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Review articles

Exosomes in the context of *Toxoplasma gondii* – host communication

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ABSTRACT. Extracellular vesicles – EV's, including exosomes, are known to be essential tools of intercellular communication, enabling the exchange of information without direct contact between cells. Exosomes are secreted both *in vitro* and *in vivo* by single- and multi-cellular organisms, regardless of their type, and play an essential role in cell-to-cell communication. EV's may carry various materials and ongoing studies have provided a new insight into their potential participation in various critical biological processes, including carcinogenesis, protein trafficking, immunostimulation and pathogenesis of infectious diseases. Although knowledge of the contribution of exosomes in *Toxoplasma* invasion is still very limited, the present article discusses aspects of their involvement in the interactions between host and *T. gondii*.

Key words: exosomes, *Toxoplasma gondii*, hosts

Exosomes

Extracellular vesicles (EVs) secreted by both prokaryotic and eukaryotic cells can be divided into three classes based on their size and biogenesis: apoptotic bodies (800–5000 nm) released during programmed cell death, microvesicles (50–1000 nm) secreted by budding and exosomes (40–100 nm), which are possibly of endocytic origin [1]. Exosomes comprise a varied mix of peptides, proteins, mRNA, DNA and lipids in a stable, subcellular form. Exosomes represent the key, universal tool of communication between cells of both hematopoietic and non-hematopoietic origin; however, their biological function is still poorly understood [2].

One particularly interesting aspect of exosome function is that complex parasite-host relations have formed during long-term coevolution, and exosomes are believed to have played the main role in these phenomena [3]. As many parasites are able to successfully modify the activity of the host immune system using highly sophisticated mechanisms such as immune evasion, molecular exploitation and molecular piracy, parasitic infections are typically chronic and allow the long-lasting persistence of the parasite and survival of the host [4].

Toxoplasma gondii

Toxoplasma gondii (type Apicomplexa) is a widespread protozoan obligate parasite that infects all species of endothermic animals and humans with a high prevalence. In immunocompetent hosts, primary infections are usually asymptomatic; under the pressure of specific Th1 lymphocyte-dependent immunity, fast replicating tachyzoites become slow-replicating bradyzoites which enclose themselves in the tissue cysts, located preferentially in the brain, muscles and eyes. The formation of tissue cysts acts as a starting point for establishing a chronic, latent toxoplasmosis lasting for the lifetime of the host. Latent toxoplasmosis represents a fine-tuned balance between the parasite and the host, and the reactivation of chronic infection is possible mainly due to the impairment of the immune system causad e.g. by AIDS or the use of immunosuppressive drugs [5]. Furthermore, long-term occupation of the brain by the parasite is believed to be associated

with several behavioural changes of the infected intermediate hosts, including humans [6–9]. The most striking example of parasite-induced changes in host behaviour is a loss of aversion toward the smell of felids, the definitive hosts of the parasite, which has been demonstrated in rodents, mostly mice and rats [10,11], humans [12] and recently in chimpanzees [13]. Primary infection may lead to foetal malformation or even death in pregnant women, and congenital toxoplasmosis in farm animals, typically sheep and goats, causes great economic losses.

Knowledge of the exosomes secreted by *T. gondii* or *T. gondii*-infected host cells is still very limited. However, some pioneering research on *T. gondii* exosomes suggests that host exosomes may be employed as a potential vaccine against toxoplasmosis [14]. In this study, dendritic cells of the DC2.4 (H-2b) line were stimulated with soluble *T. gondii* antigens. Exosomes from the culture medium, injected intravenously to C57BL/6 mice (H-2b), moved preferentially to the spleen ("homing") and induced a strong immune response of the Th1 type characterized by an intense *in vitro* proliferative response of splenocytes after stimulation with native *T. gondii* antigens. Furthermore, vaccination with exosomes led to a pronounced increase in IFN-γ and IL-2 (Th1 cytokines) synthesis by splenocytes, which was accompanied by a decrease in the secretion of Th2 cytokines (IL-10 and IL-5). This strong Th1-polarized immune response protected C57BL/6 mice from the development of acute and chronic toxoplasmosis: 67% of vaccinated animals survived the lethal challenge with *T. gondii* cysts, and vaccine administration resulted in the decrease of the parasite cyst burden by 75%. These results indicate that exosomes could be a very effective non-cellular vaccine material exhibiting both antigen and adjuvant properties.

However, the exact mechanism of T lymphocyte stimulation remains unclear. Exosomes may present directly specific *T. gondii* antigens or they could first be taken by antigen-presenting cells (APC, indirect pathway) [15]. Further studies have revealed that exosomes secreted by dendritic cells induce protection in both syngeneic and allogeneic mice [16]. In the allogenic mice, exosomes are probably first taken by other APCs where the antigen stimulus is enhanced. Vaccination triggers systemic and local cell-mediated immunity characterized by the Th1/Th2 cytokine balance in syngeneic CBA/J mice and the Th1 cytokine profile in allogenic C57BL/6 mice. Strong cytokine and proliferative responses were accompanied by the production of specific circulating IgG antibodies in both mouse strains and secretory IgA present in the intestinal secretions of CBA/J mice. The resulting immunity protected allogeneic mice from acute infection after *T. gondii* challenge and led to a 60% decrease in brain parasite burden in syngeneic CBA/J mice. Considering the possible fatal effects of intrauterine infection, the dendritic cell-derived *T. gondii* exosomes were also tested for their immunoprevention of congenital toxoplasmosis [17]. Using an experimental mouse model, it was found that mice vaccinated with exosomes before pregnancy delivered more offspring then the mice injected with soluble *T. gondii* antigens. Furthermore, both the body mass and survival rates of pups from exosome-vaccinated mothers were higher than those of the infected control animals. Although vaccination with exosomes did not completely prevent transmission of the parasite *via* placenta, it led to reduced cyst burden in the offspring: the tissue cyst burden in newborns from vaccinated pregnant mice was 65% lower than that observed in pups from non-vaccinated infected pregnant animals.

Finally, Martínez-Gómez et al. [18] used cytoskeleton proteins isolated from the virulent RH strain of *T. gondii* to immunize mice. Intraperitoneal administration of three doses of the vaccine resulted in a significant reduction of parasite brain cyst number (88%) in mice challenged with *T. gondii* ME49 strain, and the protection provided by cytoskeleton proteins was higher than that conferred by the TLA antigen obtained from sonicated RH tachyzoites.

Taking into account *T. gondii* neurotropism and neuropathogenicity very interesting results were obtained by Pope and Lässer [19]. The authors infected *in vitro* epithelial HFF cells with *T. gondii*, Prugniaud strain. After 24 to 72 hours of incubation, microvesicles of a diameter < 100 nm with an exosome-like morphology were detected in the culture medium. Using uninfected HFF cells as a control (stimulated to the release of exosomes by serum-free medium) they compared mRNA and miRNA profiles in both types of exosomes. The infection caused a highly significant increase in mRNA level, up to several dozen times, for four factors displaying neurologic activity: Rab-13, EEF1A1 (eukaryotic translation elongation factor

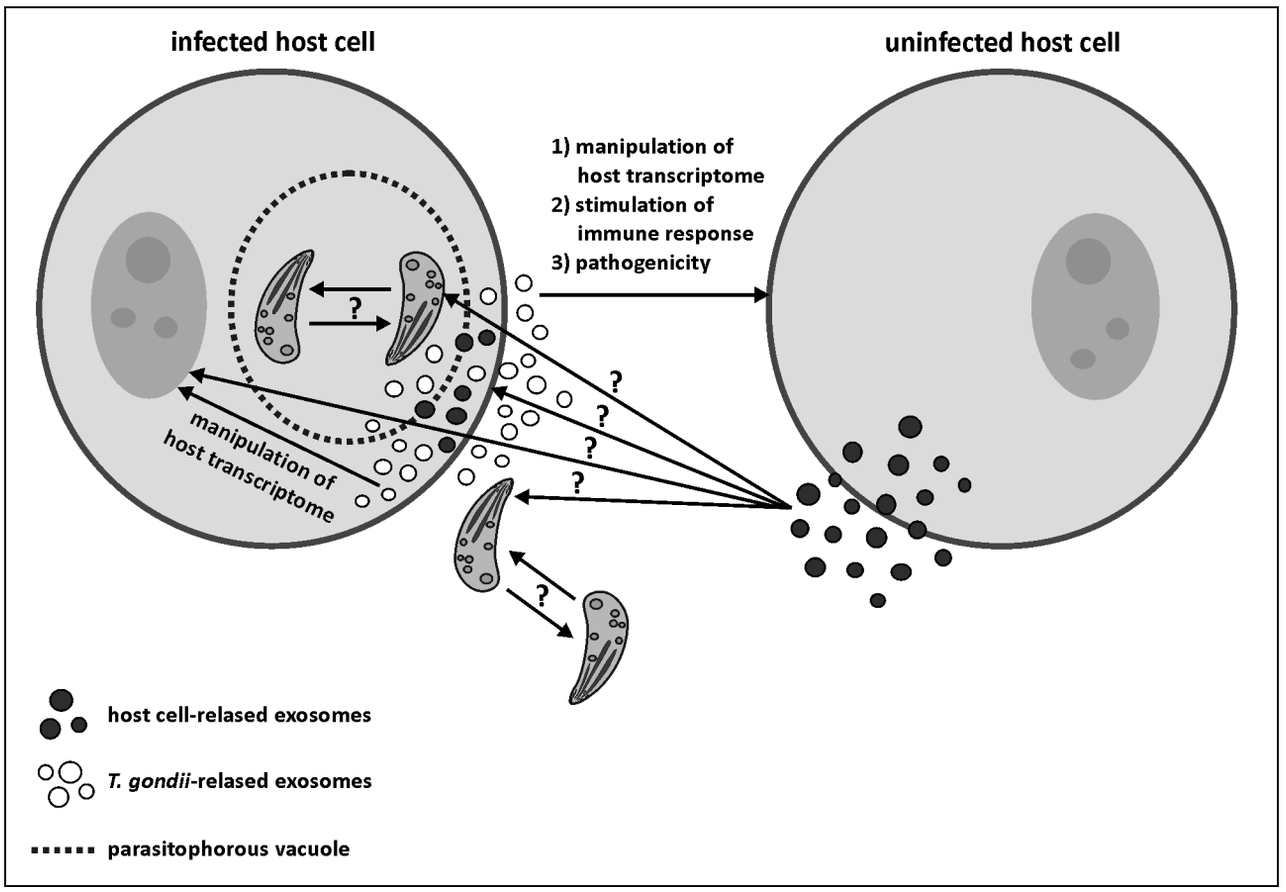

Fig. 1. Possible involvement of *T. gondii* and host cell exosomes in parasite-host interactions

alpha 1), thymosin and LLP protein homologue. In addition, an increase in the expression of miR-23b, an anti-inflammatory mediator suppressing IL-17 production and response, was also noted. Interestingly, a recent finding noted that chronic *T. gondii* infection induces the expression of miR-146a and miR-155 in brain tissue, a pair of immunoregulatory microRNAs which constitute a characteristic fingerprint for infection by type II strains which are highly cystogenic and most prevalent in Europe and North America [20]. Both miRNAs are regulated by in part by rhoptry kinase ROP16. The appearance of miR-155 and miR-146A coincides with the presence of "mature" cysts at day 20 post infection, suggesting that their level might be regulated bradyzoite-specific effector(s). Remarkably, miR-146a-deficient mice displayed lower brain colonization and a higher survival rate than control mice. A less severe course of infection is likely consequence of lower parasite burden at an early infection stage rather than a direct effect of miR-146a in the brain.

Taken together, these findings hint at an intriguing relationship between these molecular changes and parasite-induced behavioral alterations in animals and humans. The exosomes expressed by parasitic protozoa may cause a tolerance to the parasite and favor its long term survival or conversely, they may induce pro-inflammatory pathways contributing to control and clearance of infection by the host [21,22].

The protozoan *Toxoplasma gondii* is believed to be the most successful parasite worldwide, with a very wide range of potential hosts and high prevalence. The parasite manipulates the host immune system, promotes parasite persistence and prevents host death. Leroux et al. [23] have recently found that proteins secreted *in vitro* by extracellular *T. gondii* tachyzoites (ESA – excreted/secreted antigens) inhibit IFN-γ-induced expression of major histocompatibility complex class II on the surface of infected cells, thus disturbing the antigen presentation process and damping the specific CD4+ T cell response. Additionally, ESA reduced

the expression and release of TNF-α by macrophages. The great diversity of the secreted proteins present in the ESA preparation, as determined by liquid chromatography-tandem mass spectrometry (LC-MS/MS), suggests that *T. gondii* relies on numerous effector molecules to modulate host cellular functions. While it is possible that these multi-component complexes may be involved in the observed phenomena, this hypothesis needs further experimental confirmation.

The parasite is known to use the exosome-based pathway as a means of protein secretion *in vivo* [24], since vesicles possessing biochemical and morphological features of exosomes containing specific parasite proteins (SAG, MIC, GRA, GPI, ubiquitin and cyclophilin) were isolated from peritoneal fluid of *Toxoplasma*-infected mice. *Toxoplasma* is also capable of secreting molecular switches which can selectively shape the transcriptional regulatory machinery of the host in a strain-specific manner. Primary host targets for the switches include NF-κB (nuclear factor κB), c-Fos (transcription factor of Fos family), STAT3/6 (signal transducer and activator of transcription 3/6) and c-Myc (transcription factor c-Myc).

The major sources of parasite effector molecules seem to be rhoptries (ROP16, ROP18, ROP38 etc.) and dense granules (GRA15, GRA24 etc.) [25,26].

In addition, it has been demonstrated that exosomes released by the parasite can influence the proliferation and cell cycle of host cells, and that various exosomal miRNAs are involved in the regulation of target genes related to cell division [27]. However, like other eukaryotes, including unicellular apicomplexan protozoans, *Toxoplasma* maintains both the levels and localization of cytoplasmic mRNAs throughout its life as part of the gene regulation which occurs *via* assembly and disassembly of mRNPs (messenger ribonucleoproteins). Once exported from the nucleus into the cytosol, the mRNAs interact with various proteins, and the composition of mRNP complexes is permanently and dynamically changed. Cytoplasmic mRNA granules became microscopically more visible under stress conditions and translational arrest. Their marker appears to be a novel DEAD-box helicase (named TgHoD1) which is involved in the fate of transcripts and the formation of cytoplasmic stress granules [28].

The analysis of gene expression and regulation in *Toxoplasma* may facilitate a deeper understanding of the mechanisms responsible for their pathogenic action in the host. The ability to influence gene expression in distant cells through endosomes has many potential scientific and therapeutic applications. The parasite has evolved numerous means to prevent such imbalances in the gene regulatory network that could compromise host survival, which in turn would eventually prevent parasite persistence [26]. There are many questions regarding unknown aspects concerning the involvement of host and *T. gondii* exosomes in the course of acute and chronic infection. Exosomes secreted by the parasite are able to stimulate the immune response directly or indirectly, *via* dendritic cells and other APCs, but possibly they could also induce pathological processes in the host. On the other hand, *T. gondii*-derived exosomes, may well be tools of intraspecies, or even interspecies, quorum sensing. Future research should address these questions, as they might be the key to understanding the complex mechanisms behind the pathogenicity of *T. gondii*. Furthermore, considering the importance of exosomes in cell-to-cell communication [29], the role of exosomes originating from both infected and uninfected host cells in the performance of the host cell during a parasite invasion should also be considered. Finally, the influence of host cell exosomes on extracellular and intracellular parasites needs to be clarified, particularly in the case of tissues and organs preferentially occupied by *T. gondii*, such as the brain, muscles and eyes. One further, potentially valuable, use of exosomes is that of diagnostic markers. Recently, Exosome Diagnostics, Inc. (http://www.exosomedx.com/) has developed a revolutionary liquid biopsy platform that enables non-invasive detection of clinical biomarkers, one notable use being the recognition of prostate cancer from a simple urine sample.

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