VECTOR-BORNE DISEASES

Sending a message: extracellular vesicles of pathogenic protozoan parasites

Anthony J. Szempruch, Lauren Dennison, Rudo Kieft, John M. Harrington and Stephen L. Hajduk

Abstract | Parasitic unicellular eukaryotes use extracellular vesicles (EVs) as vehicles for intercellular communication and host manipulation. By using various mechanisms to generate EVs and by transferring a wide range of molecules through EVs, pathogenic protozoans are able to establish infective niches, modulate the immune system of the host and cause disease. In addition to effects on the host, EVs are able to transfer virulence factors, drug-resistance genes and differentiation factors between parasites. In this Progress article, we explore recent insights into the biology of EVs from human infectious protozoan parasites, including *Trichomonas vaginalis*, *Plasmodium* spp. and kinetoplastids, such as *Trypanosoma* spp. and *Leishmania* spp.

Pathogenic protozoans are responsible for a wide range of human and animal diseases globally, and they cause a substantial socioeconomic burden in many developing nations. These parasites use diverse mechanisms for survival and persistence within their hosts. Recent research has shown that many of these parasites use extracellular vesicles (EVs) to deliver biologically active effector molecules. EVs can be classified into two major classes of secreted vesicle: exosomes, which are generated within multivesicular bodies (MVBs), and ectosomes, which are produced through budding of the plasma membrane. Both exosomes and ectosomes can be described as EVs, which serves as a general term for all secreted vesicles[1]. The secretion of EVs has profound biological effects that result from the transfer of proteins, lipids and nucleic acids to both adjacent and distant cells[2,3]. EVs interact with target membranes through receptor-dependent and receptor-independent processes[4]. A single organism may use several different mechanisms to produce and interact with EVs and may generate a population of EVs with various cargos and functions[5].

Although our understanding of the biology of EVs in mammals and other eukaryotes has rapidly expanded, parasitology has largely lagged behind. The mechanisms of production, interaction and function of eukaryotic EVs have been reviewed thoroughly[3,6]. Recent research has identified EVs that are produced by many human pathogenic protozoan parasites, including members of the phyla Metamonada and Apicomplexa, and the class Kinetoplastida. Other pathogens, including parasitic worms, viruses and fungi, also use EVs during infection[5,7–13]. The production of EVs by parasites has been observed *in vitro* and *in vivo* in animal models, human hosts and insect vectors[5,11,12,14].

The urogenital parasite *Trichomonas vaginalis* is a member of the phylum Metamonada and is the most prevalent non-viral sexually transmitted human pathogen[15]. These extracellular parasites adhere to the epithelium of the urogenital tract in both males and females, and EVs are important for adherence and pathogenesis (see below).

The phylum Apicomplexa includes the genus *Plasmodium,* the members of which are responsible for human malaria. *Plasmodium* spp. are transmitted through the bite of an infected mosquito, which results in the injection of sporozoites, the liver infectious form. Following the invasion of hepatocytes, the parasites divide, differentiate, egress and subsequently infect circulating erythrocytes[16]. The most common forms of human malaria are caused by *Plasmodium falciparum, Plasmodium vivax* and *Plasmodium malariae,* but the mouse-specific *Plasmodium berghei* and *Plasmodium yoelii* are often used experimentally because they are more amenable to studying the complete life cycle of the parasite[16]. EVs have been detected in all of the *Plasmodium* spp. mentioned above and *in vitro* studies have focused on the inter-erythrocytic stages of the parasite life cycle. To date, the capacity of extracellular forms of the parasite to produce EVs has not been analysed.

The class Kinetoplastida contains a diverse group of parasites that cause a wide range of important diseases in humans and animals[17]. This group includes *Trypanosoma brucei,* the causative agent of African sleeping sickness and Nagana in cattle; *Trypanosoma cruzi,* which causes Chagas disease; and *Leishmania* spp., which cause human leishmaniasis[17,18]. All of these kinetoplastids are transmitted by insect vectors to their mammalian host and undergo a series of differentiation steps during their life cycles[17]. Kinetoplastid EVs are some of the best-studied parasite EVs.

Many recent studies on pathogenic protozoans have focused on how EVs modulate the immune system of the host and how they elicit a pro-inflammatory response (BOX 1). For example, treatment with EVs from *T. vaginalis* induces changes in interleukin levels that resemble changes that are observed during infection with the parasite[19]. Similarly, both purified EVs from *Leishmania donovani* and infection with the parasite induce a T helper 1 (T_H1) response in $CD4^+$ T cells[20]. Proteins that were purified from EVs have also been shown to mirror the effect of infection with *L. donovani* on liver function, that is, both reduce the levels of circulating cholesterol[21]. In addition, purified EVs cause pathological changes that resemble the

Box 1 | **Inflammatory response of the host**

The inflammatory response is part of the host innate immunity against invading microorganisms, but can also contribute to and cause disease. In general, the inflammatory response is caused by the recruitment and activation of leukocytes[71,72]. Inflammation is often associated with the increased secretion of cytokines, including interleukin-1β (IL-1β), IL-4, IL-6, IL-8, IL-10, IL-12, tumour necrosis factor (TNF) and interferon-γ (IFNγ)[71–73]. The activation of immune cells also requires interactions with cluster of differentiation (CD) proteins and the stimulation of CD proteins can act as a marker for pro-inflammatory responses[74]. Stimulation of CD40, CD54 and CD86 are all associated with this process and the downregulation of the anti-inflammatory CD163 molecule is pro-inflammatory[72,73]. These factors are part of a T helper 1 (T_H1) cell response that results in the activation and recruitment of macrophages, monocytes and other leukocytes to the site of infection[71,75].

effects of *T. brucei in vivo*[22]. In addition to causing pathology or triggering an immune response in the host, EVs can substantially change parasite populations; for example, EVs have been shown to cause the cellular differentiation of *P. falciparum*[23,24], which is crucial for the continuation of the life cycle of the parasite. EVs might also have a role in the progression of the life cycle of *T. cruzi*[25]. In addition, EVs can transfer virulence factors between parasites, which enables *T. brucei* to spread resistance to innate immune factors of the host to the whole parasite population[22].

In this Progress article, we present the most compelling evidence for the role of EVs from pathogenic protozoans in sending messages within the parasite population and to the host. Although the messages have not been fully deciphered, what we do know is that they have profound implications for parasite development, disease progression and pathology in the host.

Assembling the message

Exosomes. Exosomes were first described in eukaryotes in the early 1980s and the mechanism of their formation and secretion has been the subject of several reviews[3,26–28]. Exosomes are membrane-bound structures of homogeneous size that are derived from MVBs. MVBs are specialized late endosomes that contain exosomes and can traffic to the plasma membrane, with which they fuse to release their exosome cargo[3,27]. Exosomes are formed through the invagination of endosomal membranes, which creates vesicles that display cell surface lipids and proteins on their exterior face[3] (FIG. 1a). Released exosomes interact with target cells through three major mechanisms: receptor-mediated binding, membrane fusion and bulk-phase nonspecific entry through the endocytic pathway and fusion with endosomal membranes[3] (FIG. 1b). Although exosomes contain cell surface proteins and lipids, they are often enriched in unique molecules and show a differential distribution of proteins and lipids compared with the cell surface membrane[27]. Exosomes are also enriched in luminal cargo molecules, which include proteins and nucleic acids[2,27]. During endosomal recycling, MVBs fuse with the cell surface membrane, which results in the release of exosomes[26–28]. Initial electron microscopy studies showed that erythrocytes produce MVBs and release EVs as a mechanism of membrane maturation and homeostasis[26]. Light and fluorescence microscopy showed that *T. vaginalis* produces large MVB-like structures[19]. In addition, electron microscopy studies have shown that the kinetoplastids *T. cruzi* and *Leishmania* spp. produce MVBs to secrete vesicles[29,30]. However, the mechanisms that regulate the formation of MVBs and the subsequent release of exosomes in parasites are unknown.

Ectosomes. Ectosomes are important products of secretion that are formed through budding of the plasma membrane and encompass a wide range of vesicle types (reviewed extensively in REFS 3,6). Ectosomes have a membrane-bound structure and are often of heterogeneous size[3,6]. Ectosomes are derived from the entire cell membrane or from specialized regions of the membrane, such as the cilium and flagellum or from membrane nanotubes[3,6] (FIG. 1a). Ecotosomes have been shown to interact with target cells through similar mechanisms to exosomes[3] (FIG. 1b). For example, erythrocytes that are infected with *Plasmodium* spp. produce EVs that contain parasite proteins through budding of the plasma membrane[23,24]. Kinetoplastids use several mechanisms to produce and release EVs. Both *T. cruzi* and *Leishmania* spp. release EVs through budding along the cell body and at flagellar membranes[29,30]. The *T. brucei* flagellum gives rise to membrane nanotubes that can break down and release EVs[22]. Thus, a single parasite can use several mechanisms to produce EVs. The methods to dissect the functions of individual subpopulations of EVs have not been well established; therefore, it is crucial to note that most experiments with EVs involve a mixture of subpopulations[31].

Sending the message

***Adherence of* T. vaginalis.** *T. vaginalis* produces large MVB structures, which are visible by fluorescence microscopy, when exposed to ectocervical cells, but not in the absence of host cells[19]. EVs that are derived from *T. vaginalis* share physical characteristics with mammalian exosomes, including size, density and protein composition. Short-term incubation of the *T. vaginalis* strain G3, which has low levels of adherence, with purified EVs from other *T. vaginalis* isolates showed that EVs can increase the adherence of this strain to ectocervical cells (FIG. 2). The addition of purified EVs from parasites with preferential adherence to male prostate epithelium cells or female ectocervical cells could transfer this phenotype of tissue-tropic adherence to G3 parasites. This observation has profound implications for *T. vaginalis* infection, as it might enable mixed populations to survive in both male and female hosts and might affect the severity of disease.

Drug resistance and sexual differentiation of* Plasmodium *spp. While inside host erythrocytes, *Plasmodium* spp. can alter the quantity and composition of erythrocyte-derived EVs[32–34]. These changes include the incorporation of parasite proteins, lipids and nucleic acids, including drug-resistance genes[23,24]. Recently, it was shown that EVs from resistant *P. falciparum* could transfer drug resistance to sensitive parasites through episomal DNA and the authors of this study speculated that this mechanism facilitates the transfer of drug resistance during human infection[23] (FIG. 2). This effect was observed both during the co-cultivation of parasites that were separated by semi-permeable transwells and when purified EVs were directly added to cultures of sensitive parasites with a titratable response. The *P. falciparum* erythrocyte membrane protein 1 trafficking protein 2 (PfPTP2) was localized to structures that budded from the surface of infected erythrocytes. Deletion of PfPTP2 substantially decreased the amount of released vesicles and abolished the ability of *P. falciparum* to transfer drug resistance. In addition, EVs have been shown to have a role in the differentiation of *P. falciparum*[23,24]. Prior to egress from erythrocytes, during an important stage in the parasite life cycle for sexual commitment

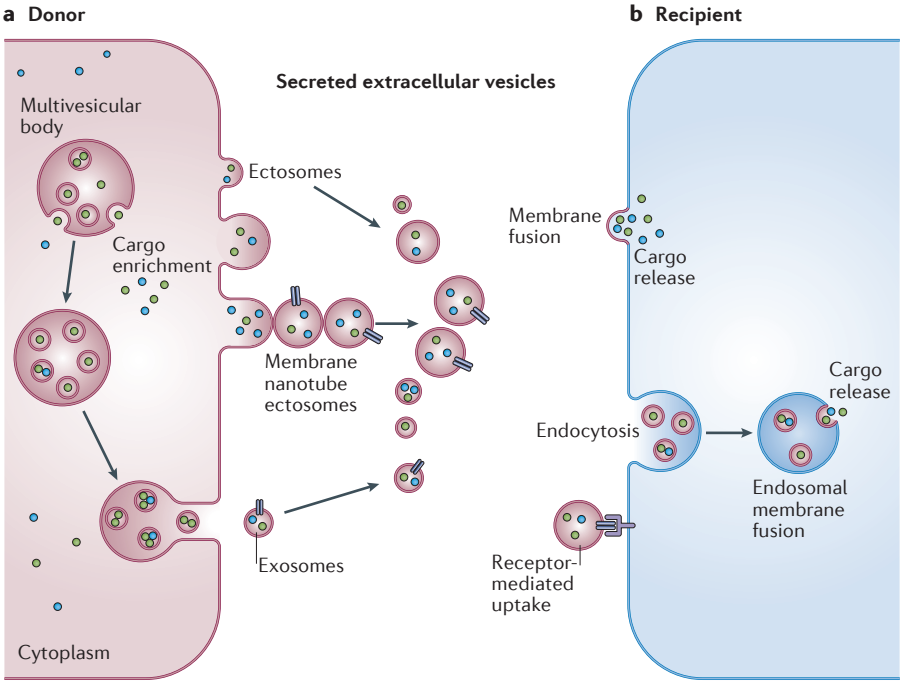

Figure 1 | **Formation and mechanisms of interaction of parasite EVs. a** | Donor cells produce extracellular vesicles (EVs) that are enriched with specific cargo molecules, which include lipids, proteins and nucleic acids[2,3,27]. The composition of EVs does not just depend on the packaging of abundant molecules, but rather depends on functional sorting of cargo molecules. The formation of multivesicular bodies (MVBs), through invagination of the endosomal membrane, results in the production of exosomes of homogeneous size that have components of the cell surface membrane on their exterior surface[3]. The formation of ectosomes occurs from budding at the plasma membrane or along specialized portions of the membrane, such as membrane nanotubes. Many cells use several mechanisms to produce EVs, which results in a mixed population of secreted EVs[3,5,6]. **b** | Interactions of EVs with recipient cells occur through three major mechanisms: membrane fusion, receptor-mediated binding, membrane fusion and bulk-phase nonspecific entry trough the endocytic pathway and fusion with endosomal membranes[64,65]. These interactions can result in cargo delivery and the incorporation of lipids and proteins from the EVs into the recipient membrane[70].

and differentiation, the levels of released EVs substantially increase[23,24,33]. *In vitro*, the addition of purified EVs stimulates the differentiation of asexual *P. falciparum* into gametes, which are the parasites that infect mosquitoes, and thus are essential for the continuation of the life cycle[23,24]. This suggests that alterations to the levels of secreted EVs *in vivo* could directly affect the frequency at which *P. falciparum* infects mosquitoes by increasing the number of gametes.

Virulence and development of* T. brucei, T. cruzi *and* Leishmania *spp. Bloodstream forms of *T. brucei* produce membrane nanotubes through budding and extension of the flagellar membrane[22]. These nanotubes vesicularize, which produces free EVs that are approximately 80 nm in size and that are enriched in flagellar proteins. This process resembles the ciliary release of EVs in the unicellular alga *Chlamydomonas reinhardtii*[6,35]. The animal pathogen *Trypanosoma brucei brucei* does not infect humans and is readily killed in human serum by primate-specific innate immune molecules, which are known as trypanosome lytic factors (TLFs). The closely related subspecies *Trypanosoma brucei rhodesiense* has evolved a mechanism of resistance to TLFs through the expression of the serum resistance-associated protein (SRA), which can bind to TLFs and enables the parasite to persist in a primate host[36,37]. Co-cultivation or the direct addition of EVs from SRA-expressing parasites results in the transfer of SRA to *T. b. brucei* and subsequent resistance to TLF (FIG. 2). These data may explain the report of mixed trypanosome infections in humans[38]. *T. brucei* undergoes density-dependent differentiation in the bloodstream, in which EVs have been proposed to have a role[39]. It has been shown that *T. brucei* can achieve larger culture densities when grown in transwells, by exchanging media that diffuses across a 400 nm membrane[40]. This may suggest that the diffusion of EVs through the membrane might decrease the concentration of EVs and thus also any stimulatory effect that they have on differentiation.

T. cruzi produces EVs through the secretion of MVB-derived exosomes and the shedding of ectosomes at the cell surface membrane. Early work on the *T. cruzi* secretome showed that mucin proteins were released as components of EVs[41,42]. Proteomic analysis of *T. cruzi* EVs showed an enrichment of immunogenic proteins and further fractionation detected the presence of tRNA-derived small RNAs (tsRNAs), which have been proposed to have functions that are similar to small interfering RNAs (siRNAs) in other organisms[43,44]. These tsRNAs colocalize with the *T. cruzi*-specific Argonaute protein TcPIWI-tryp and were transferred between cells in transwell co-cultivation experiments[43]. The addition of purified EVs resulted in the differentiation of *T. cruzi*[25] (FIG. 2). Similarly, EVs that were purified from the related kinetoplastids *L. donovani* and *Leishmania braziliensis* also showed enrichment of tsRNAs, although their role in *Leishmania* spp. interactions have yet to be investigated[45] (FIG. 2). The transfer of small regulatory RNAs by EVs may change the transcriptional landscape of a parasite population during infection and in response to stress.

Sending the message

T. vaginalis *and pro-inflammatory cytokines.* *T. vaginalis* EVs interact with host ectocervical cells through fusion at the plasma membrane, which results in the transfer of lipids and luminal cargo proteins into host cells[19]. Incubation of EVs with ectocervical cells also results in the secretion of the pro-inflammatory cytokines interleukin-6 (IL-6) and IL-8 (REF. 19) (FIG. 3). However, pre-treatment of ectocervical cells with EVs followed by infection with *T. vaginalis* showed an overall dampening of the IL-8 response, which may enable increased parasite growth and pathology without causing a strong early immune response[19]. These results suggest that EVs have a role in immunomodulation of the host, which may dampen the immune response and enable increased parasite attachment.

Plasmodium *spp. and immune activation.* Human and murine infections with *Plasmodium* spp. have been shown to increase the overall number of circulating EVs that are derived from erythrocytes and other cell types in the host[32–34]. The increased

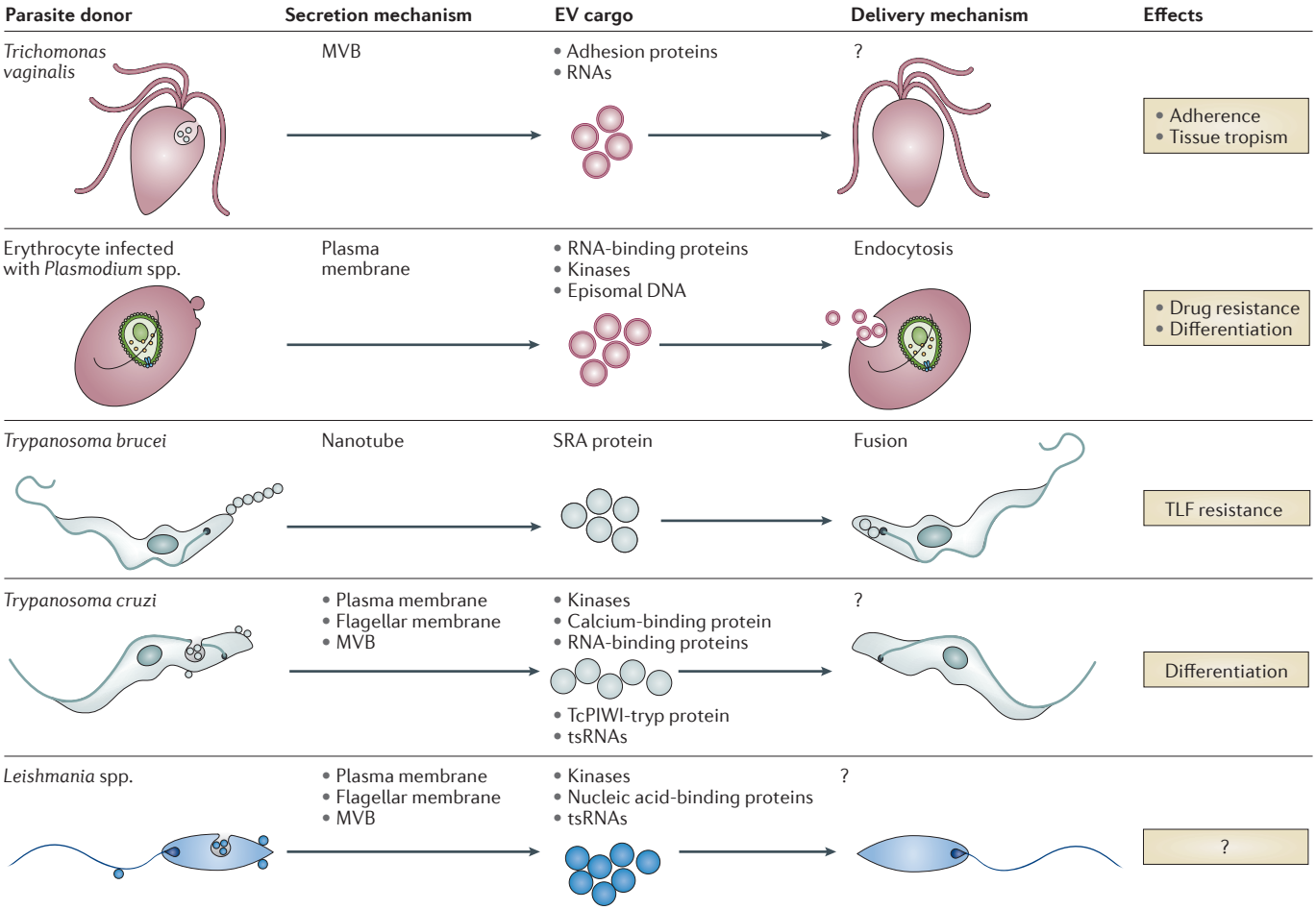

Figure 2 | **Interactions of EVs in the parasite population.** Extracellular vesicles (EVs) can transport cargo proteins, lipids and nucleic acids that mediate interactions within a parasite population. In many cases it remains unclear which mechanism protozoan parasite-derived EVs use to interact with target cells. However, it has been shown that EVs from *Plasmodium falciparum* are endocytosed by infected erythrocytes and that EVs from *Trypanosoma brucei* interact within the parasite population through membrane fusion. EVs mediate a wide range of effects on parasites including alterations to tissue tropism, differentiation, drug resistance and resistance to host immune factors. MVB, multivesicular body; SRA, serum resistance-associated protein; TLF, trypanosome lytic factor; tsRNAs, tRNA-derived small RNAs.

production of EVs during infection has also been correlated with the severity of malarial disease in humans and animals[32,34]. Using rodent malaria models, it was shown that EVs have broad immunomodulatory effects, which often result in a pro-inflammatory response[5,11,12] (FIG. 3). EVs that were isolated from mice with cerebral malaria caused by infection with *P. berghei* activated cultured macrophages and increased the expression of tumour necrosis factor (TNF) and the TNF receptor superfamily protein CD40 (REF. 46). EVs that are produced during infection with *P. berghei* adhere to blood vessels in the brain and may have a role in disease development[32]. Knockout of host ATP-binding cassette transporter 1, which is responsible for the distribution of phosphatidylserine in the plasma membrane, substantially decreased the levels of EVs, prolonged survival and reduced cerebral pathology during infection with *P. berghei*[47]. EVs may also be important for the cell-type tropism of *Plasmodium* spp.[48]. It has been shown that the transfer of EVs from a *P. yoelii* strain that preferentially infects reticulocytes, which are immature erythrocytes, can change the tropism of other *P. yoelii* strains from mature erythrocytes to reticulocytes[48]. EVs derived from erythrocytes that were infected *in vitro* with *P. falciparum* activated monocytes that were isolated from naive human peripheral blood mononuclear cells. Activated cells showed an upregulation of the inflammatory response markers CD40, CD54 and CD86 and a downregulation of CD163 (REF. 24). These EVs also activated human macrophages and stimulated the production of IL-10 and the pro-inflammatory cytokines IL-6, IL-12 and IL-1β[24]. These studies show that EVs that are produced during malaria activate an inflammatory response in the host and broadly modulate immune cells.

T. brucei, T. cruzi *and* Leishmania *spp.* *pathology, immunomodulation and host metabolism.* It has been suggested that differences in the secretome of *T. brucei* may directly affect disease progression and immune responses[49]. Experiments with bloodstream forms of *T. brucei* have shown that nanotube-derived EVs fuse with host erythrocyte membranes and that fusion is mediated by an unidentified surface-exposed protein on the EVs[22]. Fusion results in the transfer of lipids and parasite-specific antigens, including the immunogenic variant surface glycoprotein (VSG), to the erythrocyte surface. This interaction also alters the physical properties

of the erythrocyte membrane and may cause clearance of infected erythrocytes by macrophages in the liver and spleen[22]. Infection of non-primate mammals with *T. brucei* causes anaemia and often results in the death of the host[50,51]. When erythrocytes that were treated *ex vivo* with purified EVs were injected into naive mice they were rapidly cleared and the injection of EVs resulted in anaemia in two different mouse strains[22] (FIG. 3). These observations suggest that the severe anaemia that is observed during infection with *T. brucei* might be caused by biochemical and biophysical remodelling of erythrocytes by EVs.

Proteomic analysis of EVs that are secreted by *T. cruzi* showed an enrichment of proteins that have been implicated in host–parasite interactions, immunomodulation and cell signalling[30]. Injection of purified EVs into naive mice increased parasitaemia and decreased the survival of the mice when they were challenged with *T. cruzi*[52]. Mice that were treated with EVs before infection had more parasites in cardiac tissue and increased mortality than controls, and injection of EVs increased IL-4 and IL-10 mRNA levels in cardiac tissue[52] (FIG. 3). Increased mortality was linked to the secretion of IL-4 and IL-10 as treatment with monoclonal antibodies against both cytokines restored survival to the levels of controls[52]. EVs from *T. cruzi* also caused splenocytes to produce IL-10 and the pro-inflammatory cytokines TNF, interferon-γ (IFNγ) and IL-6, with some parasite strain-specific variation[53] (FIG. 3). *T. cruzi* EVs that were produced *in vitro* contained a class of antigens known as *T. cruzi* surface membrane proteins (TcSMPs)[54]. Purified TcSMP alters calcium signalling in host cells, which inhibits the invasion of host cells by the parasite[4]. EVs may locally disperse TcSMP to limit parasite invasion of cells that are immediately adjacent to sites of high parasite burden[54] and thereby also limit tissue destruction and the recruitment of immune cells. These findings suggest that *T. cruzi* EVs regulate organ tropism and provide some site-specific protection.

The first evidence for the secretion of EVs from *L. donovani, Leishmania mexicana* and *Leishmania major* came from the analysis of secreted proteins. Proteomic analysis showed a substantial enrichment of proteins that had been identified previously in EVs from other eukaryotes including, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), cyclophilin A and the surface metalloproteinase GP63 (also known as leishmanolysin), which is a known parasite

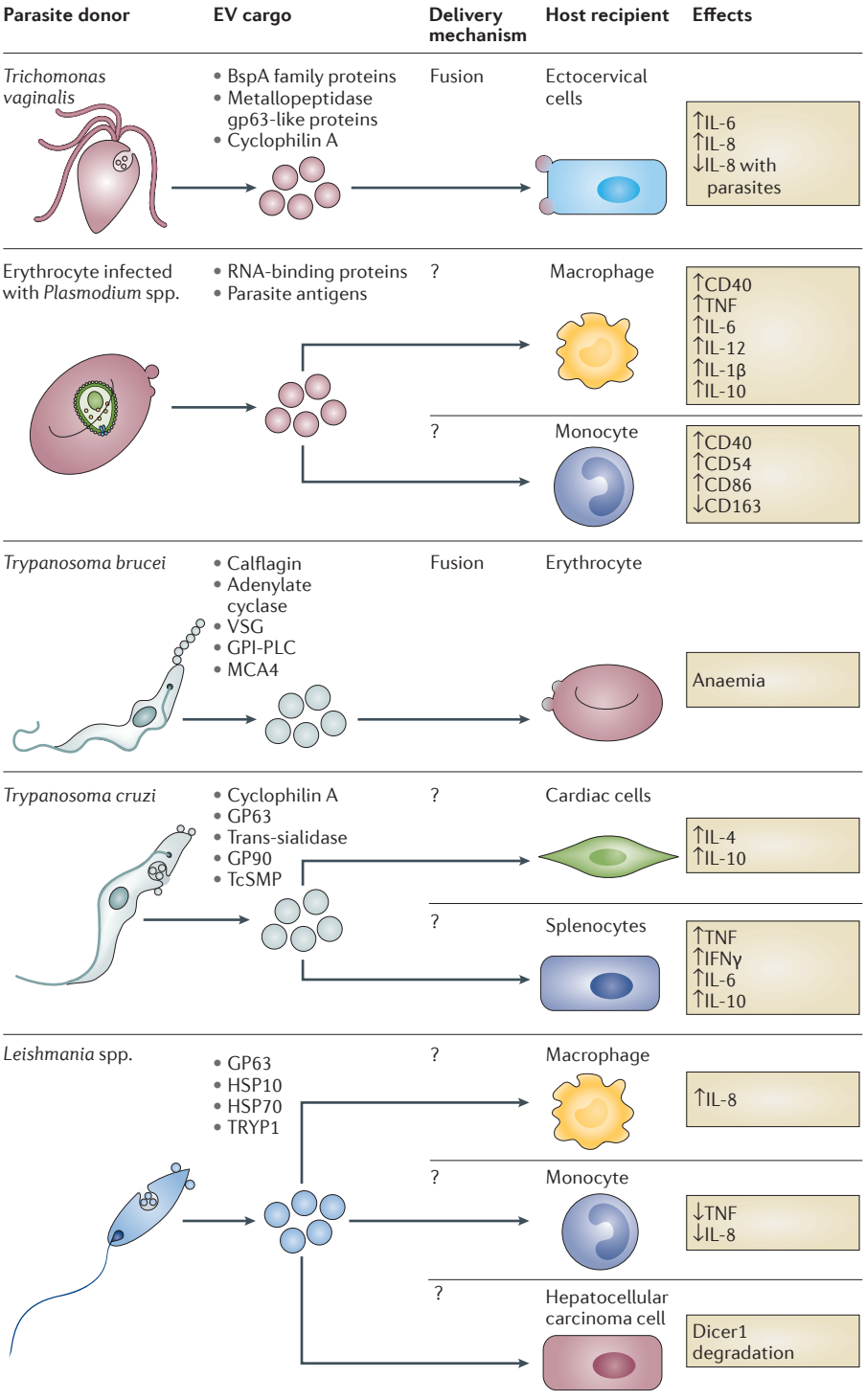

Figure 3 | **Interactions of EVs with host cells.** Parasite extracellular vesicles (EVs) contain cargo proteins and nucleic acids that have been implicated in immunomodulation and virulence within a host. The EVs from *Trichomonas vaginalis* and *Trypanosoma brucei* have been shown to fuse with host cells, which results in the transfer of cargo proteins and lipids. A broad range of target host cells, types of interaction and resulting effects have been identified. EVs often elicit a pro-inflammatory response, which increases the parasite burden in the host and/or disease. Upward arrows denote an increase in expression or secretion and downward arrows denote a decrease in expression or secretion. GPI-PLC, glycosylphosphatidylinositol-specific phospholipase C; HSP70, heat shock protein 70; IFNγ, interferon-γ; IL-6, interleukin-6; MCA4, metacaspase 4; TcSMP, *Trypanosoma cruzi* surface membrane protein; TNF, tumour necrosis factor; VSG, variant surface glycoprotein.

virulence factor[29,55,56]. *L. donovani* EVs contain several immunomodulatory proteins that are known to be T cell antigens, as well as virulence factors that are required for survival during the invasion of host cells[57]. The addition of purified *L. donovani* EVs to macrophages *in vitro* initially increases the production of IL-8; however, long-term treatment of monocytes with purified EVs inhibits the production of TNF and IL-8 (REF. 57) (FIG. 3). Consistent with these *in vitro* results, mice that were infected 3 weeks after the injection of EVs from *L. donovani* and *L. major* had higher parasite loads than control mice[20]. Analysis of *Leishmania infantum* and *L. major* within the sand fly vector showed that EVs are produced and transferred, together with the parasite inoculum, to the mammalian host, in which they stimulate an inflammatory response[14]. In mice, *L. donovani* can invade liver cells, which changes lipid metabolism in the liver and decreases serum cholesterol levels[21]. The decrease in serum cholesterol levels was shown to be due to the inhibition of microRNA-122 (miR-122)-mediated gene repression[21]. The treatment of hepatocellular carcinoma cells with purified EVs recapitulated these changes to host miR-122 and was shown to depend on the EV metalloproteinase GP63 (REF. 21). Purified GP63 degraded Dicer1 *in vitro* and the overexpression of Dicer1 or the expression of an miR-122 variant that does not require processing by Dicer1 rescued EV-mediated changes to miR-122 regulation. Treatment of mice with GP63 decreased levels of Dicer1 *in vivo* and subsequently also the levels of miR-122 processing[21]. These studies show that EVs from *Leishmania* spp. have a wide range of targets in mammalian hosts and have broad immune-dampening roles (FIG. 3).

Intercepting the message

By identifying the message that is delivered by EVs within a host it may be possible to detect even low levels of the parasite that broadcasts that message. Many parasites have developed sophisticated mechanisms to evade the innate and adaptive immune responses through alterations to their surface proteins[58–60]. However, parasite-derived EVs are enriched in invariant immunogenic proteins and nucleic acids, many of which can be found in the circulation of infected hosts[22,24,29,30]. It may be possible to screen individuals for the presence of cargo molecules as a first line of detection. EVs that are produced by mammalian cells as biomarkers for diseases such as cancer and cardiovascular diseases are also subjects of active research[31,61].

As discussed above, parasite EVs have two broad effects: immunomodulation, which often elicits a pro-inflammatory response, and the modulation of disease development through direct interactions with tissues and specific cell types. Blocking the interaction of EVs with target host cells could prevent immune activation and pathogenesis; for example, through decreased immune dampening and reduced parasite burden and disease severity during *Plasmodium* spp. and *Leishmania* spp. infection. In the case of the cattle disease Nagana, which is caused by *T. brucei*, it has been proposed that the ability to resist anaemia is more important for survival than the control of parasitaemia[50,51]. *T. brucei*-derived EVs interact with target membranes through proteins that are exposed on the surface of EVs[22]. Future efforts to identify these fusogens may provide a powerful tool for preventing disease, including anaemia, and possibly the migration of *T. brucei* to specific host tissues.

In addition to therapeutic and diagnostic applications during an active infection, EVs may provide a powerful new tool for vaccination. Immunization with EVs from *Plasmodium* spp. showed promising results in an animal model[48]. In mouse studies of *L. major* and the protozoan parasite *Toxoplasma gondii,* the use of host cell-derived EVs that were pre-treated with parasite antigens or parasite cells elicited a protective immune response to parasite challenge[62,63]. Using parasite-derived and host-derived EVs are promising new therapeutic options.

Conclusions

The biology of EVs from protozoan parasites has only recently started to be unravelled. This delay compared with research in other organisms may be due to the difficulty associated with identifying and characterizing the production of EVs by parasites in a multicellular host[64–67]. Another challenge has been to determine whether there is tissue-specific and cell-specific targeting of EVs that are produced *in vivo*. In a cancer animal model, it has been shown that cancer-derived EVs are important for tissue-specific metastatic niche formation[68]. A recent mouse study used Cre mRNA that was secreted into EVs to identify target cells of EVs, organ-specific interactions and cell reprogramming mediated by metastatic tumour cells[69]. Similar approaches could be used to identify the targets of parasite-derived EVs *in vivo*. Research on EVs in other organisms has resulted in the generation of powerful community databases that contain extensive data sets (for example, Vesiclepedia and ExoCarta). Recently, data of parasite-specific EVs have also begun to be incorporated into EuPathDB. Ample evidence suggests that parasites, similarly to other eukaryotes, use EVs to send a message[70]. With powerful new techniques being developed to understand the interactions and effects of EVs *in vivo* it is only a matter of time before the field of parasitology catches up to cancer biology and other fields. Deciphering, intercepting and blocking the messages that are carried by EVs may change the way we think about and how we treat parasitic diseases in the future.

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Competing interests statement
The authors declare no competing interests.

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