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Eimeria bovis-induced modulation of the host cell proteome at the meront I stage

Kathleen Lutz[a], Sigrid Schmitt[b], Monica Linder[b], Carlos Hermosilla[c,*], Horst Zahner[a], Anja Taubert[a]

[a] *Institute of Parasitology, Justus Liebig University Giessen, 35392 Giessen, Germany*
[b] *Institute of Biochemistry, Justus Liebig University Giessen, 35392 Giessen, Germany*
[c] *Department of Pathology and Infectious Diseases, Royal Veterinary College, Hawkshead Lane, North Mymms, Hatfield, Herts AL9 7TA, UK*

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ABSTRACT

The proteome of *Eimeria bovis* meront I-carrying host cells was analyzed by two-dimensional gel electrophoresis (2DE) at 14 days p.i. and compared to non-infected control cells. A total of 221 protein spots were modulated in their abundance in *E. bovis*-infected host cells and were subsequently analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectometry (MALDI-TOF-MS). These analyses identified 104 proteins in total with 25 host cell proteins being up-regulated and 79 proteins being down-regulated in *E. bovis*-infected host cells. Moreover, 20 newly expressed proteins were identified exclusively in *E. bovis*-infected host cells and were most likely of parasite origin. Parasite-induced differences in protein abundance concerned distinct functional categories, with most proteins being involved in host cell metabolism, cell structure, protein fate and gene transcription. Some of the modulated molecules also indicated regulatory processes on the level of host cell stress response (HSP70, HSP90), host cell apoptosis (caspase 8) and actin elongation/depolymerization (α-actinin-1, gelsonin, tropomodulin-3, transgelin). Since merozoites I were already released shortly after cell sampling, the current data reflect the situation at the end of first merogony. This is the first proteomic approach on *E. bovis*-infected host cells that was undertaken to gain a rather broad insight into *Eimeria*-induced host cell modulation. The data processed in this investigation should provide a useful basis for more detailed analyses concerning *Eimeria*–host cell interactions.

1. Introduction

Eimeria bovis is an obligate intracellular apicomplexan parasite which represents one of the most pathogenic species in cattle coccidiosis, causing severe haemorrhagic typhlocolitis in calves and high economic losses worldwide [1]. Within the gut, freely released *E. bovis* sporozoites traverse the epithelium in order to invade endothelial cells of the central lymph capillaries of the ileal villi, where they undergo the first merogony [2]. Enclosed by a parasitophorous vacuole (PV), the sporozoites of *E. bovis* develop into ≥300 μm sized macromeronts within 14–18 days [3,4]. It appears likely that once the parasite begins growth and proliferation it must acquire nutrients from the host cell as reported for other intracellular apicomplexans [5,6]. Furthermore, the massive replication and the final enlargement of the host cell far beyond the physiological size may cause considerable stress to the host cell [7,8], and cell stress, in turn, is well known to trigger host cell defense mechanisms and apoptosis [9]. In consequence, the obligate intracellular parasite *E. bovis* must rely on several regulatory processes to actively modulate the host cell proteome to guarantee survival and first merogony development.

The general capacity of apicomplexan parasites to manipulate infected host cells for their advantage is well-documented in case of *Toxoplasma gondii*. This particular parasite acquires auxotroph nutrients from infected host cells, interacts with the host cell mitochrondria, endoplasmic reticulum and cytoskeleton [10–12] and actively interferes with the apoptotic capacity of the host cell [10,13–15].

In the case of *E. bovis* only few reports deal with its capacity for host cell modulation, although its merogony takes much longer and results in much larger intracellular meronts than *T. gondii*. Some evidence is reported on the level of distinct modulation of host cell cytoskeleton [16] and inhibition of host cell apoptosis by enhanced expression of anti-apoptotic factors [17], the latter of which is in agreement with other *Eimeria* spp. [18]. Activation of NFκB in sporozoite-infected, non-permissive epithelial host cells was shown for *E. bovis* as well [19].

* Corresponding author. Tel.: +44 1707666040; fax: +44 1707667052.
E-mail address: chermosilla@rvc.ac.uk (C. Hermosilla).

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E. bovis also appears to modulate host endothelial cell-mediated immune reactions [20–23]. At least generally transcript levels of genes encoding for pro-inflammatory molecules in *E. bovis*-infected endothelial cells were significantly lower when compared with T. gondii- or N. caninum- infected cells [22].

The aim of this study was to gain a broad insight into *E. bovis*-induced host cell proteome modulation at the meront I stage. Therefore, whole cell proteomes of *E. bovis*-infected host cells and non-infected controls were analyzed by two-dimensional gel electrophoresis (2DE) followed by the identification of individual spots by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). Overall, 27 host cell proteins were found up-regulated and 82 proteins down-regulated in host cells carrying mature *E. bovis* meronts I, the majority of them being involved in cell metabolism, cell structure, protein synthesis and gene transcription. In addition, we identified 20 proteins in infected cells which were most probably of parasite origin. With these data we create a basis for more detailed analyses targeting functional *E. bovis*–host cell interactions.

2. Materials and methods

2.1. Parasite

The *E. bovis* strain H used in the present study was isolated in the field in northern Germany [24] and maintained by passages in parasite-free Holstein Friesian calves. For oocysts production, calves were infected orally at the age of 10 weeks with 5×10^5 sporulated *E. bovis* oocysts. Excreted oocysts were isolated from the faeces beginning 18 days p.i. according to the method of Jackson [25]. Sporulation of oocysts was achieved by incubation in a 2% (w/v) potassium dichromate (Merck) solution at room temperature (RT). Sporulated oocysts were stored in this solution at 4 °C until further use.

Sporozoites were excysted from sporulated oocysts as previously described [26]. Free sporozoites were washed three times in phosphate buffered-solution (PBS), resuspended in complete Iscove's modified Dulbecco medium (IMDM; Gibco) and counted in a Neubauer haemocytometre as described elsewhere [27].

2.2. Host cells and parasite infection

Bovine foetal gastrointestinal cells (BFGC), an immortalized primary cell line originally isolated from *in utero* foetuses 4–6 months after conception [26], were cultured in complete IMDM medium supplemented with 500 U/ml penicillin, 50 μg/ml streptomycin (Sigma), 1% L-glutamine (w/v) and 10% heat-inactivated foetal calf serum [(v/v); FCS; Gibco]. Cells were cultured in 75 cm^2 tissue culture flasks (Nunc) and incubated (37 °C, 5% CO_2 atmosphere) until confluency. BFGC monolayers were infected with 5×10^5 freshly excysted sporozoites. Sporozoite viability was determined by trypan blue exclusion test according to Lang et al. [17]. Cell culture medium was changed 1 day after infection and thereafter every third day. One-day p.i. infection rates of the BFGC cultures were determined microscopically.

2.3. Sample preparation for the two-dimensional gel electrophoresis (2DE)

Previous transcriptome studies on *E. bovis*-infected host cells revealed most molecules being modulated at 14 days p.i. when compared to earlier time points of infection. In accordance to these results we restricted the current analysis to this time point. At 14 days p.i. whole cell preparations of *E. bovis*-infected and non-infected BFGC were subjected to 2DE. BFGC layers were washed three times with PBS. Cells were scraped off the tissue culture flasks using a sterile rubber policeman (Nunc) and resuspended in PBS. The cell suspension was pelleted (600 × *g*, 15 min) and washed two times with sorbitol–buffer [25 mM sorbitol, 10 mM Tris-base (v/v, pH 7); all Sigma]. Cells were lyzed in a lysis buffer [8 M urea, 2 M thiourea, 4% CHAPS, 30 mM DTT, 20 mM Tris, 2% pharmalyte buffer (v/v, pH 3–10); all Sigma] and stored at −80 °C.

For 2DE 375 U of benzoase (Calbiochem) and 8 μl protease inhibitor (Sigma) for mammalian cells were added to each sample and samples were incubated (30 min, RT), sonicated twice (200 W, working time 10 s, interval time 15 s, 50 cycles, ice bath) and centrifuged (21,000 × *g*, 30 min, 20 °C). The supernatant was precipitated with acetone [1:7; (Merck)] and centrifuged (8510 × *g*, 30 min, 20 °C). The resulting pellet was washed in acetone (Sigma) and resuspended in the above-described lysis buffer. Solubilized proteins were then quantified using the Plus One™ 2D Quant Kit® technique (GE Healthcare Life Sciences) and subjected immediately to 2DE.

2.4. Iso-electro focusing (IEF) and 2DE

Proteins were solubilized by incubating the washed pellet at least 1 h at RT in iso-electro focusing rehydration buffer onto immobilized pH gradient (IPG) strips [pH 3–11 non-linear; length 11 cm; DryStrip® (GE Healthcare Life Science)]. Each strip was rehydrated overnight with 300 μg of protein sample in 220 μl lysis buffer. The IEF was carried out in a Multiphor chamber® (Amersham Biosciences) at 20 °C applying the following conditions: *Phase 1*: gradient from 0 V to 50 V for 0.25 kVh; *Phase 2*: gradient from 100 V to 3500 V for 11 kVh; *Phase 3*: 3500 V for 21 kVh. Current was limited to 0.25 mA per strip. For the second dimension the protein-loaded IPG strips were equilibrated for 15 min by rocking in DTT-equilibration buffer [10 mg/ml 1,4-dithio-DL-threitol, 20 mg/ml sodium dodecyl sulfate (SDS) in 6 M urea (all Fluka), 30% (v/v) glycerin (Sigma), 50 mM Tris–HCl (Merck), pH 8.8, 4% (w/v) SDS 0.01% bromophenol blue (Fluka)] and then washed for 15 min in jodacetamid-solution [40 mg/ml jodacetamid (Fluka), 20 mg/ml SDS in 6 M urea, 30% (v/v) glycerin, 50 mM Tris–HCl, pH 8.8, 4% (w/v) SDS 0.01% bromophenol blue (all Fluka)]. The strips were then embedded on a precast separating gel [12.5%, v/v acrylamide (Fluka)] and sealed into place with agarose-solution (Fluka). Gel electrophoresis was carried out at 25 °C applying a constant voltage of 500 V for 4.5 h in a 14.5 cm × 14.5 cm vertical gel electrophoresis chamber (Hoefer 600®; Amersham Biosciences).

The gels were then stained by colloidal Coomassie (Fluka). Thereafter, the 2DE gels were agitated for 60 min in staining solution [0.2% (w/v) Coomassie R 250, 50% (v/v) MeOH, 50% (v/v) ddH_2O] and then overnight in stripping solution [50% (v/v) MeOH, 6% (v/v) acetic acid, 44% (v/v) ddH_2O]. Analysis of 2DE gels was performed using a flatbed scanner (Powerlook 2100 XL®; Umax) with a resolution of 600 dpi. For each sample, four gels were run and analyzed in parallel.

2.5. Image analysis

Differences in protein abundance between non-infected and *E. bovis*-infected BFGC samples were detected by image analysis using the software Proteomweaver® (Version 4.0; BioRad). To prevent that proteins originating from *E. bovis*-macromeronts may falsify the total amount of host cell protein, a consistently expressed "house-keeping" protein was needed for calibration. Since recent studies showed that vimentin intermediate filaments are not altered in their abundance in *E. bovis*-infected host cells during the first merogony [16] we chose the bovine vimentin spot as internal reference to normalize the 2DE gels of infected and non-infected BFGC. Therefore five vimentin spots were identified in 2DE gels by immunoblotting (anti-vimentin, clone Vim