantification (Cq) ([14]; http://www.rdm.org) was determined in an iQ5 Thermo Cycler (Bio-Rad) using the same protocol and conditions previously described [12]. Primer pairs used for PCR amplification can be found as Supporting information File S1. The relative gene expression was assessed by the 2−ΔΔCq method [15] using cyclophilin-A gene as reference. The Mann–Whitney U test was used to compare differences between the groups of S. Typhimurium-infected and non-infected animals using SPSS 15.0 software (SPSS Inc.). Values of P < 0.05 were considered as being significant.

3. Results and discussion

3.1. Salmonella Typhimurium detection by immunohistochemistry

In this study we aimed to uncover host responses due to host-pathogen interaction. In order to ensure that there was an interaction between S. Typhimurium and ileum mucosa we checked bacteria presence by immunohistochemical
detection using a specific antibody. As shown in Fig. 1, widespread colonization of S. Typhimurium was observed in ileum samples from all infected animals being the bacteria distribution mostly restricted to the apical area of the mucosa.

3.2. Differential protein expression analysis by 2-DE

2-DE was used to identify differences in the proteome of porcine ileum mucosa after oral challenge with S. Typhimurium. Differences in spot intensity between gels from control and inoculated samples were identified in 71 of the 543 matched spots. Fifty nine spots were identified by MS/MS as being differentially regulated after S. Typhimurium infection (Fig. 2 and Table 1). Most proteins were up-regulated with only 7 proteins down-regulated. For 9 proteins, two spots with differences in Mw and/or pl, were unambiguously identified as the same protein (Table 1). This could be explained by different posttranslational modifications, but also by the existence of different isoforms or a chemical modification of the protein during sample preparation [16]. In all cases, except for TXNDC5, the sense of regulation of the two spots was the same (up-regulation). Proteins of a bacterial origin were not identified.

3.3. Western blot analysis

To validate our proteomic results, we analyzed by Western blot the up-regulation of the FK-506 binding protein 4 (FKBP4) in all animals studied. The results are shown on Fig. 3 and, consistent with the 2-DE, Western blot analysis confirmed increased levels of the protein after inoculation with Salmonella as compared with control samples. Moreover, we checked the functional effect of FKBP4 protein up-regulation based on its role as an inhibitor of the transactivation activity of IRF4, a protein which regulates Th2 response and Th17 differentiation. For this, we quantified by RT-qPCR the mRNA expression of a set of genes coding for representative cytokines of Th1, Th2 and Th17 response (Table 2). Most of these genes showed a trend towards down-regulation (see ahead).

3.4. Functional analysis of differentially expressed proteins after S. Typhimurium challenge

As shown in Table 1, differentially regulated proteins upon infection were grouped according to their biological functions. Some of them could be included in more than one headline and their involvement in different aspects of the host–pathogen interaction has been discussed.

3.4.1. Acute phase response proteins

Changes in the concentration of several plasma proteins (acute phase proteins, APP) are known to occur as a consequence of an infection and largely due to a change in hepatic metabolism. In addition, changes at local level in extrahepatic tissues and cell types are also identified [17]. In ileum mucosa

Fig. 1 – Immunohistochemical detection of Salmonella Typhimurium in porcine ileum of control animals (A and B) and infected animals (C and D) at 2 days after oral inoculation. Original magnification: 50×.
we identified seven APP differentially regulated upon S. Typhimurium infection (Table 1). According to their serum levels, APP are classified as positive if their concentration increases or negative if it decreases after the infection. In porcine ileum, we have found a similar regulation to that expected in serum, except for two negative APP (transferrin and albumin) which have been found up-regulated.

3.4.2. Immunoregulatory proteins: inflammation and innate immune response
Several proteins implicated in inflammation and innate immune response have been found differentially expressed in this study (Table 1). Some of them are proteins with anti-inflammatory effects such as APOA4 and ANXA1 that were up-regulated. The anti-inflammatory effects of APOA4 on intestinal mucosa and its possible role in innate immune response modulation have been shown in patients with Crohn’s disease [18] and in the mouse model of acute colitis [19]. Also, ANXA1 is a potent anti-inflammatory effector implicated in the regenerative mucosal response during periods of inflammation [20]. In the same way, three porcine serine proteinase inhibitors (SERPIN) were found up-expressed in our study. These proteins might act by limiting the cell damage of proteases, such as elastase, during the inflammatory response [21]. For two of these porcine serpins (alpha-1-antichymotrypsins 1 and 3) two different spots by protein were identified as being the same protein (Table 1 and Fig. 2). This might be due to different posttranslational modifications since these are glycosylated proteins, although this could not be confirmed with our data.

Another up-regulated immunoregulator was vitamin D-binding protein (Gc-globulin, GC), a chemottractant of circulating neutrophils and immunocompetent cells to inflammatory foci, which can be converted into a macrophage-activating factor (Gc-MAF) through partial deglycosylation [22]. MS identification of the spots in the gel did not permit us to know the glycosylation status of the proteins identified; therefore, it is not possible to know if we identified GC or Gc-MAF.

As shown in Table 1, infection induced up-regulation of tryptophanyl-tRNA synthetase (WARS), which could be related to apoptosis induction in ileum but also with increased inflammation due to its role as an inflammatory cytokine [23]. However, its cytokine activity relies on its cleavage for extracellular proteases such as elastase [23], whose activity could be reduced due to up-regulation of serpins, as it has been described above. An increased expression of the gene coding for this protein has been previously reported after S. Typhimurium infection in porcine mesenteric lymph nodes [24]. Finally, it is interesting to note the down-regulation of FAM49B, a protein whose function is not yet well established, but interacts with human IκB kinase epsilon [25], is a key regulator of NF-κB activity [26], and could be implicated in the inflammatory response upon Salmonella infection.

Altogether, up-regulation of WARS and GC would favor an inflammatory process. On the contrary, the up-regulation of APOA4, ANXA1, SERPIN-3 1 and 3 and SERPINB1 might be indicating a reduction of the inflammatory response in ileum mucosa infected by S. Typhimurium. From our experimental design we cannot concluded whether this is a ‘normal’
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<th>( \text{pl} )</th>
<th>Theoretical ( \text{Mr} )</th>
<th>( \text{pl} )</th>
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physiological regulatory mechanism to prevent excessive immune reactions that can be harmful to the host or whether it is an attenuation of the host response induced by the pathogen.

Also, S. Typhimurium infection induces the up-regulation of the antimicrobial protein intelectin-2 (Table 1). As part of the innate immune defenses, intelectin deposition in the epithelial brush border is supposed to shield against microbial infection [27]. Whether a higher content of intelectin-2 prevents S. Typhimurium efficient attachment to epithelial cells is not known.

3.4.3. Protein folding and posttranslational modifications: regulation of the immune response and apoptosis

S. Typhimurium infection induced the up-regulation of proteins involved in protein folding and posttranslational modifications (Table 1). Increased demand of these proteins may occur due to a high diversity of cell processes. Nevertheless, we have identified the regulation of some proteins with an important role in host immune response and which could be involved in Salmonella pathogenesis.

It is worth mentioning the pathogen-induced up-regulation of FKBP4, a protein with an important role in immune response since it acts as a regulator of the transcription factor IRF4 [28]. IRF4 regulates Th2 response but it is also a crucial regulator of Th17 differentiation [29,30]. Moreover, IRF4 activity is essential to dendritic cells (DC) development [31] and it has been reported that Salmonella can impair host adaptive immunity through interference with DC function [32]. FKBP4 inhibits IRF4 DNA binding and transactivation functions by a posttranslational modification of IRF4 [28]. Recently, it has been shown that an IRF4 binding protein inhibits IL-17 and IL-21 production by controlling the activity of IRF4 [33]. Therefore, FKBP4 protein up-regulation identified in our study could be inhibiting both Th2 and Th17 response in ileum mucosa upon S. Typhimurium infection. However, it is currently established that S. Typhimurium infection does not induce a Th2 type response, so could be an interesting question to find out if this bacteria affects the highly pro-inflammatory Th17 response. In order to check this, we analyzed the gene expression of two sets of cytokines involved in Th2 and Th17 response in ileum mucosa, i.e. IL-4, IL-5 and IL-13 for Th2 subset and IL-17a, IL-21 and IL-23 for Th17 subset (Table 2). Although some of the differences are not statistically significant, our RT-qPCR results indicated a general trend toward inhibition of the genes coding for both groups of cytokines (Table 2). Therefore, the down-regulation observed for cytokines of Th17 cells subset could be another specific characteristic of Salmonella infection at mucosa level.

In addition, it is worth noticing that IRF4 competes with IRF5, an inducer of pro-inflammatory cytokine gene expression, for binding MyD88, thus acting as a regulator of TLR dependent inflammatory response [34,35]. We did not find any references informing that FKBP4 modifications on IRF4 affect its affinity for MyD88. Then, we checked the mRNA regulation of three IRF5 targeted genes, i.e. IL-6, IL-12 and TNF-α [34] (Table 2). The lack of up-regulation found in these genes would support the premise that IRF4 could be efficiently carrying out the down-modulation of the inflammatory response at 2 dpi. Nevertheless, these results should be regarded with caution since this latter group of genes can also be regulated for other transcription factors (e.g. IRF1, IRF8 or NFκB). Moreover, further functional research is needed to prove that an effective hijack of MyD88 by IRF4 in competition with IRF5 relies on FKBP4 posttranslational modifications of IRF4. At this point it is of interest to remark that we found an up-regulation in two different spots identified as FKBP4 (Table 1 and Fig. 2).

| Table 2 – Regulation of the expression of Th1, Th2 and Th17 cytokine genes in porcine ileum mucosa at 2 days after Salmonella Typhimurium infection. |
|---|---|---|---|---|---|---|
| Th2 | Fold-change | P | Th17 | Fold-change | P | Th1 (IRF5 targeted genes) | Fold-change | P |
| IL-4 | -1.3 | 0.114 | IL-17A | -21.0 | 0.047 | TNF | 1.4 | 0.343 |
| IL-5 | -6.2 | 0.029 | IL-17A | -7.4 | 0.029 | IL-12p40 | -3.7 | 0.047 |
| IL-13 | -34.7 | 0.029 | IL-23 | -1.6 | 0.343 | IL-6 | -1.2 | 0.486 |

* Mann–Whitney U test P value between infected and non-infected animals. P values < 0.05 were considered significant.
These could represent two different posttranslational modifications that could be involved in the functionality of this protein.

Finally, and due to the intracellular location of S. Typhimurium during pathogenesis, it is interesting to highlight that Myd88-mediated IRF4 activation is signaled through TLR-7 and TLR-9, both of them located in the endosomes [34]. Therefore, the S. Typhimurium-induced up-regulation of FKBP4 could in addition be a mechanism of TLR-7 and 9 signaling blockage.

Apoptosis is a common host-response to limit bacterial infection [36]. It is known that S. Typhimurium induce cell death in epithelial cells, macrophages, and DC [37,38], but it is also known that bacteria have developed strategies to subvert apoptotic signaling pathways in host cells as a survival mechanism [39]. In this proteomic approach, we have identified the up-regulation, after S. Typhimurium infection, of some proteins such as heat shock protein beta-1 (HSPB1), heat shock cognate 71 kDa protein (HSPA8), thymidylate synthase and the protein thioredoxin domain containing 5 (TXNDC5), known to be involved in anti-apoptotic signaling and in cell survival promotion and proliferation [40–43]. In this sense, the up-regulation of two glycolytic enzymes (GPD2 and G6PD) and a subunit of the ATP synthase (ATP5B) could indicate that an active production of energy would be taking place in the mucosa upon S. Typhimurium infection. Thus, it has been reported that activated macrophages during inflammation utilize significant amounts of glycolytic-generated ATP to maintain mitochondrial membrane potential and prevent apoptosis [44]. Taken together, these results and the regulations of ANX1, WARS and FCSN1 mentioned above, might be in agreement, on the one hand, with the existence of stimuli inducing apoptosis in intestinal mucosa after pathogen challenge and, on the other, with the activation of cell mechanisms to prevent it.

3.4.4. Cytoskeleton related proteins
The induction of host-cytoskeleton modifications by Salmonella effectors has been a subject of extensive study [45–47]. In our in vivo infection, all cytoskeletal structural proteins identified were up-regulated 2 days after the S. Typhimurium challenge (Table 1). In turn, a variable regulation occurred for proteins involved in cytoskeleton assembly, dynamics and signaling such as villin, fascin homolog 1 (FSCN1) and debrin-like protein (DBNL). Thus, the up-regulation of DBNL could be implicated in cytoskeleton changes related to immune response, since this protein is fundamental for successful phagocytosis of S. Typhimurium by neutrophils, endocytosis and antigen presentation by B cells [48,49]. Also, DBNL is a key component of the immunological synapses that modulates T-cell activation [50]. On the other hand, the down-regulation of FSCN1 might be related to the induction of apoptosis in neutrophils [51], although it could also lead to a reduced maturation of DC and impaired NK cell activation by DC [52,53]. Other cytoskeletal proteins found as differentially expressed in our study might play an important role in SCV dynamics since RA6 mediates dynactin recruitment to Golgi membranes and is involved in cargo transport through microtubules [59]. Other protein which has been found up regulates after infection is MAPRE1 that could be also involved in the perinuclear migration of SCV through microtubules because it is implicated in microtubule stabilization and associates with members of the dynactin complex [60,61].

Although most bacteria reside in the SCV, a fraction of the intracellular population can also be found in the cytosol [45]. It has been shown that both cytosolic bacteria and SCV-associated S. Typhimurium are targeted by the autophagy system associated with ubiquitinated proteins [62,63]. In this work we found an up-regulation of two proteins with ubiquitin-conjugating activity (E2Z and E2K, Table 1) that might be involved in these processes, but also in apoptotic ones.

Related to intracellular survival of the bacteria could be the up-regulation of leukocyte elastase inhibitor (SERPINB1) (Table 1), a fast acting inhibitor of neutrophil elastase, involved in the degradation of the bacterium during phagocytosis. Neutrophil elastase efficiently inhibits virulence factors of Shigella, Yersinia and Salmonella [64]. An important increment in
the intracellular SERPINB1 protein occurs in vitro after Yersinia pestis and Y. pseudotuberculosis exposure [65]. These and our results would support the up-regulation of SERPINB1 as a common strategy of Yersinia and Salmonella to evade host immune defense.

4. Concluding remarks

In this study we show proteomics changes due to the interaction in vivo between the enteropathogenic bacteria S. Typhimurium and porcine ileum mucosa. Our general pattern reflects an anti-inflammatory response, regulation of apoptotic signaling and a possible attenuation of antigen presentation and engagement of acquired response. Proteome changes due to pathogen cell entrance and/or increased phagocytosis were also identified. Although our approach did not let us assign proteome changes directly to Salmonella effectors, some identified regulation suggests an active modulation of the porcine immune response by the pathogen. Finally, results derived from this study would also be of relevance as an in vivo model for human salmonellosis.

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The authors declare no conflict of interests.

REFERENCES


