Published in final edited form as:

Trends Parasitol. 2016 June ; 32(6): 435–436. doi:10.1016/j.pt.2016.03.001.

Vesicles as Vehicles for Virulence

Monica R. Mugnier^{1,2}, F. Nina Papavasiliou¹, and Danae Schulz^{1,3}

¹Laboratory of Lymphocyte Biology, The Rockefeller University, 1230 York Avenue, New York, NY, 10065

²The Rockefeller Graduate Program, The Rockefeller University, 1230 York Avenue, New York, NY, 10065

Abstract

Parasites have long been known to influence host responses to infection through the secretion of virulence factors. Extracellular vesicles are emerging as an important mediator of these manipulations, and a new study by Szempruch et al suggests they could have a critical role in host responses to African trypanosome infections.

Extracellular vesicles (EVs) are produced by nearly every cell type, including most parasites [1]. The transfer of their contents, whether protein, lipid, or nucleic acid, from one cell to the next can alter the functioning of the recipient cell, making these vesicles crucial mediators of cell signaling with huge potential as therapeutic and diagnostic tools. EVs were recently reported in African trypanosomes in *Trypanosoma brucei gambiense*, a form of *T. brucei* responsible for chronic African sleeping sickness in humans [2]. A new study by Szempruch and Sykes et al identifies EVs in an animal infective form of the parasite, *T. b. brucei* [3], which appear quite similar to those described in *T. b. gambiense*, at least morphologically. The authors extend upon the findings in *T. b. gambiense* by identifying parasite “membrane nanotubes”, which had been described in *T. brucei* in the 1960s and referred to as “plasmanemes” at the time [4]. The role of plasmanemes was unclear then, but Szempruch and colleagues suggest that these nanotubes/plasmanemes may be the origin of EVs in African trypanosomes. They microscopically observe the formation of EVs during the dissociation of membrane nanotubes, though it is not clear if this is the exclusive mechanism of EV formation in *T. brucei*. Interestingly, membrane nanotubes appear to transiently interact with neighboring parasites. If functional, these structures might mediate cell-to-cell communication.

One of the most intriguing aspects of EVs in the context of infectious disease is their potential to fuse with and alter the function of host cells. Szempruch et al show that, indeed, parasite EVs fuse with host erythrocytes, transferring parasite variant surface glycoproteins

*Correspondence can be addressed to any of the authors (; Email: mmugnier@rockefeller.edu, ; Email: papavasiliou@rockefeller.edu, ; Email: dschulz@rockefeller.edu)

³Present address: Department of Biology, Harvey Mudd College, 301 Platt Blvd, Claremont, CA 91711 USA

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

(VSG). The transfer of VSG to host erythrocytes was previously observed by [5] but the mechanism behind it and its functional relevance remained a mystery. Szempruch et al demonstrate that fusion of EVs with host erythrocytes affects erythrocyte rigidity and size and postulate that this results in preferential clearance by host myeloid cells [6], though this is not tested directly. Consistent with this model, the intravenous injection of parasite EVs into naïve mice results in a 5–10% decrease in the number of host erythrocytes within 1hr, demonstrating a potential role for parasite EVs in infection-associated anemia. The transfer of VSG from the parasite surface to the recipient erythrocyte raises the possibility that erythrocytes could become targets of antibody responses directed against parasite VSGs, which might also be expected to cause increased erythrocyte clearance, though [6] show preferential clearance of trypanosome-altered erythrocytes even in uninfected mice. It remains to be seen whether *T. brucei* EVs fuse with other host cell types, and what, if any, modulatory effect they have.

In addition to the interaction between EVs and erythrocytes, Szempruch and colleagues demonstrated that African trypanosome EVs were able to transfer virulence factors from one parasite to another. One such factor is the serum resistance associated (SRA) gene product, which allows *T. b. rhodesiense* to escape lysis by blocking the action of the trypanosome lytic factor (TLF) found in human blood [7]. *T. b. brucei* lacks the SRA gene and is thus usually susceptible to lysis by TLF. In an elegant series of experiments, the authors demonstrated that co-culturing *T. b. brucei* in transwells with either *T. b. rhodesiense* or with *T. b. brucei* carrying an SRA transgene conferred resistance to killing by TLF. They went on to show that resistance to TLF could be conferred through the addition of EVs alone, provided that they were harvested from either SRA transgenic *T. b. brucei* or from *T. b. rhodesiense*. The authors also demonstrated that SRA and other vesicular membrane proteins from EVs enter the endocytic pathway at the flagellar pocket of the recipient parasite, with no evidence for receptor-mediated internalization. This raises interesting questions as to whether co-infections with *T. b. brucei* and *T. b. rhodesiense* could lead to enhanced ability of *T. b. brucei* to survive within a primate reservoir.

In all scenarios tested by Szempruch and colleagues, the EV-mediated interaction between trypanosomes, or between trypanosomes and host cells, leads to the transfer of proteins. Thus, the question remains open as to whether EVs also carry DNA or RNA cargo, the delivery of which could effectively reprogram the recipient cell, as happens with *P. falciparum* [1]. This type of reprogramming could lead to immune modulation in the host, which has been demonstrated in a number of other parasitic systems [1].

Additionally, it remains to be seen whether *T. brucei* EVs contain cargo that mediate communication or quorum sensing. While trypanosomes are single-celled eukaryotes, their behavior within their insect and mammalian hosts suggests that they communicate with one another, and the means by which this occurs has been a longstanding mystery in the field. In the insect form, *T. brucei* exhibits social motility, which has been shown to be important for colonization of the fly midgut [8]. Colonies of migrating parasites repel one another, but the nature and function of the repellants are unknown. Within the mammal, *T. brucei* appears to possess a quorum-sensing mechanism that results in density-dependent differentiation from long slender to short stumpy forms that are transmitted to the fly midgut, but the mysterious

stumpy induction factor (SIF) has yet to be identified [9]. Additionally, the authors note that stress from RNAi-mediated depletion of an essential gene or from complement active serum increases the frequency of nanotube formation. Assuming that nanotubes do result in an increase in the number of EVs, one could imagine a scenario where generalized stress induced by high levels of parasitemia within the mammal could lead to increased nanotube formation, followed by delivery of SIF via EVs, culminating in differentiation from long slender to short stumpy parasites that are pre-adapted for survival within the fly midgut. The involvement of EVs as carriers of signals for differentiation to stumpy forms or for social motility is now an open and exciting area of inquiry.

This work ties together the mysterious observations of plasmanemes and parasite-to-host transfer of VSG, while at the same time suggesting new mechanisms for parasite communication. With these findings, *T. brucei* joins the ranks of the numerous parasites that use these unique structures as mediators of cell-to-cell communication, both between parasites and with their intimately associated hosts [1].

References

1. Coakley G, et al. Exosomes and Other Extracellular Vesicles: The New Communicators in Parasite Infections. *Trends Parasitol.* 2015; 31:477–489. [PubMed: 26433251]
2. Geiger A, et al. Exocytosis and protein secretion in *Trypanosoma*. *BMC Microbiol.* 2010; 10:20. [PubMed: 20102621]
3. Szempruch AJ, et al. Extracellular Vesicles from *Trypanosoma brucei* Mediate Virulence Factor Transfer and Cause Host Anemia. *Cell.* 2016; 164:246–257. [PubMed: 26771494]
4. Vickerman K, Luckins AG. Localization of variable antigens in the surface coat of *Trypanosoma brucei* using ferritin conjugated antibody. *Nature.* 1969; 224:1125–1126. [PubMed: 5353729]
5. Rifkin MR, Landsberger FR. Trypanosome variant surface glycoprotein transfer to target membranes: a model for the pathogenesis of trypanosomiasis. *PNAS.* 1990; 87:801–805. [PubMed: 2300563]
6. Stijlemans B, et al. Development of a pHrodo-based assay for the assessment of *in vitro* and *in vivo* erythrophagocytosis during experimental trypanosomosis. *PLoS Negl Trop Dis.* 2015; 9:e0003561. [PubMed: 25742307]
7. Capewell P, et al. A co-evolutionary arms race: trypanosomes shaping the human genome, humans shaping the trypanosome genome. *Parasitology.* 2015; 142:S108–19. [PubMed: 25656360]
8. Imhof S, Roditi I. The Social Life of African Trypanosomes. *Trends Parasitol.* 2015; 31:490–498. [PubMed: 26433252]
9. Mony BM, Matthews KR. Assembling the components of the quorum sensing pathway in African trypanosomes. *Mol Microbiol.* 2015; 96:220–232. [PubMed: 25630552]

Figure 1. Extracellular vesicles could mediate a variety of processes in *Trypanosoma brucei* infection

Szempruch et al demonstrate that *Trypanosoma brucei*-generated extracellular vesicles (EVs) have the ability to transfer protein to other parasites and to host cells. This could be important for modulating the immune response to parasite infection both in the mammal and the fly. EVs could also be the mechanism by which repellants or other factors important for social motility are transferred between parasites in the fly. Finally, EVs could play a role in quorum sensing that mediates density-dependent differentiation from long slender to stumpy forms within the mammal.