v. 3, n. 8, 2023

International Journal of Biological and Natural Sciences

PARTICIPATION OF EXTRACELLULAR VESICLES OF EXTRACELLULAR PATHOGENS IN CONTACT-INDEPENDENT PATHOGENIC MECHANISMS

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Abstract: Extracellular vesicles (EVs) are spherical particles of different sizes and contents that participate in physiological as well as pathogenic processes. According to their size and biogenesis, they are classified into exosomes, ectosomes or microvesicles and apoptotic bodies. The emission of these vesicles is an evolutionarily conserved physiological process carried out by both prokaryotic and eukaryotic cells. This review focuses on EVs emitted by extracellular pathogenic organisms; bacteria, fungi, protozoa, and helminths, which mediate pathogen-host and pathogen-pathogen communication, playing a fundamental role in the pathogenesis of these organisms, forming part of contact-independent pathogenic mechanisms. The participation of EVs in adhesion, cell invasion, immunomodulation, induction of cell damage and death, as well as the transfer of resistance factors, is described through the review of articles published on the PUBMED platform from 2010 to date using the keyword "extracellular vesicles".
Keywords: Exosome, Microvesicle, Pathogens

INTRODUCTION

Extracellular vesicles (EVs) are spherical particles delimited by a lipid bilayer which are released into the extracellular environment by prokaryotic and eukaryotic cells. They are mainly classified into three categories according to their size and biogenesis: exosomes (30-150 nm), which originate from multivesicular bodies, ectosomes or microvesicles (100-1000 nm) produced through outpouchings of the plasma membrane, and apoptotic bodies (50-5000 nm) emitted by dying cells [24]. The content of EVs is varied and depends on the emitting cell and the microenvironment [24,64], so they can contain biomolecules such as proteins [28,52], lipids [20], nucleic acids [53] and/or carbohydrates. [47].

EVs carry out an important role in intercellular communication mainly due to the ability to transfer their content [24,39]. Upon release, EVs move through the extracellular medium the process is carried out at different times, reaching different distances[16]. Subsequently, when interacting with target cells, EVs can be internalized by endocytosis (phagocytosis, pinocytosis) [39], they can also discharge their content through fusion with the plasma membrane [39,43] or binding to surface receptors to activate signaling pathways [17], resulting in changes in cell physiology.

In recent years, the participation of EVs in physiological and pathogenic processes [11,55] has been reported, both in non-infectious diseases [45], as well as in infectious diseases [54]. Specifically, EVs emitted by bacteria, fungus, helminths and protozoa are relevant for their survival, as well as for the progression of the disease in the host. This work aims to describe the current state of the art of research focused on understanding the role of EVs emitted by various pathogenic organisms as part of their contact-independent pathogenicity mechanisms.

EXTRACELLULAR VESICLES IN PATHOGENIC PROCESSES

Some bacteria, protozoa, fungus and helminths are etiological agents of several diseases in humans and other animals. Within the host, they use diverse mechanisms to survive and invade cells and tissues [27,37], in which EVs are important mediators between pathogen-host and pathogen-pathogen [31,36]. EVs play a fundamental role in various processes such as: pathogen adhesion and invasion, modulation of the immune response, in cell damage and death, as well as in the transmission of resistance and survival factors between pathogens (Table 1) (Figure 1). The inducing effect of EVs depends on factors such as the type of target cell [20], the

species and even the strain in study [7,42], as well as the amount and time in which the vesicles interact [4].

INCREASED ADHERENCE AND INVASION OF PATHOGENS

Pathogenic microorganisms carry out mechanisms to cause damage to the host, of which adhesion and/or invasion of cells and tissues are key points to achieve this [3,58].

Recent reports demonstrate that bacteria [14], fungi [67] and protozoa EVs [19,51,61] increase the adherence of these microorganisms to monolayers of epithelial cells and, in some cases, increase invasion or internalization to cells. [14,22,51], Likewise, an increase in the invasion of macrophages by fungi [23] or protozoa [36] has been described. As mention before, the increase in cell adhesion and invasion varies depending on the time and concentration of the EVs [14,22,23], in others, it depends on the emitting strain, as reported in the protozoan *T. vaginalis* where the EVs of high adherence strains transfer factors that favor this characteristic to those with low adherence capacity [61]. In addition, Gavinho and collaborators (2020) [19] report that the ability of the protozoan *G. intestinalis* to adhere may also depend on the type of vesicle, since they observed that these properties are found in the large vesicles of the parasite.

Similar results have been reported through *in vivo* assays, where it was demonstrated that the interaction of EVs emitted by pathogenic organisms interacting with mice is related to greater cellular invasion. For example, exposing mice to EVs days before infection with the fungi *S. brasilensis* [23] or *P. brasilensis* [42] increased the fungal load in skin and lung lesions, respectively. On the other hand, the heart and blood of mice treated with EVs prior to infection with the protozoan *T. cruzi*, shown a higher parasite load compared to mice without exposure to EVs [36].

Simultaneous inoculation of EVs and pathogens also increases cellular invasion. In skin lesions of mice caused by *L. amazonensis*, a higher parasite load is recorded when they induce simultaneous inoculation with EVs [2]. Similarly, parasitemia of mice inoculated with *T. gondii* increases when they are simultaneously inoculated with EVs [48]. Finally, the cerebrospinal fluid and brain of mice simultaneously inoculated with EVs and *C. neoformans* also register higher fungal load [22].

The arguments presented in previous paragraphs, suggests that EVs have an important role since the early stages of infection, increasing cell adhesion and invasion processes of different pathogens, which could favor the establishment and development of the infection.

IMMUNOMODULATION

EVs may contain immunogenic components that interact with various epithelial cells and cells of the immune system, which are essential for host defense during an infection [23,41]. This complex interaction stimulates the host response to eliminate the infectious agent or the pathogen can modulate the immune system to evade its response and survive [12].

In macrophages, various adverse effects have been reported, for example, EVs emmited by *P. aeruginosa* can reduce the expression of molecules associated with the major histocompatibility complex (MHC) [1], similarly, EVs from *Leishmania* spp. reduce the expression of MHC, in addition to the co-stimulatory molecule CD86 and the production of nitric oxide [57], Besides, it increases the production of anti-inflammatory cytokines in leukocytes [56] which can affect the innate and adaptive immune response, decreasing the inflammatory process and antigen presentation to T lymphocytes, and

FUNCTION	SPECIES
INCREASE IN CELL ADHERENCE AND INVASION	**BACTERIA:** *Campylobacter jejuni* [14]. **FUNGUS:** *Paracoccidioides brasilensis* [42], *Candida auris* [67], *Sporothrix brasilensis* [23], *Cryptococcus neofarmans* [22]. **PROTOZOA:** *Leishmania amazonensis* [2], *Trypanosoma cruzi* [36,51], *Trichomonas vaginalis* [61], *Toxoplasma gondii* [48], *Giardia intestinalis* [19].
IMMUNOMODULATION	**BACTERIA:** *Streptococcus pneumoniae* [43], *Staphylococcus aureus* [21,25], *Pseudomonas aeruginosa* [1], *C. jejuni* [15], *Escherichia coli* [26,46,59]. **FUNGUS:** *Aspergillus flavus* [4] *C. Auris* [67], *P. brasilensis* [8,42], *Malassezia furfur* [70], *Candida albicans* [38], *Candida parapsilosis* [30], *Candida glabrata* [30], *Candida tropicalis* [30], *Talaromyces marneffei* [66]. **PROTOZOA:** *Trypanosoma brucei* [10], *T. cruzi* [36], *Acanthamoeba* spp. [7], *Acanthamoeba castellanii [*34], *L. amazonensis* [2,50], *Leishmania donovani* [56], *Leishmania infantum* [5], *Leishmania mexicana* [57], *T. vaginalis* [44,61], *T. gondii* [33], *Naegleria fowleri* [32], *G. intestinalis* [71], *Entamoeba histolytica [*12]. **HELMINTHS:** *Trichinella spiralis* [18,28], *Brugia malayi* [53], *Fasciola hepática* [40], *Schistosoma mansoni* [29], *Schistosoma japonicum* [35,62], *Opisthorchis viverrini* [6], *Clonorchis sinensis* [65].
INDUCTION OF CELL DAMAGE AND DEATH	**BACTERIA:** *S. pneumoniae* [43], *C. jejuni* [14,15], *E. coli* [26,46,59]. **FUNGUS:** *S. brasilensis* [23], *C. neofarmans* [22], *C. albicans* [38]. **PROTOZOA:** *A. castellanii* [20,34], *T. brucei* [60], *T. cruzi* [51]. **HELMINTHS:** *O. viverrini* [6], *C. sinensis* [65], *S. japonicum* [63].
TRANSFER OF DRUG RESISTANCE OR SURVIVAL FACTORS	**BACTERIA:** *S. aureus* [31], *Klebsiella pneumoniae* [9]. **FUNGUS:** *P. brasilensis* [42], *C. tropicalis* [30], *C. albicans* [68,69]. **PROTOZOA:** *Leishmania major* [13], *L. infantum* [13].

Table 1: Extracellular vesicles function emitted by pathogenic agents (Bacteria, helminths, Fungus and protozoa).

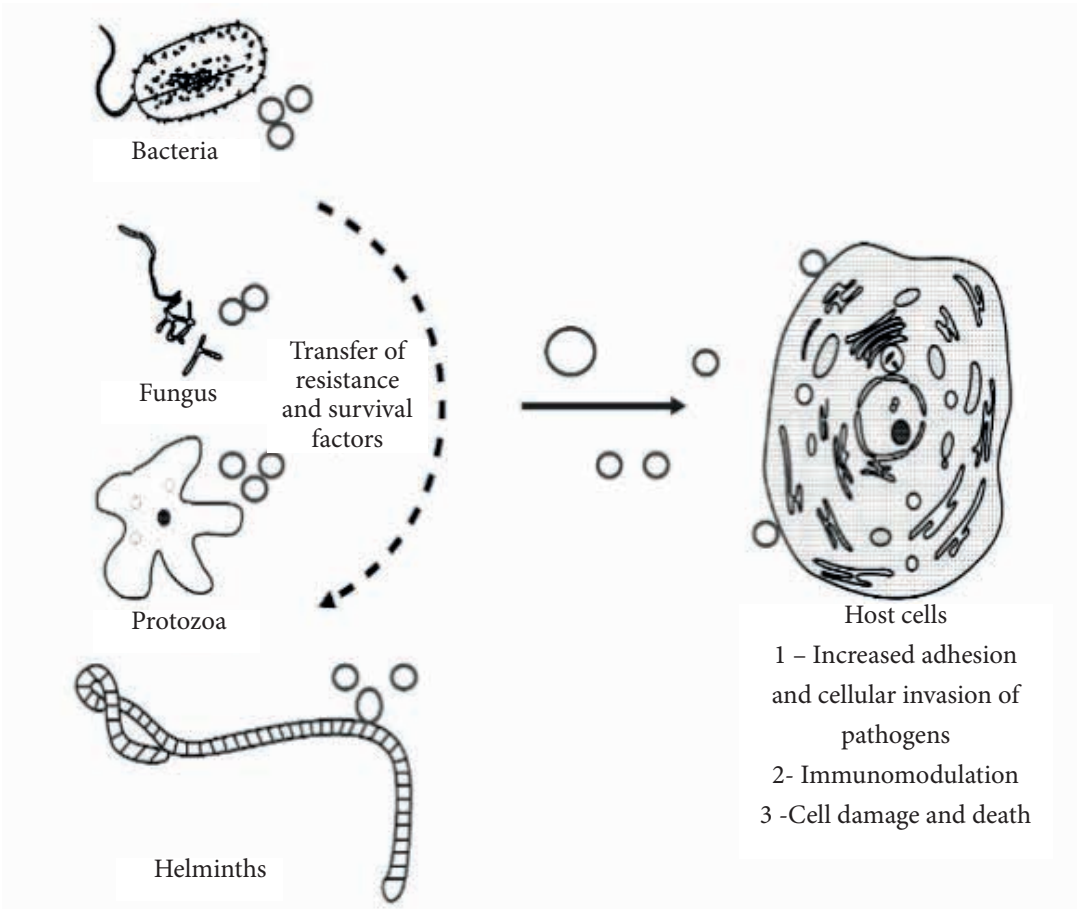

Figure 1: Participation of extracellular vesicles in contact-independent pathogenic mechanisms. Bacteria, fungi, protozoa and helminths emit extracellular vesicles that interact with host cells (solid arrow) playing a fundamental role in various processes such as: increasing pathogen cell adhesion and invasion, modulating the immune response, as well as as in the induction of cell damage and death. As well, extracellular vesicles have a role in intra- and interspecific communication between pathogens (dashed arrow), favoring the transmission of drug resistance and survival factors. The effects of extracellular vesicles can favor the establishment and development of infection.

therefore the elimination of the pathogen. EVs released by *T. cruzi* modulate the immune response *in vivo* and *in vitro* through direct effects on macrophages, where they induce the formation of lipid bodies and the production of prostaglandin E2, affecting the host's immune response [36]. Similarly, *L. infantum* EVs have a chemotactic effect on macrophages, which would favor the invasion of the parasite into them and therefore the establishment of the infection [5].

The negative effects of EVs emitted by diverse pathogens on other immune system cells have also been observed; EVs from *E. histolytica* reduce the respiratory burst and a delay in the initial stages of the release of extracellular traps in neutrophils (NETs) stimulated by the amoeba [12]. Furthermore, EVs from the helminth *B. malayi* affect dendritic cell signaling pathways involved in the immune response [53]. EVs from *F. hepatica* induce a unique phenotype in dendritic cells capable of suppressing the secretion of IL-2 from T cells [40]. Through murine models, has been reported that the inoculation of mice with EVs prior to infection with the protozoan *L. donovani* increases the production of anti-inflammatory cytokines, exacerbating the infection [56]. Similarly, EVs from *T. vaginalis* reduce inflammatory processes, allowing the persistence of the parasite in the host [44].

The above suggests that EVs produced by diverse pathogens can affect or modify the response of the host immune system cells, which favors the survival of infectious agents.

On the contrary, an important immunomodulatory role of EVs is also suggested from early stages of infection, which has been reported *in vitro* in keratinocytes [70] as well as in epithelial cell lines monolayers [6,15,25,61].

EVs also induce dendritic cells and macrophages response that could favor the elimination of pathogens. Particularly, in dendritic cells, EVs can stimulate the production of several cytokines and regulate the expression of costimulatory molecules such as CD80, CD86 and MHC [29,67], benefiting the induction of the adaptive immune response. Similarly, macrophages that internalize EVs secreted by pathogens, produce components such as chemokines and proinflammatory cytokines [7,8,25,32,34,42,43,59], nitric oxide [7,8,10,42,66] and reactive oxygen species [66], in addition to increasing the expression of costimulatory molecules CD80, CD86, MHC-II [66] and MHC-I [10]. The stimulation of macrophages with EVs emitted by different species of *Candida* genus as well as the protozoan *T. gondii*, not only induces an increase in the production of proinflammatory cytokines, but also decreases the secretion of IL-10, an anti-inflammatory cytokine [30,33]. The interaction of EVs from the fungi *A. flavus* [4], *P. brasilensis* [8] or the helminth *S. japonicum* with macrophages induces the production of proinflammatory molecules that lead to the polarization of macrophages to an M1 phenotype [8, 62], which would favor the elimination of infectious agents.

Moreover, the interaction of *T. brucei* EVs with peripheral blood mononuclear cells leads to the differentiation of regulatory T cells [10]. Likewise, EVs from the protozoan *L. amazonensis* modulate the activation and differentiation of B-1 cells [2,50]

The innate and adaptive inflammatory response are an essential protective mechanism against pathogens; however, the exacerbated secretion of proinflammatory components can also cause damage directly to the host and allow the persistence of the pathogen. For example, the constant exposure of mice to EVs produced by the bacteria *S. aureus* or *E. coli* by airway causes neutrophilic lung inflammation [25,26], in addition, *E. coli* produce EVs that induce host responses that resemble to a sepsis-like

condition characterized by the production of proinflammatory cytokines, in addition to hypothermia, tachypnea, leukopenia, lung dysfunction, and hypotension [46,59]. EVs from the helminth *C. sinensis* favor a pro-inflammatory hepatic microenvironment that aggravates biliary lesions [65]. Similarly, it has been reported that EVs from the helminth *S. japonicum*, which are internalized mainly by macrophages, induce their proliferation and secretion of TNF-α; however, it is suggested that this condition favors the persistence of the parasite [35].

Although in some cases a production of proinflammatory cytokines is reported in mice inoculated with EVs of various pathogens [33,42,59], further *in vivo* studies are required in various conditions to know if EVs trigger an excessive proinflammatory response that could affect the host.

CELL DAMAGE AND DEATH

Divers cytotoxic molecules have been identified in the cargo of EVs emitted by pathogens [14,34,43] which, when internalized by target cells, can cause some damage or alteration and even induce cell death.

A reduction of the proteins e-cadherin and occludin has been reported in epithelial cell cultures, when intecting EVs produced by the *C. Jenuni* [14], in addition, fusion between host cells has been observed due to the interaction with EVs of *C. neofarmans* [22], processes that are probably related to the adhesion and internalization of EVs. Cell permeabilization is modified in epithelial cells due to the effect of EVs from *T. cruzi* [51], resulting in the plasma membrane allowing the passage of molecules that could affect the host cells into the cytoplasm. In helminths, negative effects of EVs on cells have also been reported, for example, when *O. viverrini* EVs are internalized by biliary cells, they induce pro-tumorgenic changes [6], suggesting to be the key to the development of cancer in infected people. Similarly, EVs released by *S. japonicum* induce changes in hepatic cells causing fibrosis [63]. It has been reported that EVs secreted by pathogens are capable of inducing death [59] of epithelial cells and macrophages, mainly through necrotic [15,38] or apoptotic processes [43]; EVs from the protozoan *A. castellanii* can induce both [20,34].

Through *in vivo* models, it has been demonstrated that EVs induce cell damage, for example, *S. aureus* EVs can cause skin disruption mainly by inducing keratinocyte cell death [21], EVs emitted by the helminth *C. sinensis* induce and exacerbate biliary lesions [65]. Similarly, lesions induced in in a murine model of *S. brasilensis infection* has been observed [23].

Interestingly, inoculation of *E. coli* EVs in murine models, induced a severe inflammatory response, and also cause cardiac lesions [59], pulmonary emphysema [26] and even death [46]. On the other hand, studies with the protozoan *T. brucei* demonstrate that its EVs fuse with mammalian erythrocytes, causing physical changes in the membranes that lead to erythrophagocytosis, decreasing the number of blood cells and causing anemia during acute trypanosomiasis [60].

The reports of cell damage and cell death caused by EVs released by pathogen, evidence their importance in the establishment of infection. It is important to highlight that the damage caused through these independent contact mechanisms can also favor the development of collateral diseases.

TRANSFER OF DRUG RESISTANCE AND SURVIVAL FACTORS

Most research focuses on determining the effect of EVs emitted by pathogens on host cells. However, it is important to emphasize the role that vesicles play among pathogens,

where not only has it been reported an intra-species communication, but also an inter-species communication [13,31].

Drug resistance considered one of the of the most current public health crises [49], can be acquired through EVs. Lee et al. [31] reported that EVs from *S. aureus* contain β-lactamase, which can be transferred to gram-negative and gram-positive bacteria to confer resistance to ampicillin. Interestingly, drug resistance can not only be transferred by proteins present in EVs, but also by genes; Dell'Annunziata et al. [9] and Douanne et al. [13] reported genes related to drug resistance present in the EVs of *K. pneumoniae* [9] and in *Leishmania* spp. [13], which can be transferred to other microorganisms.

EVs can also promote the survival of pathogens in adverse conditions. EVs from the highly virulent strain of the fungus *P. brasilensis* [42] and the drug-resistant strain of *Leishmania* [13] have been determined to transfer molecules to susceptible strains that help resist oxidative stress conditions [13,42]. EVs produced by *Candida* spp., in addition to participating in drugs resistance, they also intervene in the synthesis and regulation of biofilms [30,68,69].

It is important to continue the study of EVs as a mechanism of intra- and interspecific communication given their potential relationship with the emergence of new strains of pathogenic microorganisms resistant to drugs and with characteristics to survive in hostile environments, which increases the risk of infections, making it difficult treatments and prognosis of pathologies that cause the diverse microorganisms.

CONCLUSIONS

In the last decade, the study of extracellular vesicles released by bacteria, fungi, protozoa and helminths of medical importance has gained importance due to their implications in contact-independent pathogenic mechanisms. To date, it has been shown that EVs derived from pathogens can increase the adherence and invasion of these organisms to host cells, and can damage and induce cell death, favoring the invasion and establishment of infectious and parasitic diseases. Furthermore, in parallel, the immunomodulatory effect of EVs produced by these organisms has been demonstrated, some with the capacity to suppress the host's immune response and favor the survival of the pathogen; or inducing and exacerbating inflammatory response and causing damage to host tissues. In addition, it is recognized that EVs participate in intra- and inter-species communication through which they can transfer virulence factors that allow the pathogen to survive adverse conditions.

Despite the reported advances, the functions of EVs emitted by pathogenic bacteria, fungi, protozoa and helminths are still unknown. Furthermore, as carriers of various components, additional studies are required to accurately determine the content of EVs secreted in response to different stimuli and whether the host-pathogen and pathogen-pathogen effect is modified. The study of the EVs participation as part of pathogenic mechanisms will expose new information that could provide information to create strategies that interfere with the functions of EVs with the purpose of avoiding the establishment of the pathogen in the target tissue and thereby preventing damage to the host.

ACKNOWLEDGMENTS

This review is part of the documentary research carried out as part of the theoretical support of the PhD. studies of the Master of Sciences Ismael Castelan Ramirez, who thanks to CONAHCYT for the scholarship awarded for his postgraduate studies (no. 858867).

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