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Schistosoma mansoni infection affects the proteome and lipidome of circulating extracellular vesicles in the host

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ABSTRACT

Eggs, schistosomula and adult *Schistosoma* worms are known to release extracellular vesicles (EV) during *in vitro* incubations and these EVs are postulated to affect the host responses. So far only those EVs released during *in vitro* incubations of schistosomes have been studied and it is unknown whether in blood of infected hosts the schistosomal EVs can be detected amidst all the circulating EVs of the host itself. In this study we analyzed the protein as well as the phospholipid composition of EVs circulating in blood plasma of *S. mansoni* infected hamsters and compared those with the EVs circulating in blood of non-infected hamsters. Although neither proteins nor lipids specific for schistosomes could be detected in the circulating EVs of the infected hamsters, the infection with schistosomes had a marked effect on the circulating EVs of the host, as the protein as well as the lipid composition of EVs circulating in infected hamsters were different from the EVs of uninfected hamsters. The observed changes in the EV lipid and protein content suggest that more EVs are released by the diseased liver, the affected erythrocytes and activated immune cells.

1. Introduction

Extracellular vesicles (EVs) have been defined by the International Society for Extracellular Vesicles (ISEV) as the generic term for particles naturally released from the cell, that are delimited by a lipid bilayer and cannot replicate, *i.e.* do not contain a functional nucleus [1]. EVs vary substantially in size (*ca.* 30–1000 nm in diameter), density (*ca.* 1.13–1.19 g/ml) and biochemical composition (*e.g.* proteome, lipidome, DNA and RNA content, *etc.*) [2]. EVs are released by most types of cells and organisms, including parasites [3]. Three types of EVs can be distinguished, 1) Exosomes are approximately 40 − 100 nm in diameter and are released by most cell types; they are formed within the endosomal network and their secretion is the result of inward budding and fusion of multi-vesicular bodies with the plasma membrane, 2) Microvesicles/microparticles can be difficult to distinguish from exosomes and can be up to 1 μm. They are formed by outward budding of the plasma membrane, incorporating lipids, proteins and other compounds before budding, 3) Apoptotic bodies are the largest vesicles with a size of 1–5 μm and they are formed as blebs of cells undergoing apoptosis [4]. Were EVs first regarded to be involved in cellular waste management, EVs are now thought to be important as mediators of intercellular communication [1–3]. In recent years, the knowledge on EVs has increased exponentially and it has been demonstrated that the plasma concentration of EVs changes during disease, and that EVs may function as biomarker for various diseases [5,6]. In addition, there is accumulating evidence that the host immune system is affected by the lipids, nucleic acids and/or glycans contained in, or present on, pathogen derived EVs [7,8]. From a host-pathogen interaction

Abbreviations: EVs, extracellular vesicles; PC, phosphatidylcholine; PCA, Principle Component Analysis; PE, phosphatidylethanolamine; PS, phosphatidylserine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SM, sphingomyelin; TEM, transmission electron microscopy

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perspective, schistosomiasis is an intriguing disease as it is caused by blood-dwelling flukes. Despite the hostile environment that comprises all types of host immune cells and antibodies, the adult worm pairs have an average life-span of 5–10 years [9]. Adult worm pairs of *Schistosoma mansoni*, the best studied *Schistosoma* species, inhabit the mesenteric veins of the host and here the adult female produces up to 300 eggs per day. Eggs are either shed into the environment *via* the faeces, or are by chance retained in host tissues where they induce inflammation and subsequently granuloma formation [10]. Eggs as well as adult *S. mansoni* excrete factors that manipulate the host immune system and host haemostasis for their own survival [11–13]. However, the mechanisms applied by schistosomes in the interaction with their hosts, are not yet fully understood.

Previous research has shown that *S. mansoni* schistosomula and adult worms release EVs during *in vitro* incubations and that these EVs contain microRNAs (miRNAs) and proteins [14–16]. These reports demonstrated that schistosome derived EVs comprise common EV markers, such as heat shock proteins, enzymes involved in energy metabolism and cytoskeletal proteins. Furthermore, adult worms and eggs of *Schistosoma japonicum* are known to release EVs containing miRNAs and proteins during *in vitro* incubations [17,18]. Analysis of the contents of these EVs suggests that schistosome-derived EVs may affect the host responses [19–21].

So far only those EVs released during *in vitro* incubations of schistosomes have been studied and it is unknown whether in blood of infected hosts the schistosomal EVs can be detected amidst all the circulating EVs of the host itself. In our study we analyzed the protein as well as the phospholipid composition of EVs circulating in blood plasma of *S. mansoni* infected hamsters and compared those with the EVs circulating in blood of non-infected hamsters. In these investigations neither proteins nor lipids specific for schistosomes could be detected in the circulating EVs of the infected hamsters. However, infection with schistosomes had a marked effect on the circulating EVs of the host, as the protein as well as the lipid composition of EVs circulating in infected hamsters were different from the EVs of uninfected hamsters.

2. Material and methods

Puerto Rican strain of *S. mansoni* was maintained in Golden hamsters (*Mesocricetus auratus)* for which animal ethics was approved (license EUR1860-11709). Animal care and maintenance was in accordance with institutional and governmental guidelines. In this study five hamsters infected with *S. mansoni* were used and five control hamsters, which were housed and fed identically to the infected hamsters. Blood was collected seven weeks after infection with approximately 600 cercariae per hamster. Infected and control hamsters were housed and fed identically. After seven weeks, the hamsters were anesthetized with isoflurane and whole blood was obtained by heart puncture and collected in tubes with trisodium citrate as an anticoagulant. Citrated platelet poor plasma was obtained by two-fold centrifugation of citrated blood at 2000*g* for 15 min at room temperature. Subsequently, EVs were isolated by a well-established method using size-exclusion chromatography with a cut-off of 70 nm as described earlier [22]. In short, platelet poor plasma was loaded on a Sepharose CL-2B column (30 mL, GE Healthcare; Uppsala, Sweden), that was washed with PBS containing 0.32 % trisodium citrate (pH 7.4). Subsequently, fractions were eluted with PBS/0.32 % citrate (pH 7.4). The collected fractions were further analyzed using electron microscopy after which the EV containing fractions were stored at —80 °C until further analysis.

To determine the protein composition of EVs, 3 samples of infected and 3 samples of control hamsters and individually processed for proteome analysis. The proteins were first separated by SDS-PAGE, after which the lanes of the SDS-PAGE were subsequently divided into 10 slices and the proteins were then subjected to in-gel tryptic digestion as described by Shevchenko et al. [23]. After digestion the samples were analyzed on an Orbitrap Q-Exactive (Thermo Fisher Scientific, Waltham, MA, USA) connected to a UHPLC Proxeon Easy-nLC 1000 (Thermo Fisher Scientific, Waltham, MA, USA). Peptides were trapped on a double-fritted trap column (Dr. Maisch Reprosil C18, 3 µm, 2 cm × 100 µm (Dr. Maisch HPLC GmbH, Ammerbuch-Entringen, Germany)) and separated on an analytical column (Agilent Zorbax SB-C18, 1.8 µm, 40 cm × 75 µm (Agilent, Santa Clara, CA, USA)). Solvent A consisted of 0.1 M acetic acid, solvent B of 0.1 M acetic acid in 80 % acetonitrile. Samples were loaded at a pressure of 800 bar with 100 % solvent A. Peptides were separated by a 30 min gradient of 10–30 % buffer B followed by 30–100 % B in 2 min, 100 % B for 2.5 min at a flow rate of 150 nL/min. Full scan MS spectra were acquired in the Orbitrap ($350-1500$ m/z, resolution 35,000, AGC target 3e6, maximum injection time 250 ms). The 20 most intense precursors were selected for HCD fragmentation (isolation window 1.2 Da, resolution 17,500, AGC target 5e4, maximum injection time 120 ms, first m/z 100, NCE 33 %, dynamic exclusion 60 s). The results were normalized on the Total Ion Count, then pooled for the 3 infected and the 3 control hamsters and subsequently filtered using Percolator [24,25] to a false discovery rate (FDR) below 1%. We further only accepted peptides with at least six amino acid residues, a Mascot ion score of at least 20, and search engine rank of 1 and at least 2 identified peptides for protein identification. Scores (Mascot ion score) and peptide spectrum matches (PSM; total number of identified peptide spectra matched for the protein) were used to compare the samples. Analysis of the data to annotate the peptides was performed with Scaffold using the *S. mansoni* database (Schistosoma_mansoni_v5.2.fa, Wellcome Trust Sanger Institute, Hinxton, UK, accessible from: ftp://ftp.sanger.ac.uk/pub/project/pathogens/Schistosoma/mansoni/Archive/S.mansoni/genome/) and the genomic information available for the golden or Syrian hamster (*Mesocricetus auratus*) accessible from ftp://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/349/665/GCF_000349665.1_MesAur1.0 The set of identified proteins in EVs derived from infected and control hamster plasma was normalized on the albumin score and further analyzed using the "FunRich" software package, which allows functional and comparative analysis with EV proteome data from other studies [26]. As FunRich does not contain hamster proteins, gene analogs coding for the identified proteins in hamsters were determined in the genome of *Mus musculus*. For 38 out of the 42 identified hamster proteins, gene homologues in the *M. musculus* genome could be automatically retrieved from the Uniprot database. The four remaining proteins were manually annotated by determining the most similar protein and its corresponding gene *via* BlastP analysis. The resulting 42 *M. musculus* genes (nomenclature according to NCBI gene) were used as a query in FunRich to identify whether the proteins encoded by these genes had been detected in earlier studies in EVs derived from blood plasma.

The phospholipid composition was determined at species level by Liquid Chromatography coupled to Mass Spectrometry (LC–MS). The extracted lipids were loaded on a hydrophilic interaction liquid chromatography (HILIC) column (2.6 µm HILIC 100 Å, 50 × 4.6 mm, Phenomenex, Torrance, CA) and eluted at a flow rate of 1 mL/min with a gradient from acetonitrile/acetone (9:1, v/v) to acetonitrile/H_2O (7:3, v/v) with 10 mM ammonium formate. Both elution solutions also comprised 0.1 % (v/v) formic acid. The column outlet of the LC was connected to a heated electrospray ionization (HESI) source of an LTQ-XL mass spectrometer (ThermoFisher Scientific, Waltham, MA). Full scan spectra were collected from m/z 450–1050 at a scan speed of 3 scans/s. For analysis the data were converted to mzXML format and analyzed using XCMS version 1.52.0 running under R version 3.4.3 [27]. Principle Component Analysis was provided by the R package PCA Methods [28].

3. Results

EVs were isolated from citrated plasma collected from either five control or five *S. mansoni* infected hamsters by size-exclusion

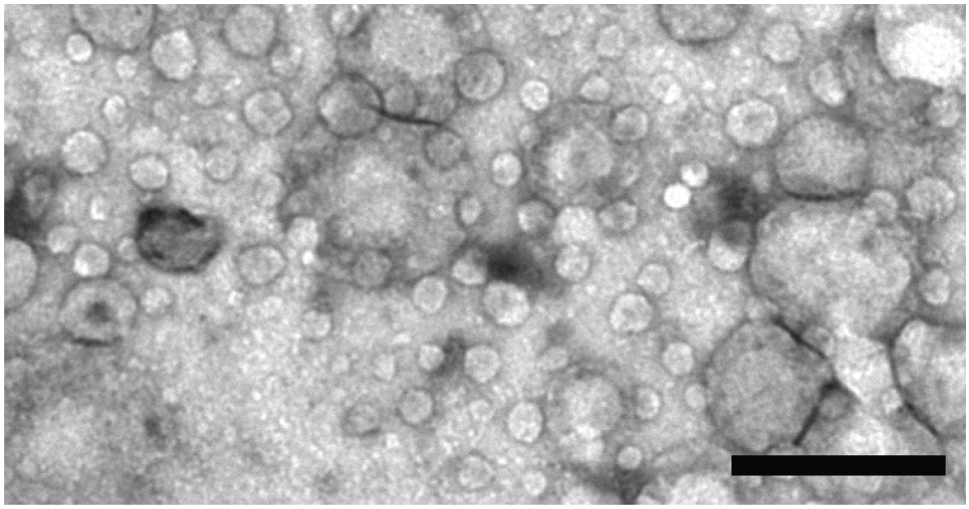

Fig. 1. EM photograph of extracellular vesicles isolated from plasma of a hamster infected with *Schistosoma mansoni*. Visible are donut-shaped extracellular vesicle (EV)-like structures ranging in size from 50 to 150 nm. The scale bar denotes 200 nm.

chromatography as described earlier [22]. The presence of EVs was confirmed by transmission electron microscopy (TEM) imaging, as shown in Fig. 1. The isolated vesicles range in size from about 50 nm–150 nm, which is in agreement with the reported sizes of EVs released by schistosomes as well as of EVs isolated from plasma [14–16,29].

The protein composition in the isolated EV fraction was determined by LC–MS/MS using a MASCOT database with hamster proteins as well as *S. mansoni* proteins. By this analysis no proteins of schistosomal origin were discovered and all identified proteins were of host origin (hamster) (Supplementary Table 1 and data are available *via* ProteomeXchange with identifier PXD019809). However, infection with *S. mansoni* had an effect on the protein content of the EVs of the host, as remarkable differences in protein composition were observed between the EVs of infected and non-infected hamsters (Fig. 2). In total 42 proteins were identified, of which 14 proteins were exclusively detected in EVs of *S. mansoni* infected hamsters, 7 proteins exclusively in EVs of control hamsters and 21 proteins were present in EVs of infected as well as control hamsters (Fig. 2). Of those 21 identified proteins present in EVs from the infected and from the control hamsters, three proteins were more than 2-fold enriched and two proteins were over 2-fold decreased in EVs of infected hamsters compared with the EVs of control hamsters (Supplementary Table 1).

In order to compare the set of EV proteins identified in our study with those identified in earlier studies, the FunRich software package was used [26]. FunRich does not contain hamster proteins, therefore gene analogs coding for the identified proteins in hamsters were determined in the genome of *Mus musculus* (see Materials and Methods for details). The resulting 42 *M. musculus* genes (nomenclature according to NCBI gene) were used as a query in FunRich to identify whether the proteins encoded by these genes had been detected in EVs derived from blood plasma in earlier studies. This analysis showed that 36 out of these 42 proteins had been detected earlier in plasma-derived EVs (See Supplementary Table 1). In one of those nine studies [30] on EVs derived from plasma of healthy human volunteers, 30 of the 42 proteins of our study were identified (Supplementary Fig. 1). In that de Menezes-Neto et al. study [30](2015) a comparison was made between different EV isolation methods. That analysis revealed a core set of 17 proteins that were more frequently detected regardless of method or sample type. Of these 17 proteins, 11 (64.7 %) were present in our EV preparation (Supplementary Fig. 1).

Most of the identified EV proteins in our study are known blood plasma proteins, membrane proteins or proteins that are part of the cytoskeleton or extracellular matrix and they have a function in hemostasis, immunity or transport (Supplementary Table 1). Of the 14 proteins exclusively found in EVs derived from infected hamsters, four function in hemostasis (fibronectin, fibrinogen gamma chain, alpha-2 antiplasmin, thrombospondin-1), three are extracellular matrix proteins (collagen alpha-1, 2 and 3) and two are present in the cytoskeleton of erythrocytes (spectrin alpha chain, ankyrin-1).

We analyzed not only the protein content of the EVs, but also the lipid content of EVs derived from plasma of infected as well as control hamsters. This analysis demonstrated that the membranes of EVs of both infected and control hamsters comprised many distinct phospholipid species in all investigated major phospholipid classes: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylglycerol (PG) and phosphatidylinositol (PI) and sphingomyelin (SM) (Fig. 3 and Supplementary Data 1). Our earlier research demonstrated that the outer surface of adult *S. mansoni* contains several specific phospholipids, such as lyso-PS 20:1, PC 16:0/18:1 Δ5, PC 16:0/20:1, PI 16:0/18:0 and PI 18:0/20:1, that are not present in their mammalian host [31]. Not a single one of these schistosome-specific lipids was detected in the EVs derived from infected or control hamsters. However, principal component analysis (PCA) of the top 150 lipid signals demonstrated that the membrane lipid composition of EVs derived from infected hamsters differed consistently from that of control hamsters (Fig. 3, top left panel). Clear differences in PC species were observed between EVs derived from infected and control hamsters, as PCs 36:4 and 33:0 were enriched in infected hamsters, while PCs 36:2, 36:3 and 34:2 were decreased compared to control hamsters (Fig. 3, bottom panel). However, the most striking difference was the relative increase in nearly all PS and SM species and decrease of most PI and lyso-phospholipid species in EV membranes derived from infected hamsters compared to control hamsters.

4. Discussion

Host-parasite interactions in schistosomiasis have been a subject of research for decades and recently several studies focused on a possible role for EVs in this interaction. It has been shown that adult schistosomes, schistosomula and eggs, three life cycle stages of *S. mansoni* that are present in the mammalian host, release EVs when they are cultured *in vitro*, outside the host [15,16,32]. Using exponential amplification *via* quantitative reverse transcription polymerase chain reaction of microRNAs (miRNAs) the presence of schistosomal miRNAs was demonstrated in the EV fraction harvested from sera of human patients infected with schistosomes [33]. This shows that schistosomes release EVs not only during *in vitro* incubations but also when living in the

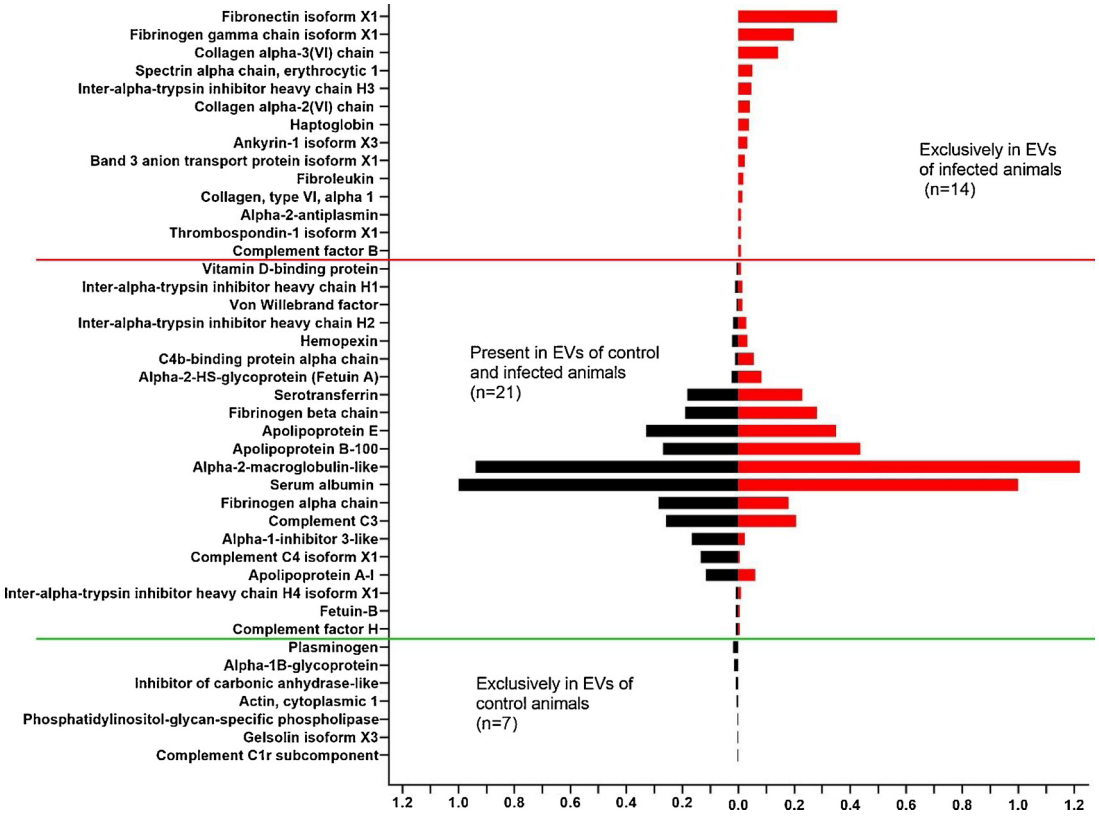

Fig. 2. Infection with *S. mansoni* modifies the protein composition of extracellular vesicles in host blood plasma. Proteins identified by LC–MS/MS in extracellular vesicles (EVs) isolated from blood plasma from five non-infected control host (black bars) *versus* five infected host (red bars). Protein content in each sample was normalized to the serum albumin content and the relative abundance of identified proteins is given in comparison to serum albumin. The top part shows the proteins only detected in EVs from infected hosts, whereas the bottom part shows the proteins detected only in EVs of non-infected, control hosts. The center box shows proteins detected in EVs of control as well as infected hosts. Proteins exclusively in EVs of infected animals OR exclusively present in EVs of control animals are ordered from high to low based on their absolute abundance. For proteins present in both samples, the ordering is centered around serum albumin (ratio = 1 infected/control). Proteins with a ratio > 1 (infected/control) are ordered low to high (absolute abundance of proteins present in vesicles isolated from infected animals), proteins with a ratio < 1 are ordered high to low (based on absolute abundance of proteins present in vesicles isolated from control animals). Supplementary Table 1 contains these and other data on the proteins in question.

bloodstream of the host. However, the effect of an *S. mansoni* infection on the composition of circulating EVs in blood of the host has not been investigated. It is not even known yet whether schistosomal proteins and lipids can be detected at all in EVs isolated from blood of a host infected with schistosomes, as amplification is not possible in those analyses in contrast to the analysis of miRNAs. Therefore, in this study we examined the EVs present in blood plasma of hamsters infected with *S. mansoni* and compared those with the EVs present in uninfected hamsters.

In our analysis of isolated EVs from plasma of *S. mansoni* infected hamsters no schistosome-specific proteins or lipids were found. Apparently, the presence in blood of the large number of EVs produced by the host [34] completely overshadows in the proteome and lipidome analysis the presence of EVs produced by the parasite. This observation correlates with the fact that despite many attempts so far no specific protein nor lipid markers of cancer-derived exosomes have been detected in plasma derived EVs, except from melanoma [35]. Although no schistosome-specific compounds were detected in EVs circulating in blood of infected hamsters, the *S. mansoni* infection resulted in an altered plasma EV profile in the host, as a consistent difference in the EV protein and lipid composition was observed between infected and non-infected hamsters (Fig. 2,3).

In our study we identified a relatively small number of proteins in the EV fractions compared to other studies [36,37] Compared to these studies many aspects of the protein identification method were similar to those used in our study, but two differences might explain the lower number of identified proteins in our study. First, the lower number of identified proteins could be caused by a difference in the quality of the genome information available of the golden hamster (*Mesocricetus auratus*) compared to that of humans and mice, as the golden hamster genome has not been assembled and curated as thoroughly as the genomes of mice and humans. These differences could have limited the identification of EV proteins in our study compared to studies performed on humans or mice. In addition, in our study the proteins in the EV fraction were separated by SDS-PAGE prior to trypsin digestion, whereas the other studies digested the proteins directly. For samples with a low protein concentration the SDS-PAGE method might be less efficient as reduced protein digestion and peptide diffusion out the gel pieces could result in a lower peptide yield.

Lipid analysis of the isolated EV fractions demonstrated that the EVs derived from plasma of *S. mansoni* infected hamsters contained relatively more PS and SM and less PI and PG compared to those of control hamsters. Phospholipid classes PS and SM are both enriched in plasma membranes [38,39] and the increased contribution of these two classes to the EVs of infected hamsters could reflect a shift from endosome- to plasma-membrane derived EVs, *i.e.* from exosomes to microvesicles. We also observed a shift in molecular species within the PC and PE classes. PC and PE are the two major phospholipid classes and contribute to all organelle membranes, albeit with different molecular species. In addition, a moderate decrease in lyso-PC content was observed in EVs derived from infected hamsters compared to control hamsters. However, lysophospholipids constitute only a minor, highly dynamic phospholipid fraction and are readily reacylated to their diacyl forms or further degraded to water soluble building blocks. Taken together, the

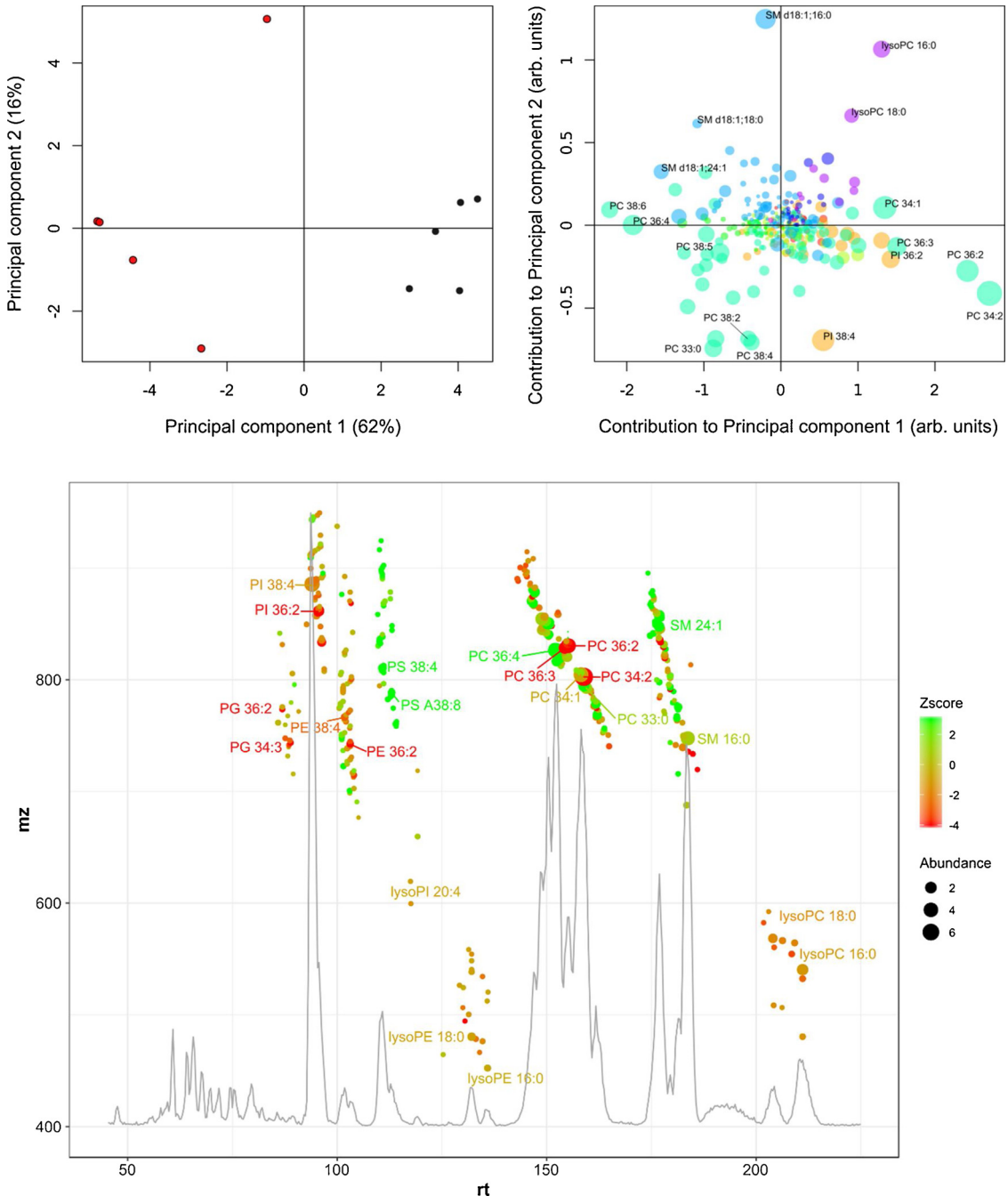

Fig. 3. Infection with *S. mansoni* modifies the phospholipid composition of extracellular vesicles in host blood plasma. Complete phospholipid analysis of extracellular vesicles (EVs) isolated from blood-plasma of five infected *versus* five non-infected hosts. The top left panel shows the score plot of PCA of lipid content from vesicles isolated from infected hosts (red) *versus* non-infected hosts (black). The top right panel shows the corresponding loadings plot of the PCA; sizes of lipid species correspond to abundance, colors depict lipid class; green (PC), orange (PI), cyan (SM) and purple (lysoPC). Bottom panel shows an overlay of a contour plot and the base peak chromatogram as recorded during the analysis of EVs isolated from an infected host. Detected lipid species are shown at their corresponding retention time (rt, in seconds) and the color scale indicates the relative increased (green) or decreased (red) abundance in vesicles isolated from infected hosts compared to non-infected hosts.

lipidomic data clearly demonstrate a shift of systemic EV composition in response to the schistosome infection.

The most abundant proteins exclusively present (Table 1), or enriched in EVs of infected hamsters, were fibronectin, fibrinogen, and collagen. These proteins play a significant role in the formation of liver fibrosis, which is known to be induced by the deposition of *S. mansoni* eggs [40]. Both fibronectin and fibrinogen were reported to be exclusively present or significantly increased in EV preparations of plasma from patients suffering from various liver diseases [41,42]. These results suggest that a substantial part of the circulating EVs in plasma of the *S. mansoni* infected host originate from the liver, probably as a response to tissue damage caused by egg deposition by the parasite.

Furthermore, the increased abundance of fibrinogen and haptoglobin could also result from an inflammatory response induced by the *S. mansoni* infection, because both proteins are so-called acute phase proteins. The exclusive detection of fibroleukin (FGL2) in EVs of infected animals further supports the inflammatory response as a cause of the changed EV proteome, as FGL2 secreted by T-cells is an important

Table 1
Protein function and possible link with schistosomiasis of proteins exclusively detected in extracellular vesicles of *S. mansoni* infected animals.

	Proteins exclusively detected in infected animals	Functional process	Possible link with the disease schistosomiasis
1	fibronectin isoform X1	extra-cellular matrix protein	liver fibrosis
2	fibrinogen gamma chain isoform X1	blood coagulation	liver fibrosis & inflammatory response (acute phase reaction)
3	collagen alpha-3(VI) chain	extra-cellular matrix protein	liver fibrosis
4	spectrin alpha chain, erythrocytic 1	linking cytoskeleton to erythrocyte plasma membrane	anaemia
5	inter-alpha-trypsin inhibitor heavy chain H3	plasma protease inhibitor	inflammatory response
6	collagen alpha-2(VI) chain	extra-cellular matrix protein	liver fibrosis
7	haptoglobin	plasma protein binding free haemoglobin	inflammatory response (acute phase reaction)
8	ankyrin-1 isoform X3	linking cytoskeleton to erythrocyte plasma membrane	anaemia
9	band 3 anion transport protein isoform X1	transporter protein in erythrocyte plasma membrane	anaemia
10	fibroleukin	immune regulatory factor secreted by T-cells	host inflammatory response
11	collagen, type VI, alpha 1	extra-cellular matrix protein	liver fibrosis
12	alpha-2-antiplasmin	haemostasis	hypo-coagulable and hyper-fibrinolytic state during schistosomiasis
13	thrombospondin-1 isoform X1	haemostasis	hypo-coagulable and hyper-fibrinolytic state during schistosomiasis
14	complement factor B	complement system	complement system activation on the surface of schistosomes

immune regulator of both innate and adaptive responses [43].

Of the other identified proteins enriched, or exclusively found in EVs derived from *S. mansoni* infected hamsters, several function in hemostasis (fibronectin, fibrinogen gamma chain, alpha-2 antiplasmin, thrombospondin-1 and Von Willebrand Factor). Hemostasis is affected by an *S. mansoni* infection, as schistosomes excrete and/or expose several factors that interfere with primary and/or secondary hemostasis as well as with fibrinolysis [12]. In addition, in hepatosplenic schistosomiasis many hemostatic abnormalities occur, which include impaired coagulation, lower levels of platelets, coagulation and anticoagulation factors, as well as increased thrombin generation, fibrinolysis and Von Willebrand Factor levels [44–46]. The coexistence of elevated thrombin and plasmin generation suggests a steady state of low-grade disseminated intravascular coagulation in hepatosplenic schistosomiasis, in which enhanced fibrin formation is coupled to enhanced fibrinolysis [44]. Since platelets are a major source of plasma EVs [34], parasite induced enhanced coagulation and thrombocytopenia is expected to result in an increase in platelet-derived EVs in *S. mansoni* infected hamsters. Indeed a substantial number of proteins was identified in EVs of *S. mansoni* infected hamsters, that have previously been reported as proteins present in EVs formed by platelets (thrombospondin-1, fibrinogen, albumin, gelsolin and actin) [47].

Also a relatively high number of complement system related proteins were identified in this study. Complement factor B and the C4b-binding protein, which has an inhibitory function, were exclusively detected, or enriched in EVs derived from *S. mansoni* infected hamsters, respectively. On the other hand complement factor C3, C4 and C1r were present in lesser amounts in EVs of infected hamsters compared to control hamsters. These results suggest that the complement system is activated by the *S. mansoni* infection, which fits with the earlier reported complement activation on the surface of adult schistosomes [48].

Finally, the detection of the specific erythrocyte plasma membrane proteins spectrin, ankyrin-1 and band 3 protein only in EVs isolated from infected animals suggests an increase in erythrocyte-derived EVs during schistosomiasis. Schistosomiasis is often associated with anemia, for which several causes have been postulated including malnutrition, blood loss and restricted erythropoiesis due to inflammation [49,50]. Thus, the increased formation of erythrocyte-derived EVs in *S. mansoni* infected animals suggests that during schistosomiasis more lysis of erythrocytes occurs and more improper erythrocytes are circulating, as EVs are thought to be shed by erythrocytes upon apoptosis or activation of cells by oxidative injury, endotoxin, cytokines, complement or high shear stress [51].

In conclusion, our results demonstrate that although *S. mansoni* eggs, schistosomula and adults are known to produce EVs, their relative contribution to the entire EV population circulating in plasma of their host is limited as no parasite-specific proteins nor lipids could be identified in EVs derived from plasma of infected hosts despite a relatively high infection dose. However, the *S. mansoni* infection does affect the EV composition in plasma of the host. Further research is necessary to determine the clinical relevance of these changes, and whether these changes are the result of the pathogenic effects of the infection or whether they have a function in the host-parasite interaction, or both.

Transparency document

The Transparency document associated with this article can be found in the online version.

CRediT authorship contribution statement

Michiel L. Bexkens: Investigation, Formal analysis, Visualization, Writing - original draft. **Renske A. van Gestel:** Methodology, Investigation, Data curation, Writing - review & editing. **Bas van Breukelen:** Methodology, Investigation, Data curation, Writing - review & editing. **Rolf T. Urbanus:** Writing - review & editing. **Jos F. Brouwers:** Methodology, Investigation, Data curation, Visualization, Writing - review & editing. **Rienk Nieuwland:** Methodology, Investigation, Writing - review & editing. **Aloysius G.M. Tielens:** Conceptualization, Methodology, Data curation, Writing - review & editing, Supervision. **Jaap J. van Hellemond:** Conceptualization, Methodology, Data curation, Writing - review & editing, Supervision.

Declaration of Competing Interest

None.

Appendix B. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.molbiopara.2020.111296.

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