REVIEW

Isolation and Functions of Extracellular Vesicles Derived from Parasites: The Promise of a New Era in Immunotherapy, Vaccination, and Diagnosis

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Abstract: Experimental and epidemiological evidence shows that parasites, particularly helminths, play a central role in balancing the host immunity. It was demonstrated that parasites can modulate immune responses via their excretory/secretory (ES) and some specific proteins. Extracellular vesicles (EVs) are nano-scale particles that are released from eukaryotic and prokaryotic cells. EVs in parasitological studies have been mostly employed for immunotherapy of autoimmune diseases, vaccination, and diagnosis. EVs can carry virulence factors and play a central role in the development of parasites in host cells. These molecules can manipulate the immune responses through transcriptional changes. Moreover, EVs derived from helminths modulate the immune system via provoking anti-inflammatory cytokines. On the other hand, EVs from parasite protozoa can induce efficient immunity, that makes them useful for probable next-generation vaccines. In addition, it seems that EVs from parasites may provide new diagnostic approaches for parasitic infections. In the current study, we reviewed isolation methods, functions, and applications of parasite's EVs in immunotherapy, vaccination, and diagnosis.

Keywords: parasites, extracellular vesicles, immunotherapy, vaccination, diagnosis

Introduction

Parasitic infections have a history as long as human living.[1,2] Despite lethal infections due to some parasites such as human-infecting species of *Leishmania*[3] and *Plasmodium*,[4,5] *Naegleria*,[6] *Entamoeba histolytica*,[7] most of them, particularly helminths, live within their hosts without considerable symptoms.[8,9] Parasitic infections are the cause of economic loss in developing and developed countries. Based on the latest reports, more than one billion people around the world suffer from parasitic diseases.[10,11] For example, it was estimated that 219 million cases in 2017 suffered from malaria with 435,000 deaths that makes this parasitic infection a global public health problem and one of the most important debilitating infectious diseases in developing countries.[12] From the economic point of view, only single malaria reduces economic growth in Africa by 1.3% per annum.[12] Leishmaniasis is the second lethal parasitic disease after malaria that the lack of effective vaccine has led to serious problems in tropical regions.[13,14] Therefore, the most important challenges facing with these infections are detection, and immunization.

However, studies on different applications of EVs for in parasitic diseases and the interesting roles of these micro/nano-particles in cellular and molecular biology are being dramatically increased. In the current study, we comprehensively gathered and

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categorized different applications of EVs in the field of parasitology that helps researchers for their future studies. For this purpose, available English electronic major libraries including: PubMed, Scopus and Web of Science were searched with keywords: "Parasite" AND "Extracellular Vesicles" AND "Immunotherapy" OR "Vaccination" OR "Diagnosis" OR "Detection".

Parasites and the Immune System

Apart from the traditional view emphasizing on the pathogenic role of parasites, a body of evidence has demonstrated the positive consequences of facing with some parasites in the immune system homeostasis,[15–18] which is famously discussed as "hygiene hypothesis". This hypothesis was supported by studies showing less exposure to the parasite infections during early childhood may increase the susceptibility to immune disorders during adulthood.[18,19] Actually, parasites, particularly helminths, can modulate the immune system through effective soluble mediators which interact with the host immune cells to escape from the immune responses.[20,21] This subject has been supported by recent studies showing the manipulation of the immune system by secreted proteins and carbohydrate molecules expressed by helminths.[22]

During the contact between the normal immune system and foreign antigens, a plethora of proteins are produced and released from immune and non-immune cells.[23,24] Accordingly, the role of some helminths and their components were practiced to ameliorate the pathological conditions and clinical manifestations of some autoimmune diseases such as asthma, inflammatory bowel diseases (IBD),[25–27] multiple sclerosis (MS)[28,29] and etc., using in vivo/in vitro studies. However, the most important limitations of these studies were either alive helminthic materials such as eggs or low efficacy of the derived proteins.[30,31]

On the other hand, during the invasion of some parasite protozoa such as *Leishmania, Plasmodium*, and *Toxoplasma gondii*, the cellular immune responses are the most important defense mechanisms. Studies on this subject have been led to engage of either total antigen or derived specific proteins of protozoan parasites as a vaccination strategy against themselves or other intracellular microorganisms.[32–36]

During the last decade, focuses on EVs released from parasites or immune cells sensed by parasites have been explosively increased. Up to now, several distinct types of EVs were known of which exosomes (size between 30 to150 nm, spherical shape with membrane bound) are an important

subtype.[37,38] Based on the growing evidence, the clinical applications of EVs in the field of parasitology are mainly categorized into three major classes.[39] A) Immunotherapy by EVs to modulate the immune responses. In this application, EVs derived from helminths are mostly employed to attenuate the immunological responses during autoimmune diseases such as IBD and allergy. B) Increasing the immunity responses against some parasitic infections, also known as vaccine. This application has majorly been practiced for parasitic protozoa in both medical and veterinary researches. C) Detection of specific EVs (particularly their contents) as a promising tool for diagnosis of parasitic diseases, particularly blood protozoa (Figure 1).

EVs in the parasitology studies have mostly been employed for immunization against a parasitic infection (8/17; 47.05%) while only four studies (23.53%), particularly in recent years, were carried out to use EVs for detection of parasitic infections (Table 1). In addition, ultracentrifugation more than 100,000 × g for at least 1 hr at 4ºC has been the most common technique for isolation and purification of EVs.

Furthermore, IBD was the autoimmune diseases that were frequently investigated for evaluation of the effects of EVs. In most of the studies, mice were the choice of host models and laboratory chickens were mostly the choice animal model for study on *Eimeria* (Table 2).[40–56]

Parasite's EVs

Parasites are eukaryotic microorganisms that during the time have learnt to communicate with their hosts to provide favor conditions in their niches.[1,2] Although some of the parasites such as human-infecting species of *Plasmodium* and *Leishmania, E. histolytica*, and some other parasites usually hurt their hosts, the hygiene hypothesis claims that some parasites, as an old friend of humans, have played an important role in evolution, improvement and homeostasis of the immune system.[31]

However, it seems that parasites are mostly companied with their host via ES products.[57,58] Numerous studies have explored the role of EVs released from either parasites or different types of host cells sensed by parasites in communication between parasites and host cells.[59] Actually, derived EVs from parasites can manipulate the target cells via delivering the pathogenic, immunomodulatory, and genetic materials to provide a pleasant condition for surviving and multiplication of parasites.[39,60] Furthermore, it was suggested that during the unpleasant environments, host cells may release EVs recruiting immune cells to defense against parasites.[39] Experimental
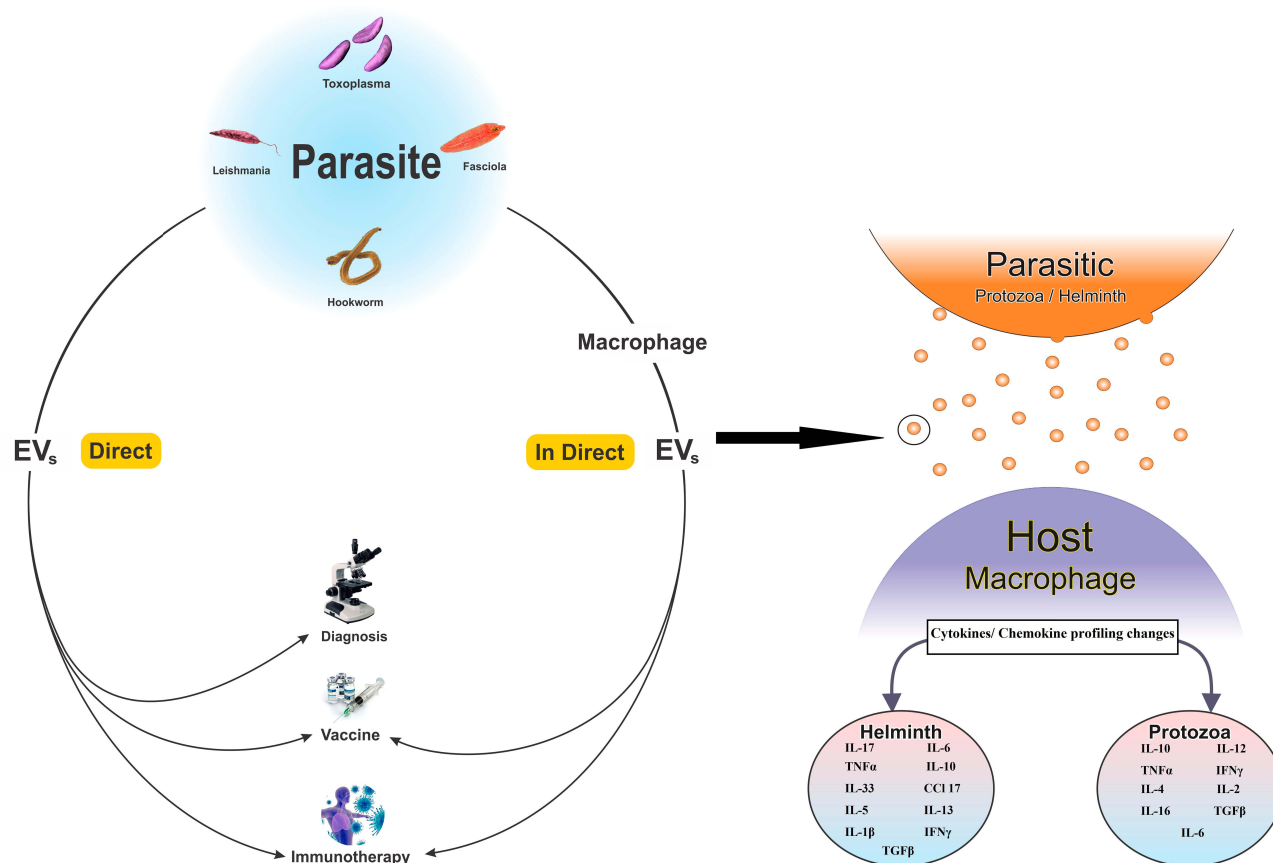
Figure 1 Extracellular vesicles (EVs) extracted directly from parasites or indirectly from host cells sensed by parasites. Indirectly released EVs from host cells sensed by parasites are mostly used for vaccination and immunotherapy while for diagnosis, EVs originated from parasites are a regular target. EVs can also change the cytokine/chemokine pattern in recipient cells. Protozoan parasites mostly increase the level of cytokine/chemokine pattern involved in polarization of cellular immunity while EVs from helminths usually increase the level of immunomodulatory cytokines such as IL-4 and TGFβ. However, there are studies showing elevated levels of immunomodulatory cytokines in host cells affected by EVs from parasite protozoa or vice versa.
Abbreviations: IL, interleukin; TNF, tumor necrosis factor; IFN- γ, interferon-gamma; TGF-β, tumor growth factor-beta; CCL, chemokine (C-C motif) ligand.

studies have demonstrated that EVs mostly contain lipid, proteins, DNA, RNA, and metabolites[61–63] (Figure 2). Apparently, EVs have also played a crucial role during transferring of drug-resistant,[64] virulence,[65,66] and regulatory genes[67–69] into the host cells.

Extracellular Vesicles: Classification and Isolation Techniques

Up to now, there is no consensus terminology on the EVs. Based on the size and origin of the mother cell, a spectrum of terms has been given to the extracted cells.[70] As a traditional nomenclature that categorized EVs based on their size and origin, microvesicles, apoptotic bodies, and exosomes are the major classes with size range from 5 μM to 30 nm.[39,70] During recent years, a class of EVs originally released from tumor cells, known as oncosomes, was described which has the size of 10 μm.[71,72] Nevertheless, two main types of EVs, endosomes, and ectosomes, based on their biogenesis have

been defined. Accordingly, endosomes are mostly smaller which as an organelle, formed within the mammalian cells. Exosomes are considered as endosomes and fused to the plasma membrane of the recipient cells. Contrary, ectosomes are formed as budding from the plasma membrane of the donor cells and included microvesicles, apoptotic cells, and oncosomes.[73,74] Over the recent years, a couple of terms have been used for the EVs based on the study context and the mother cells of the released EVs. However, EVs reflect the donor cell conditions.[70] Over the past decade, many isolation and purification techniques have been practiced based on the purpose of studies, available facilities, and the type of EVs.

Overviews of the isolation techniques represented that there are two major isolation methods: (1) size-dependent and (2) size-independent techniques.[75–77] Size-dependent techniques comprise a broad spectrum of methods which isolate EVs based on their size, buoyant density, and physical features. In this approach, differential centrifugation ranged

Table I All Eligible Studies Were Categorized Based on Methods of Isolation of EVs, Clinical Applications and Target Parasites

No.	Author	Year	Method of Isolation	Applications	Parasite
I	Roig[40]	2018	Ultracentrifugation 120,000 × g for Ih at 4°C	Immunotherapy	*F. hepatica*
2	Shears[41]	2018	Ultracentrifugation 100,000 × g for 2 h at 4°C	Vaccination	*T. muris*
3	Eichenberger[42]	2018	Ultracentrifugation 120,000 × g for 3h at 4°C	Immunotherapy	*N. brasiliensis & T. muris*
4	Wang[43]	2018	Ultracentrifugation 105,000 × g for 1.5h at 4°C	Diagnosis	*A. suum*
5	Bautista-López[44]	2017	Ultracentrifugation 100,000 × g for 16h at 4°C	Diagnosis	*T. cruzi*
6	Li[45]	2018	ExoEasy Maxi Kit	Vaccination	*T. gondii*
7	Coakley[46]	2017	Ultracentrifugation 100,000 × g for 2 h at 4°C	Vaccination	*H. polygyrus*
8	del Cacho[47]	2011	Ultracentrifugation 100,000 × g for I h at 4°C	Vaccination	*E. tenella*
9	Meningher[48]	2017	Ultracentrifugation 100,000 × g at 4°C	Diagnosis	*Schistosoma* spp.
10	Trelis[49]	2016	Ultracentrifugation 120,000 × g for I h at 4°C	Immunotherapy	*E. caproni*
II	Olmos-Ortiz[50]	2017	Ultracentrifugation 100,000 × g for 2 h	Immunotherapy	*T. vaginalis*
12	Martin-Jaular[51]	2011	Ultracentrifugation 100,000 × g for 2 h at 4°C	Vaccination	*P. yoelii*
13	Buck[52]	2014	Ultracentrifugation 100,000 × g for 2 h at 4°C	Immunotherapy	*H. polygyrus*
14	Schnitzer[53]	2010	Ultracentrifugation 100,000 × g for 5 h	Vaccination	*L. major*
15	del Cacho[54]	2012	Ultracentrifugation 100,000 × g for I h at 4°C	Vaccination	*E. tenella, E. maxima*, and *E. acervulina*
16	del Cacho[55]	2016	Total Exosome Isolation Reagent (from serum)	Vaccination	*E. tenella*
17	Antwi-Baffour[56]	2019	Ultracentrifugation 100,000 × g for 90 min	Diagnosis	*P. falciparum*

Abbreviation: EVs, extracellular vesicles.

from 300 × g to 200,000 × g is the most frequently used method.[75,78] In this technique, cell debris and dead cells sediment in 300 × g, apoptotic bodies sediment in 2000 × g, microvesicles sediment in 10,000 × g, and exosomes sediment in 100,000–200,000 × g.[79] Although searching among literature reveals that most of the studies employed differential centrifugation to recover exosomes, it seems that the recovery rate and purity of this technique are insufficient.[80] However, utilizing differential centrifugation followed by floatation using sucrose or iodixanol gradient improves the purity and recovery rate of EVs.[77,81,82]

Precipitation-based techniques using polyethylene glycol (PEG), protamine, acetate, and precipitation of proteins with organic solvent (PROSPR) are used to cover the limitations of differential centrifugation-based techniques.[75,83] Although precipitation-based methods improve the recovery rate of EVs, the purity of EVs remains as a challenge.

Ultrafiltration is used to separate EVs based on their molecular weight and size, and increases recovery of EVs compared to the conventional differential centrifugation. However, this technique is not able to differentiate EVs type in a sample based on their size; therefore, the purity of EVs is still insufficient.[77] To overcome the limitations of conventional ultrafiltration, size-exclusion chromatography (SEC), and asymmetrical flow field-flow fractionation (AF4) were developed.[76,77] Recent studies performed by Mol et al[84] and Gamez-Valero et al[85] represented the higher functionality and lower alteration of EVs isolated by SEC compared to

ultracentrifugation and precipitating agents in clinical practices, respectively. EVs separation based on the microfluidic technology was recently developed and practiced. This technique isolates EVs according to their size. However, this method has not been standardized and needs to be compared to the available common isolation techniques.[43,86-88]

Although many techniques based on the size of EVs have been developed, improved, and practiced, two major size-independent EVs isolation methods were described during the recent years. In this regard, immunoaffinity isolation and flow cytometry are two techniques which isolate EVs regarding the expressed proteins on their surface, not their physical criteria. These techniques are usually performed to characterize EVs based on the expressed proteins on their surface in a small volume of samples. However, immunoaffinity isolation followed by a magnetic isolation was described to increase the volume of isolated EVs for further analyses.[89,90] There are available commercial kits which employ specific antibodies to detect CD markers and EVs proteins such as heat shock proteins (HSP).[90–92] Some studies designed and developed flow cytometry-based method for detection of EVs in which the most important limitations for this technique are the particles smaller than 600 nm and low refraction index.[90,93]

The Major Content of EVs

It is widely accepted that EVs contain divergent molecules such as lipids, proteins, genetic materials, and even metabolites which affect the recipient cell's functions.[70] EVs released

Table 2 Targeted Diseases, Cytokine Profile, Cell Line and Animal Models Employed in Each Study

No.	Helminths /Protozoa	Parasite	Diseases	Cytokine/ Chemokine	In vitro/Cell Line	In vivo/Host	Refs
I	Helminths	*F. hepatica*	IBD	IL-17 (Dec) IL-6 (Dec) IL-10 (NC) TNFα (Dec)	-	Female C57BL/6 mice	[40]
		A. suum	Ascariasis	-	Murine macrophage cell line J774A.1	-	[43]
		H. polygyrus	Heligmosomoidesis	IL-10 (Dec) IL-33(Dec) IL-6 (Dec) CCL 17 (Dec) TNF (Dec)	RAW246.7 macrophage cell line, MODE-K (small intestinal epithelial cell line)	Female C57BL/6 mice, BALB/c mice	[46]
		H. polygyrus	Allergy due to *Alternaria*	IL-5 (Dec) IL-13 (Dec) IL-33R (Dec)	MODE-K cells	BALB/c mice	[52]
		N. brasiliensis, T. muris	IBD	IL-6 (Dec) IL-1β (Dec) IFNγ (Dec) IL-17a (Dec) IL-10 (Inc) TGFβ (Dec)	-	B10.BR mice	[42]
		T. muris	Trichuriasis	-	-	C57BL/6 and SCID mice	[41]
2	Protozoa	*T. cruzi*	Chagas	-	-	-	[44]
		T. gondii	Toxoplasmosis	IL-10 (Dec) IL-12 (Inc) TNF-α (Inc) IFN-γ (Inc) IL-4 (NC)	HEK293T cell line, RAW 264.7	Female BALB/c mice	[45]
		E. tenella	Coccidiosis	IL-2 (Inc) IL-16 (Inc) IFN-γ (Inc)	-	White Leghorn chickens	[47]
		Schistosoma spp.	Human Schistosomiasis	-	-	-	[48]
		E. caproni	-	IFN-c (Inc) IL-4 (Inc) TGF-β (Inc) IL-10 (Inc)		BALB/c mice	[49]
		T. vaginalis	Trichomoniasis	*	RAW264.7	BALB/c mice	[50]
		P. yoelii	Malaria	-	-	BALB/c mice	[51]
		L. major	Leishmaniasis	*	-	Female BALB/c mice	[53]
		E. tenella, E. maxima, and E. acervulina	Coccidiosis	IL-2 (Inc) IL-16 (Inc) IFN- γ (Inc) IL-4 (Dec) IL10 (Dec)	-	White Leghorn chickens	[54]

(*Continued*)

Table 2 (Continued).

No.	Helminths /Protozoa	Parasite	Diseases	Cytokine/ Chemokine	In vitro/Cell Line	In vivo/Host	Refs
		E. tenella	Coccidiosis	IL-2 (Inc) IL-6 (Inc) IFN- γ (Inc) IL-4 (Inc)	-	Cobb 500 broilers	55

Note: *In these studies, several times and combinations were investigated. For details, please see the cited references.
Abbreviations: IBD, inflammatory bowel diseases; Dec, decreased; Inc, increased; NC, no changes; IL, interleukin; TNF, tumor necrosis factor; IFN- γ, interferon-gamma; TGF-β, tumor growth factor-beta; CCL, chemokine (C-C motif) ligand.

from parasites contain a complex of molecules ranged from genetic materials to functional proteins. Firstly, Couper et al[94] demonstrated that microparticles with size <1 µm derived from the plasma of mice infected with *P. berghei* were able to activate macrophages. Although it seems that the isolated microparticles were EVs, one of the first studies which showed the presence of EVs in parasites was performed by Marcilla et al[95] who demonstrated the presence of EVs in ESP of *Echinostoma caproni* and *Fasciola hepatica*. Moreover, the proteome analysis of the isolated EVs indicated the similarity of 54% and 52% of protein content of isolated EVs from *E. caproni* and *F. hepatica* with their secretome, respectively. Furthermore, the protein content of *F. hepatica* comprised a higher number of proteases (such as cathepsins and leucine aminopeptidase) and detoxifying enzymes compared to *E. caproni*. Later, Bayer-Santos et al[96] described two types of EVs which were derived from the plasma membrane and within the flagellar pocket of both epimastigotes and metacyclic trypomastigotes stages of *Trypanosoma cruzi*, respectively. The proteome analysis showed that there was EVs content exclusive for either epimastigote or metacyclic trypomastigote, while major identified proteins were known to play crucial roles in metabolism, signaling, nucleic acid binding, parasite survival, and virulence of the parasite.

Later, a couple of small RNAs were characterized within the EVs derived from *T. cruzi* and it was suggested that the parasite can modulate its communication with the host cells via the small RNAs.[97–99] During recent years, focusing on the non-coding small RNA content of the EVs derived from both helminths and protozoa demonstrated a couple of unique small RNAs together with common small RNAs which previously identified in hosts.[100–102] Juntao Liu et al,[103] determined the RNAs content of EVs released by *Schistosoma japonicum* using high-throughput sequencing and then evaluated up taking the EVs by peripheral blood immune cells. Accordingly, they demonstrated that approximately 32% of the RNA content was micro RNA consisted of mir-125b, mir-

61, mir-3505, and a helminth-specific micro RNA, *bantam*. However, they concluded that *S. japonicum* derived EVs increased macrophage proliferation and TNF-α production in host cells via mir-125b and *bantam*.

The Role of EVs in Pathogenicity of Parasites

Parasite–host interaction studies have elucidated that invasive parasites usually release EVs in response to the environmental changes to provide suitable conditions for their pathogenicity. It was shown that EVs are able to modulate the immune system and carry virulence factors to the target- or the related cells in order to manipulate their life cycle using switching on/off the signaling pathways involved in cell death.[104] Recently, it was reported that promastigotes of *L. infantum* release major surface proteases (MSP), an important virulence factor, via EVs.[66]

Furthermore, it was shown that the released EVs can induce transcriptional changes in the target cells, modulate the immune responses, and affect the severity of diseases. Sampaio et al[105] elucidated that monocytes sensed with EVs derived from PfEMP1 transport knock-out *P. falciparum*-infected RBC, induced more transcriptional changes. Furthermore, it was demonstrated that the released EVs carried drug-resistant and virulence genes, and affect the expression level of some host genes. Studies in animal models showed that the released EVs from parasites may affect the pathogenicity of them. Lovo-Martins et al[106] showed that EVs released from *T. cruzi* led to parasitemia and reduced the level of inflammatory biomarkers such as nitric oxide (NO), IL6, and TNFα. It was reported that EVs released from *L. amazonensis* could provide favor conditions in host cells which led to the recruitment of Th2 responses and higher parasite burden.[107] However, it seems that EVs play a central role in the development of a parasite in the host cells.[108]

EVs as a Promising Target for Immunotherapy of Autoimmune Diseases

History of the role of parasites, particularly helminths, in the modulation of the immune system in autoimmune diseases backs to the theory firstly described by Strachan, so-called "Hygiene hypothesis".[109,110] Regarding the chronic infections due to helminth parasites, distracting the immune system seems to be the most important defense tool of these parasites. Actually, helminths modulate the host's immunity via changing the surface antigen or releasing the immunomodulatory components. The ES components are also the well-known mechanism used by helminths to escape from the immune system.[22,58] Until know, ESP of helminths were described to be able to manipulate the immune responses in humans.[111–113] In fact, helminth parasites provoke the polarization of the Th2 responses that lead to the secretion of anti-inflammatory cytokines such as IL4, IL5, IL10, IL13, as well as regulatory cytokine, transforming growth factor (TGFβ).[31] These unique features of helminth parasites were established based on the use of non-human- or human-infecting worms and their eggs,[114,115] released ESP from helminths[111,112] and specific synthesized proteins,[116,117] which are originally secreted from helminths.

The most important autoimmune diseases which have been included in worm therapy plans are IBD, rheumatoid arthritis (RA), MS, diabetes type 1 and asthma.[118] Despite the promising results of worm therapy in almost all of these autoimmune diseases, the major challenge was working with alive worm/egg in humans. Therefore, recent studies employed either natural proteins from the worms or recombinant proteins modeled based on their specific secreted proteins. Tissue inhibitory metalloproteinase-2 (TIMP-2), which is currently known as anti-inflammatory protein-2 (AIP-2), is a major protein secreted in ESP of hookworms that showed favorable results in the attenuation of the clinical manifestations of asthma and IBD in animal models.[119]

During the recent decade, it has been focused on the applications of EVs extracted from either helminth ESP or macrophages stimulated by helminths and their products to ameliorate the clinical manifestations of Th1 autoimmune diseases. EVs as a cargo carry a heterogenic complex of genetic and metabolic materials which are deployed by parasites to communicate with hosts.[39,120] Recently, a growing number of studies experienced both in vitro and in vivo applications of EVs derived from helminths or immune cells stimulated with parasites to cure the symptoms of autoimmune diseases, particularly IBD, that the results of most of them have been satisfying.[40,42,52] However, there is still no clinical trial to demonstrate the pleasant consequents of parasite-released EVs to control the clinical manifestations of autoimmune diseases.

EVs as a New Hope for Designing Next-Generation Vaccines

EVs-based vaccines were widely introduced, practiced, and even proved for bacterial infections and some cancer types, until now. Among bacterial infections, EVs extracted from *Helicobacter pylori*,[121] *Salmonella enterica* (*typhimurium*),[122] *Neisseria meningitis*,[123] *Staphylococcus aureus*,[124,125] *Escherichia coli*[126] and *Acinetobacter*[127] have been studied. Notably, a vaccination strategy based on EVs with trade name "Bexcero" for *N. meningitis* was performed in some countries.[128,129] There is evidence showing that EVs released from tumor cells may control the growth of tumor cells via presenting MHC I and MHC II, and immune system stimulatory molecules.[130,131] Furthermore, during recent years, bioengineered EVs using interference RNA (iRNA) have motivated scientists around the world to design a cost-benefit and effective vaccination strategy for cancers.[132,133]

In case of parasitic diseases, immunization with attenuated parasites or specific synthetic proteins has a long history while there are few studies focusing on the ability of EVs to induce protection against parasites. Although protective effects of EVs were reported in *Trichuris muris*,[41] these experiences were mostly successful in case of parasitic protozoa due to the immunological pathways involved in defending against protozoa.[45,47,55]

Macrophages and dendritic cells, as the progeny of monocytes, stimulated with EVs are able to manage the innate and adaptive immunity against a pathogen. Macrophages and dendritic cells act as antigen-presenting cells (APCs) and are the first barrier against microbial pathogens. These cells after up taking EVs derived from parasites and presenting antigen can initiate cellular or humoral immune responses. However, it seems that shifting towards Th1 responses together with releasing some cytokines, such as IL2, IL12, IL16, and IFNγ, is the main immune system's arms against parasitic protozoa. However, successful induction of protective immunity against *Leishmania, Toxoplasma,* and *Eimeria* suggest EVs and their contents as the next generation vaccines[45,47,53,55] (Figure 1).
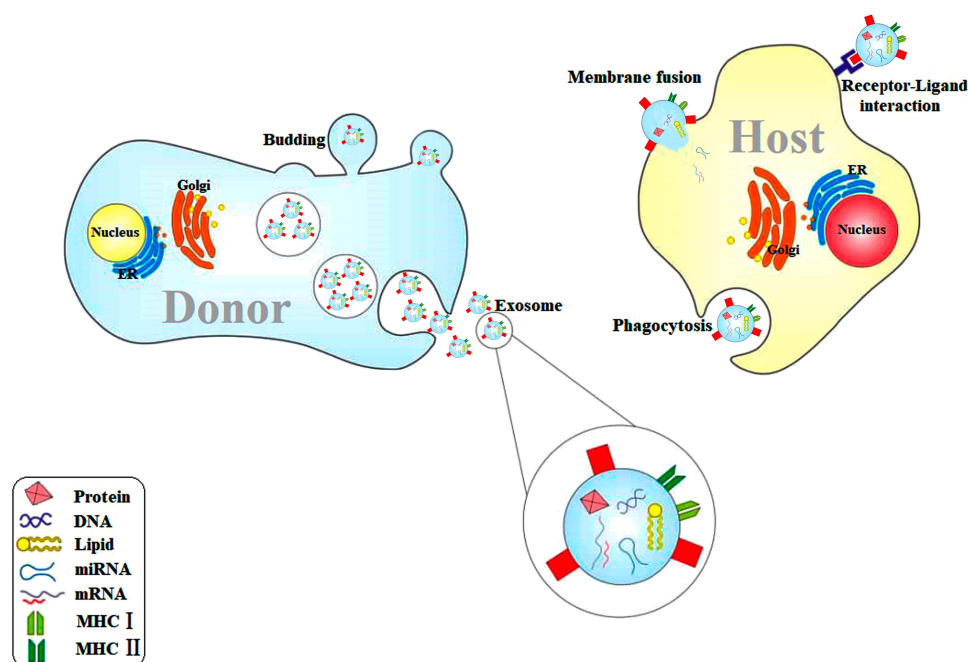
Figure 2 Host cells usually release EVs in response to the environmental changes such as contact with parasites or their products. EVs contain DNA, messenger RNA, MicroRNA, proteins and lipids, and express MHC I and II. EVs are released via budding and vesicle cargo. EVs may affect the recipient cell through direct fusion, receptor– ligand interaction, as well as phagocytosis.
Abbreviations: EVs, extracellular vesicles; MHC, major histocompatibility complex; ER, endoplasmic reticulum; mRNA, messenger RNA; miRNA, micro RNA.

EVs are Specific Targets for Detection of Parasitic Diseases

Rapid and reliable diagnosis of the infectious agents provides all choices on the table for clinicians for on-time interventions. Since EVs represent the features of their mother's cell, these particles have been considered as new biomarkers for diagnosis of different types of cancers, and also detection of infectious diseases. Although it is controversial,[134] based on the infrastructure of the envelope of EVs that is similar to liposomes, it seems that these particles remain stable enough to employ as a diagnostic biomarker. This feature of EVs makes them more applicable to be a biomarker than miRNAs and specific proteins.[133]

A meta-analysis conducted by Zhou showed that the prediction of exosomal miRNA such as miR21, miR-451a, miR-1290, and miR-638 were significantly correlated with the prognosis of solid tumors.[135] EVs (in this study exosome) due to carrying oncogenic materials, were suggested as a prediction tool for human malignant mesothelioma.[136] Studies on EVs for diagnosis of infectious diseases have suggested these interesting tiny particles as a biomarker for the detection of infectious diseases. Anyanwu et al[137] observed human immunodeficiency virus (HIV) protein in EVs released in urine of infected patients and concluded that EVs might be a diagnostic tool for the detection of

HIV. EVs as biomarkers were recently employed for parasitic infections as well. In this regard, released EVs carrying ESP of *T. cruzi*, from mammalian cells were suggested as reliable diagnostic tools.[44] EVs for diagnosis of parasitic infections were later investigated and proposed for *Ascaris*[43] and *Schistosoma*.[48]

However, regarding the high specificity and reliability of EVs, particularly their contents, for the detection of infectious diseases, it seems that a diagnostic panel based on EVs would be a useful, applicable and rapid method for the detection of infectious diseases.

Conclusion and Future Perspectives

During the recent decade, the number of studies that investigated the potential applications of EVs has explosively grown. The most important reason of this growth backs to the interesting results of studies on EVs. Many studies characterized the EV's content in human- and animal-parasites. However, characterization of the specific contents such as micro RNAs and proteins can provide a reliable panel for diagnosis of parasitic diseases, particularly lethal infections. Although there are well-successful attempts for diagnosis of malaria, leishmaniasis, and schistosomiasis using EVs-based approaches, we think that there is a gap for rapid and reliable diagnosis of primary amoebic meningoencephalitis (PAM)

caused by free-living amoeba (FLA), *Naegleria fowleri*. The disease caused by this FLA is almost always fatal and because of the rapid progress of the disease, diagnosis mostly happens post-mortem. Therefore, a rapid and reliable diagnosis panel using the parasite-specific EVs probably improves on-time intervention and prognosis.

There are only a few studies evaluating the effects of parasite-derived EVs on the host cells in vivo or in vitro. Since infections caused by parasites are mostly chronic, parasites communicate with their host for a long time. Therefore, the role of parasites-derived EVs in the modulation of the immune system seems to be an important portion of host-parasite interaction. This fact makes parasite's EVs a terra incognita that needs to explore, particularly in the correlation with autoimmune diseases.

Furthermore, it seems that together with increasing our knowledge on the content of released EVs from parasites, new opportunities will be opened in the field of EVs bioengineering. In the other words, parasites can communicate with the hosts using their EVs; therefore, characterization of the EVs content may describe new useful parasite-specific molecules which makes packaging of EVs using some parasite-specific proteins, metabolites, and iRNA (such as micro RNA, siRNA and Inc RNA) achievable interesting target during the next years, particularly for immunotherapy and vaccination.

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Disclosure

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