

Exosomes or microvesicles? Two kinds of extracellular vesicles with different routes to modify protozoan-host cell interaction

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Abstract Parasite-host cell interaction can be modulated by a dynamic communication between extracellular vesicles (EVs). They should play key roles in cell-cell communications transferring biomolecules (miRNA, proteins, soluble factors) from one cell to another cell. While many names have been used to denominate EVs, a better comprehension to understand these vesicles is raised when we classify it according to biogenesis: originated from multivesicular bodies, named exosomes, and from plasmatic membranes, denominated microvesicles. Here, we have reviewed EV participation during the protozoan-host cell interaction and reinforced the differences and similarities between exosomes and microvesicles, suggesting different intracellular routes and functions. We also discussed perspectives to study EVs and the role of EVs in diagnosis and chemotherapies of infectious diseases.

Keywords Microvesicles · Parasite host cell-interaction · Evasion mechanism · Exosomes · Extracellular vesicles

Extracellular vesicles (EVs) are being considered a new element during parasite-host cell interaction. EVs are released by a wide number of cells including blood cells, immune system cells, tumour cells, epithelial and endothelial cells, and adult and embryonic stem cells. Extracellular vesicles are a heterogeneous kind of vesicles (size ranging between 40 and 1000 nm) known by several different names including

microparticles, microvesicles, ectosomes, shedding vesicles, exosomes, and oncosomes (Tetta et al. 2012).

An interesting global initiative (The International Society for Extracellular Vesicles or ISEV) started in 2012 with a clear focus of boosting stimulation with the aim of enhancing research in this field. The ISEV meets every year, and much significant progress has been made in the study of EVs from the ideas exchanged from the members of the community. In addition to the contributions of the society, many reviews have been published in general and specific journals to summarize aspects of biogenesis, functions, and applications of extracellular vesicles in many kinds of cell (Deolindo et al. 2013; Raposo and Stoorvogel 2013; Ratajczak et al. 2006).

The participation of extracellular vesicles during parasite-host cell interaction has been the focus in recent reviews highlighting communications between parasites and host cells (Barteneva et al. 2013; Marcilla et al. 2014; Schara et al. 2009; Williams and Urbe 2007).

In this review, we have concentrated our efforts to define biological concepts explaining differences in the biogenesis of extracellular vesicles. Moreover, we have summarized most of the findings involving microvesicles and exosomes in protozoans. We also discussed new perspectives to study the role of EVs during parasites and host cell interaction and hypothesized that EVs could be useful for diagnostic and chemotherapeutic alternatives to control diseases.

Extracellular vesicle biogenesis

Because of the confusion with the nomenclature of extracellular vesicles, the scientific community has established a consensus to classify them according to the size and origin of three kinds of vesicles: exosomes (around 40–100 nm); microvesicles, also named microparticles, ectosomes, etc. (around 100 nm to

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1 μM); and apoptotic bodies (from 2 to 4 μM) as we have summarized in Fig. 1 as extracellular vesicle “world”.

Among the vesicles, the apoptotic bodies have well-defined characteristics and are easy to identify. They are of large size and originate from cells in the process of programmed cell death.

Some decades ago, different authors started to identify the product released by parasites *in vitro* or *in vivo* as shedding vesicles or secreted protein molecules. In several parasites, exo-antigens were identified, and in some cases, compartmentalized structures as vesicles were described. Later, with the advent of new technologies within genomics and post-genomics ages, the concept of “secretome” was accepted as the total product released for a cell in some period. Proteomics strategies allowed the elucidation of some secreted products and showed the presence of biomolecules (glycolipids, glycoconjugates, glycoproteins, etc.) and extracellular vesicles as an important component of the extracellular material. Nowadays, a lot of information and knowledge have been built around the concept of “extracellular vesicles” as the material released by the cells.

In general, the extracellular vesicles detectable both *in vivo* and *in vitro* correspond to a mixed population of exosomes and microvesicles which originated from different pathways and are in some occasions indistinguishable.

The exosomes originate from the endosomal membrane cell compartment where the exocytosis of multivesicular bodies occurs discharging into the extracellular space of intraluminal vesicles after fusion with the plasma membrane in a cytoskeleton-dependent activation and independent of calcium (Schara et al. 2009).

Microvesicle shedding is stimulated by sustained elevation of intracellular calcium, opening of calcium-dependent

potassium channels, activation of calcium-dependent proteases like calpain, and activation of the phospholipid transporter scramblase, driving the exposure of phosphatidylserine in the outer leaflet of the bilayer. Cytoskeletal and membrane protein as synexin seems to be involved with the protrusion of the membrane and release of the microvesicle from the plasmatic membrane (Schara et al. 2009; Silverman et al. 2010).

Communication between cells

Cells alone and within tissues have communication between them. A natural means to have communication is the physical contact by cell junctions, adhesion molecules, and soluble factors. From the endocrine point of view, the soluble factors can be autocrine acting on the same cells from which they were produced, or paracrine affecting neighbouring cells or even work a long distance. The emerging extracellular vesicle world has been considered a new mechanism of cell communication. As mentioned before, EVs are released by numerous cell types and also found in body fluids such as serum, urine, amniotic fluid, and breast milk in physiological and pathological conditions. The release of EVs is being considered a universal communication between cells. In this way, cells can modify the behaviour of other cells, passing material and information through proteins, lipids, nucleic acids, and other components (Deolindo et al. 2013). EVs are cytosol fragments surrounded by a lipid bilayer and proteins derived from the plasmatic membrane. They are released constitutively *in vitro* or *in vivo* by cells or upon activation by an agonist or physical or chemical stress, like hypoxia, oxidative stress, etc. (Silverman and Reiner 2011). Another possible inductor is the physical contact of a protozoan circulating in the blood or in direct contact with host cells (Cestari et al. 2012).

New talk in protozoan-host cell interaction

Protozoa are a wide group of unicellular eukaryotic organisms that have a complex life cycle; 10,000 species of protozoa have been described, and less than 1 % produce diseases in humans. EVs can carry receptors, biomolecules, proteins, mRNAs, and miRNA which deliver information to recipient cells. Within the context of the protozoan-host cell interaction, the release of EVs is able to modify the phenotype and function of target cells. This kind of communication could reformulate concepts in parasitism and modulate the innate immune system.

Here we have pointed out the main findings of EVs in some protozoan-related diseases.

In protozoan-host cell interaction, it has long been hypothesized that secretion of effector molecules should be important

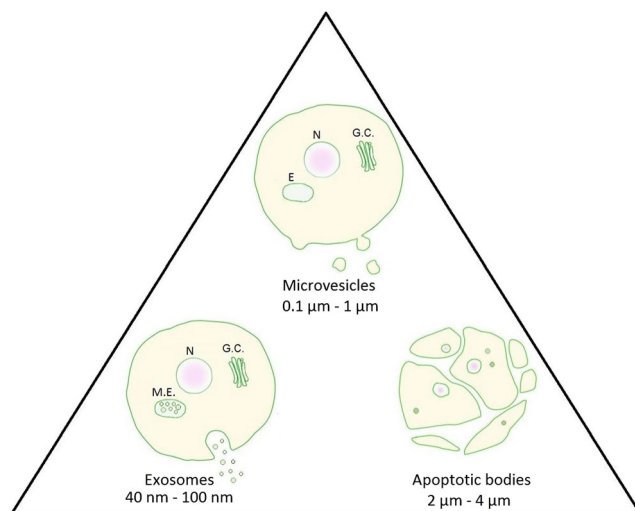


Fig. 1 Extracellular vesicle world. The diagram shows three big groups of extracellular vesicles classified by origin and size. Exosomes are from multivesicular bodies and about 40–100 nm. Microvesicles originate from plasmatic membrane with size between 100 nM and 1 μm . Finally, apoptotic bodies are from programmed cell death and about 2–4 μm

for pathogenesis. Many research have been described showing putative molecules released by pathogens (secretome) (Da Silveira et al. 1979; Goncalves et al. 1991; Barteneva et al. 2013).

Advances in high-throughput mass spectrometry allowed secretome analysis from several single-cell pathogens. Some works have been done identifying extracellular vesicles as the product of secretome. The enormous amount of data has contributed to understanding some strategies used by protozoans to interact with other cells. However, the assays have showed a high diversity of secreted proteins according with the complexity of the infection process. Moreover, information on well-defined and identified proteins involved with translation, carbohydrate and lipid metabolism, and DNA packaging is varied regarding the function and localization within the cells. Maybe more controlled experiments and a new generation of mass spectrometry could give more accurate and reliable results (Thery et al. 2009). We have listed different manuscripts published in recent years on some protozoa, regarding extracellular vesicles and secretome (Table 1).

Intracellular protozoans

Plasmodium spp.

Malaria is a disease caused by apicomplexan protozoa of the genus *Plasmodium* and is transmitted to humans through the bite of the insect mosquito anopheles. Four species of *Plasmodium* spp. produce the disease: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, and *Plasmodium ovale*. The World Health Organization (WHO) considers the disease as the highest produced by protozoans in the world with more than 200 million cases and with a third of

the cases reaching death (Coltel et al. 2006). There are reports showing EV release during murine models and human infections. Several studies have described the circulation of EVs during *P. falciparum* and *P. vivax* infections in humans (Regev-Rudzki et al. 2013; Combes et al. 2005). In general, the first work named EVs as microparticles and not microvesicles, and recently, authors have started to identify the vesicles as exosomes and microvesicles. Important research have suggested a role of EVs at the pathogenesis of the disease because levels of circulating EVs have coincided with fever and cerebral dysfunctions (Nantakomol et al. 2011). Murine malaria caused by *Plasmodium berghei* represents the clearest protozoan model where EVs are producing an effect at pathogenesis. EVs isolated from infected animals induced a potent activation of macrophages through Toll-like receptors, while microvesicles (MVs) from native animals have no effect over macrophages. Moreover, when the ABCA transporter gene that modulates the presence of phosphatidylserine on the surface of the plasmatic membrane was knocked out from animals, the animals were protected from cerebral malaria (CM), indicating a relation between microvesicles and CM (Combes et al. 2005). Recently, an important report has showed that exosomes derived from infected reticulocytes are able to modulate the immune system. In fact, it is the first evidence that exosomes derived from reticulocytes could be used as a vaccine for malaria infections. Immunizations of balb/c with exosomes from infections raised IgG antibodies capable of recognizing *Plasmodium yoelii*-infected red blood cells. Moreover, in immunizations combined with CpG oligodeoxynucleotides, they have found a complete protection against lethal infections in more than 85 % of the mice infected (Martin-Jaular et al. 2011).

Important concepts regarding the co-participation of EV production between parasite and host cells were demonstrated

Table 1 Extracellular vesicles and protozoans

Protozoan	Intracellular	Extracellular	Exosomes	Microvesicles/microparticles/ ectosomes	Reference
<i>Plasmodium vivax</i>	+			+	Mantel et al. 2013
<i>Plasmodium berghei</i>	+		+		Regev-Rudzki et al. 2013
<i>Plasmodium yoelii</i>	+		+		Martin-Jaular et al. 2011
<i>Plasmodium falciparum</i>	+			+	Nantakomol et al. 2011; Combes et al. 2005
<i>Toxoplasma gondii</i>	+		+		Aline et al. 2004
<i>Trypanosoma cruzi</i>	+		+	+	Cestari et al. 2012; Bayer-Santos et al. 2012; da Silveira et al. 1979; Garcia-Silva et al. 2014
<i>Leishmania</i> spp.	+		+	+	Silverman et al. 2010; Bayer-Santos et al. 2012; Silverman and Reiner 2011
<i>Giardia intestinalis</i>		+	+	+	Deolindo et al. 2013
<i>Trichomonas vaginalis</i>		+	+		de Miguel et al. 2010; Twu et al. 2013
<i>Trypanosoma brucei</i>		+	+		Geiger et al. 2010

by some authors (Regev-Rudzki et al. 2013; Coltel et al. 2006; Bhatnagar et al. 2007) in *P. falciparum* infection. Regel-Rudzel et al. have showed the release of exosome-like vesicles (80 nm) by ring-stage parasites using an atomic force microscope. Moreover, they have demonstrated the transference of genetic material between infected red blood cells (iRBCs). Transgenic parasites which are resistant to one drug were co-cultured in the presence of two drugs, and doubly resistant parasites were obtained in a few days. The participation of exosomes was demonstrated by transwell assays where exosomes from one population were transferred to another, inducing a new resistance to the drug for the recipient population. In another study, some authors have shown larger-sized vesicles between 150 and 250 nm at the schizogony stage. They also showed that iRBCs produce 10 times more EVs than uninfected RBCs (Mantel et al. 2013).

Toxoplasma gondii

Toxoplasma gondii is an intracellular protozoan that causes toxoplasmosis which affects humans and domestic animals worldwide. In humans, it is associated with complications during pregnancy and in immunocompromised patients, causing severe clinical complications including death. The first report on the use of exosomes in therapy was related to *Toxoplasma*. Dendritic cells were pulsed in vitro with antigens and exosomes from *T. gondii*. Later, the exosomes from *T. gondii* were used in immunizations of mice that were challenged with lethal doses of parasites, and nearly 70 % of protection was done. Moreover, exosomes obtained from infected macrophages were able to stimulate a pro-inflammatory response (Aline et al. 2004). Some authors also reported exosomes being released from macrophages infected with *Toxoplasma*. These vesicles contain an array of miRNA (Pope and Lasser 2013).

Trypanosoma cruzi

In *Trypanosoma cruzi*, da Silveira et al. have demonstrated for the first time the release of MVs from non-infective epimastigote forms where parasites were cultured with acid pH buffers and other reagents (da Silveira et al. 1979). Later, it was described in infective cultured trypomastigotes the shedding of surface molecules in extracellular vesicles with a size smaller than 80 nm (Torrecilhas et al. 2012). Some years ago, the concept of the shedding of vesicles was used again from the same group in an interesting description. Trocoli Torrecilhas et al. have reported that mammalian tissue culture cell-derived trypomastigotes (TCTs) release vesicles (ranging from less than 100 nm to approximately 500 nm) carrying virulence factors involved in Chagas disease pathogenesis by increasing heart parasitism and inflammation (Trocoli Torrecilhas et al. 2009). Maybe this heterogeneous description from extracellular

vesicles in *T. cruzi* could be due to differences of the experimental conditions to obtain vesicles as methodology divergences among other factors. Moreover, several reports have been showing excreted/secreted proteins by *T. cruzi*, involving the shedding of vesicles (Bayer-Santos et al. 2012; da Silveira et al. 1979; Goncalves et al. 1991; Neves et al. 2014). Recently, a comprehensive proteomic analysis of *T. cruzi* showed the existence of two different extracellular vesicle populations in non-infective epimastigote and infective metacyclic trypomastigote forms. Bayer-Santos et al. have demonstrated that both forms release exosomes and microvesicles. Moreover, the infective metacyclic forms release vesicles carrying virulence factors such as GP82 glycoproteins and mucins suggesting that the parasite could use extracellular vesicles to deliver modulatory molecules into host cells (Bayer-Santos et al. 2012).

Recently, some authors have showed that *T. cruzi* in trypomastigote stage from Y and CL-Brener strains are able to release vesicles with acid phosphatase activities that increase the adhesion of the parasite to the host cell (Neves et al. 2014). The importance of the microvesicle release during *T. cruzi*-host interaction as an evasion mechanism was demonstrated before (Cestari et al. 2012). The authors have showed that microvesicles released during metacyclic forms and THP1 cell line interaction are able to inhibit the complement system by C3 convertase inhibition and increase the invasion of metacyclic forms to host cells. The role of extracellular vesicles modulating host cells seems to be associated with the presence of small RNAs as indicated recently by different groups (Bayer-Santos et al. 2012; Ghildiyal and Zamore 2009; Garcia-Silva et al. 2014; Liu et al. 2010; Twu et al. 2013). Bayer-Santos et al. show that the small RNAs contained in extracellular vesicles originate from multiple sources, including tRNAs. Their results reveal that the variety and expression of small RNAs are different between parasite stages, suggesting diverse functions. Garcia-Silva et al. analysed the effects induced by *T. cruzi* shed vesicles and their associated small tRNA cargo on gene expression of susceptible HeLa cells. The ability to transfer genetic information between different cells has provided support to understand the modulation of innate immunity and cellular communications.

We have summarized in Fig. 2 the dynamics of microvesicle release during parasite-host cell interactions. The different kinds of interaction between extracellular vesicles derived from protozoans or eukaryotic cells during the parasite-host cell relationship are shown indicating biogenesis, release of autocrine message, fusion events, and modulation of neighbouring host cells.

***Leishmania* spp.**

In leishmaniasis, the release of exosomes and microvesicles during interaction with mammalian cells has also been described (Silverman et al. 2010). Indeed, the authors have

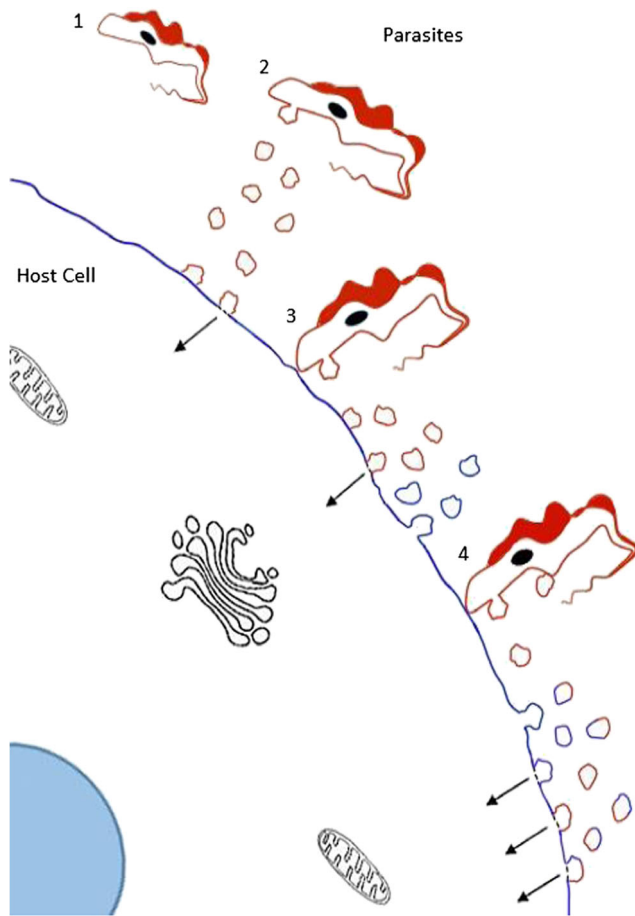


Fig. 2 Extracellular vesicle release during protozoan-host cell interaction. 1 The protozoan has reached the mammalian host. 2 The protozoan has started to release EVs. 3 The protozoan contacts with the host cell releasing EVs that can be received and internalized by the host cell, and also the proper host cell could start to release EVs. 4 Different kinds of EVs could participate in the dynamic contact between the protozoan and host cell: EVs from the protozoan, EVs from host cells, and fusion between the protozoan and host cell EVs that can produce an effect in the protozoan or host cells

concentrated their efforts to analyse the impact of extracellular vesicles on intracellular stages of *Leishmania* spp., indicating that changes in the environment seem to define the kind of release and cargo. Proteomic analysis has supported the idea that in neutral pH, the exosomes were enriched in kinase activity, while in acidic pH, they were enriched with phosphatase activity. Extracellular stages submitted to heat shock stress treatment have showed a similar kind of enzymes. Interestingly, *Leishmania* respond to specific environment changes with the release of extracellular vesicles containing a group of virulence factors such GP63, membrane proteins, and redox and heat shock proteins that should modulate the parasite-host cell interaction (Silverman and Reiner 2011).

A large number of research groups have shown that extracellular vesicles can function to modulate immune responses, including immune stimulation and immune suppression during intracellular parasite infection (reviewed by Marcilla et al.

2014; Schorey et al. 2015). The role of extraparasitic material release during the parasite-host cell interaction could be critical to maintaining parasite infection by aiding colonization and modulating the host immune response. Our current knowledge of extracellular biogenesis does not allow for specific inhibition of extracellular release; therefore, an insight to evaluate the importance of these extraparasitic materials during in vivo infection is currently not possible

Extracellular protozoans

The ability of protozoa to respond to environmental changes has been found in *Giardia intestinalis*, an extracellular parasite that produces diarrheal illness with global prevalence. *Giardia* is considered the earliest branching protist. The parasite presents a secretory pathway, including organelle biogenesis, that is limited or completely understood (Embley and Hirt 1998; Tovar et al. 2003). Some authors have described secretory vesicles associated with encystation process (Benchimol 2004; Gottig et al. 2006). Moreover, recently it was shown that *G. intestinalis* is able to release microvesicles in response to environmental changes (e.g. pH changes, bilis presence, calcium concentration, etc.). Deolindo et al. have suggested that this response could be a mechanism from the parasite to adapt to different changing conditions during the course of infection and to avoid innate immunity.

Another extracellular parasite that releases extracellular vesicles during contact with the host is *Trichomonas vaginalis* (de Miguel et al. 2010; Twu et al. 2013). Twu et al. have described that exosome secretion increases during parasite attachment to the host and modulation of the innate immunity of the host cell reducing the IL-8 expression of the host ectocervical cells. The authors have suggested that extracellular vesicles could control the regulation of IL-6 and IL-8 secretion and exosomes could be important for the establishment of chronic infection at the urogenital tract (Twu et al. 2013).

Trypanosoma brucei is the parasite that causes sleeping sickness transmitted by tsetse flies. The illness can be lethal and affect more than 60 million people in sub-Saharan Africa. *T. brucei* spends its life as an extracellular parasite, and when exposed to innate immunity, it must develop evasion mechanisms such as antigen variation and extracellular vesicle release (Brun and Blum 2012). Proteomic analysis of the secretome in *T. brucei* has allowed the characterization of proteins released by the bloodstream forms of different strains. More than 400 proteins have been identified from the secretome of *T. brucei*, and curiously, a higher percentage of proteins lack targeting for a secretory pathway. The authors have described microvesicles containing markers and proteases that could affect host cells (Geiger et al. 2010).

Similarities and differences between exosomes and microvesicles during the protozoan-host cell interaction: clarifying concepts

Many descriptions have been made in different biological systems showing an intense extracellular vesicle release of exosomes and microvesicles. This activity also has been shown at the parasite-host cell interaction and seems to be a new kind of communication between the cells. In several parasites, exo-antigens were identified, and in some cases, compartmentalized structures as vesicles were described. Later, with the advent of new technologies within genomics and post-genomics ages, the concept of “secretome” was accepted as the total product released for a cell in some period. Proteomics strategies allowed elucidation of some secreted products and showed the presence of biomolecules (glycolipids, glycoconjugates, glycoproteins, etc.) and extracellular vesicles as an important component of the extracellular material. Nowadays, a lot of information and knowledge has been built around the concept of “extracellular vesicles” as the material released by the cells.

How this intense cargo of protein, biomolecules, and genetic information (DNA, RNA, small RNA) could be modulating the host cell and the progression of the infection is still not understood. Exosomes and microvesicles have different origins, content, and probably different functions.

Highlighted points

Different biogenesis and complicated purification

Although there are several names given to extracellular vesicles, it is clear to differentiate exosomes (30–80 nm) that are formed from the other larger multivesicular bodies named microvesicles (from 100 nm to 1 μm) (also ectosome, microparticles, oncosomes) that originate from the plasmatic membrane. The ISEV recommendation to name these microvesicles will avoid confusion of vesicle nomenclature and will facilitate the understanding of the phenomenon. MVs are grouped based on size, density, method of purification, and markers. EVs represent a mixture of vesicular fractions of exosomes and microvesicles.

The understanding of extracellular vesicles has been associated with exosomes, and recently, the concept of microvesicles is being used by researchers. The processes that drive exosomes and microvesicles are completely different.

Exosomes are derived from the formation of multivesicular bodies (MVB) that are a late endosome loaded with intraluminal vesicles. The organelles fuse with lysosomes producing a fusion of the vesicles in the lysosomes. This represents a general and slow general degradative lysosomal pathway. This process is physiological and depends on GTPases rab 7, Snare system, and syntaxin (Bucci et al. 2000) and

belongs to the exocytosis process. Exosomes are not related to the plasma membrane, and many reports previously published could have included incorrect or wrong information about the location or size of exosomes, while on the other hand, vesicles are larger in size and derived from the plasmatic membrane.

Microvesicles originate from the plasma membrane with the budding of the small plasma membrane domain in an active process with participation of the scramblase enzyme and segregation of phospholipids, cholesterol, ceramide, etc. (Gottig et al. 2006). In contrast to exosomes, the release of microvesicles does not require exocytosis and larger (100–1000 nm) vesicles are released into the extracellular space at a high rate. This process is calcium-dependent where an increase of free Ca^{++} acts as a second messenger to sustain the release of microvesicles intensively in seconds (Schara et al. 2009).

Purification of the mixture of extracellular vesicles is carried out by pre-cleaning with filtration before centrifugation (differential centrifugation, sucrose gradients). There are also immune affinity beads, gel filtration, etc. Unfortunately, the standardization is not fully understood. Indeed, the physical properties and ability of vesicles to fuse with each other produce a heterogeneous population with variable sizes. There are methods such as nanoparticle tracking and X-ray scattering that are promising and are under investigation.

At least CD63 and CD61 are exosome markers, and flotillin and Annexin V represent good microvesicle markers. The markers are present in most of the subpopulations studied and could be important to distinguish between the different kinds of vesicles. It is accepted that exosomes are CD63(+) and Annexin V(-) and that microvesicles are Annexin V(+) and CD63(-). These markers and the size of the vesicles are the most important point to differentiate between the vesicles.

Different routes and functions

Recently, a manuscript has shown for the first time the differences between exosomes and microvesicles with the ability to transfer genetic information (Kanada et al. 2015). The authors have observed that only microvesicles (MV), but not exosomes, can functionally transfer loaded reporter molecules to recipient cells. It seems interesting that the membrane prevents soluble factors, proteins, and RNA against degradation. The authors have shown that exosomes and MVs are structurally and functionally distinct and could have different roles during contact with host cells.

Microvesicles as biomarkers

Microvesicles have been described in several diseases including diabetes, hypertension, atherosclerosis, and cancer. In this process, the RNA transference between cells could be involved. Recently, microvesicles have been described as a

biomarker for tumour progression (Nakano et al. 2015; Giusti et al. 2013). Recently, Chen et al. have seen the identification of circulating biomarkers in sera of *Plasmodium knowlesi*-infected malaria patients. Microvesicle detection in serum from the patient could help to diagnose the diseases and also be useful for prognosis of the diseases (Chen et al. 2015).

Content of biomolecules

Maybe at the context of cell communication, the knowledge about proteins, nucleic acids, lipids, and biomolecules would give ideas about the putative phenotypic modification produced by the biomolecules contained in the extracellular vesicles. Many reports (Marcilla et al. 2014; Montaner et al. 2014) have shown the diversity of proteins included in exosomes and microvesicles through proteomic analysis and have been included at the Evpedia (Kim et al. 2015).

Probably, exosomes and microvesicles carry part of the cytoplasm and protein which are not specific for each kind of vesicles. Differences in the nucleic acid content should indicate the different routes between the vesicles and the probable modulatory effect in neighbouring cells. The analysis of phospholipids in extracellular vesicles could indicate the redistribution of the plasma membrane and phospholipids triggered by increased cytosolic Ca^{++} (Zhou et al. 1998).

Methods for the measurement of phospholipids scrambling under different conditions and analysis of phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine, and other phospholipids at the extracellular vesicles and at the plasmatic membrane from protozoan and host cells will help to understand the mechanism of biogenesis, dynamics of lipid remodeling, and interchange between the cells.

Microvesicles at therapy

Extracellular vesicles represent a promising therapeutic delivery tool. Microvesicles have the ability to act as bioactive cargoes carrying secretory molecules such as cytokines, chemokines, and growth factors as genetic material. In cardiovascular diseases, microvesicles are reduced to normal levels in order to reduce associated deleterious signalling (Martinez et al. 2011).

In parasitic diseases, at least in toxoplasmosis, this has been used with success (Aline et al. 2004).

The understanding of the exosome or microvesicle mechanism could allow to design strategies to block the induction of exosomes/microvesicles through inhibitors. The release of microvesicles could be inhibited with RNA interference targeting the genes involved with the pathway (flipase/scramblase).

The mechanism of the releasing extracellular vesicles, intracellular trafficking, and signal transduction could be important to modulate the host cell and produce phenotype

transformation of the cells. This important issue of cell biology should be a focus to be investigated in the future. Moreover, investigation of the host cell target of small RNA could be important to understand how the extracellular vesicles could modify gene regulation and modulate the host cell.

Concluding remarks

In this review, we have summarized information about extracellular vesicles during protozoan-host cell interactions. We have focused on defining the differences between the main extracellular vesicles—exosomes and microvesicles—and on reinforcing the role of these vesicles in cell communication. The findings including the transference of biological material (microRNA, proteins, and soluble factors) from one cell to another change the concepts in parasite-host cell interactions.

We have pointed on the biogenesis and route of the vesicles during the parasite-host cell interaction. Indeed, the huge increase in the research of extracellular vesicles is a reason to think that new advances will come in the next few years. These may include understanding the mechanism of release, content, phenotypic effect, and communications between the cells. More knowledge in the physiology and pathology of extracellular vesicles will allow the exploration of the rational use of exosomes and microvesicles as biomarkers in the diagnosis and therapy of infectious diseases.

Extracellular vesicles during protozoan-host cell interactions are a fascinating issue with impact in cell biology, pharmacology, immunology, and medicine joining basic and applied sciences. New findings will help to understand the role of EVs in parasitism and cell communication.

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