Toxoplasma gondii: qualified to secrete exosomes?

Marbel Torres, Céline Ducournau, Isabelle Dimier-Poisson

Host microvesicles (MVs) help pathogens, such as the intracellular parasite, *Trypanosoma cruzi*, to evade complement attack. We have now found that the infectious metacyclic trypomastigote forms, by interacting with host integrins, lipid rafts and stretch activated channels, stimulate a calcium-mediated depolymerization of the actin cytoskeleton and MV release. The release of MVs in turn stimulates a lysosomal repair mechanism in the host cell, to plug the breach in the plasma membrane. By using specific inhibitors of lysosomal exocytosis and both pharmacological and siRNA-mediated inhibition of microvesicle release, we describe a novel entry mechanism by which the parasite opportunistically takes advantage of a host membrane repair mechanism, to execute entry before membrane integrity is fully restored.

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43. *Toxoplasma gondii*: qualified to secrete exosomes?

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Toxoplasma gondii is a protozoan parasite responsible for toxoplasmosis, a worldwide disease that leads to encephalitis in immune-compromised individuals and to congenital toxoplasmosis of infected fetus. In order to design effective vaccine strategy a more comprehensive understanding of the biology of *Toxoplasma*-host interactions is crucial. *T. gondii*, as eukaryotic cell, contains an endomembrane network including an endoplasmic reticulum and a single Golgi stack. A Rab5 protein-like, localized to tubovesicular structures adjacent to but distinct from the Golgi, is involved in the parasite cholesterol pathway. *T. gondii* apicoplast expresses Rab7 late endosomal marker. Moreover, *Toxoplasma rhoptries*, secretory organelles essential for cell invasion, present certain features of lysosomal secretory pathway. These data suggest that, among all these parasite secretory pathways, one could be dedicated to exosomal secretion. Using TEM, we observed *Toxoplasma* secretory organelles and detected MVB-like structure containing 65-nm vesicles confirming this hypothesis. We purified by sucrose cushion vesicles from peritoneal fluid of *Toxoplasma*-infected mice, which displayed biochemical and morphological characteristics of exosomes. Immunostaining with colloidal gold indicated the presence of *Toxoplasma*-specific proteins and proteomic analysis revealed the presence of exosomal markers (Tsp7, Tsp18, CD82, Rab5 and Rab11) and specific *Toxoplasma* proteins (SAG, MIC, GRA, GPI, ubiquitin and cyclophilin). These results suggest a novel exosome-based pathway as a mechanism of protein secretion used by *T. gondii*.

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44. Exosomes in the interaction host-parasitic trematodes

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The study of host-parasite interactions has increased considerably in the last decades, with many studies focusing on the identification of parasite molecules [i.e., surface or excretory/secretory proteins (ESP)] as potential targets for new specific treatments and/or diagnostic tools. In parallel, in the last few years, there have been significant advances in the field of exosome research. These vesicles carry several atypical secreted proteins in different organisms, including parasitic protozoa. Here, we present experimental evidence for the existence of exosomes in parasitic helminthes, specifically the trematodes *Echinostoma caproni* and *Fasciola hepatica*. These microvesicles are actively released by the parasites and penetrate into the host cells. Trematode exosomes contain most of the proteins previously identified as components of ESP, as confirmed by proteomic and electron microscopy studies. In addition to parasitic proteins, we also identify host proteins in these structures. The existence of exosomes explains the secretion of atypical proteins along with host proteins in trematodes, and the demonstration of their uptake by host cells suggests an important role for these structures in host-parasite communication, as described for other infectious agents.

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46. Characterization of the exosome-associated prion protein in hamster plasma

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In recent years, a number of publications have shown that both the cellular and misfolded prion proteins (PrP) are secreted in association with exosomes in vitro. These exosome preparations are in some cases infectious and capable of propagating prions by inducing de novo formation of scrapie in healthy cells and animals. Despite these findings suggest that exosomes could be involved in prion propagation and partially confirm the "Troyan exosome hypothesis" of Gould et al. (1), there is still no evidence that disease-associated PrP is carried by microvesicles in vivo. To date, in vivo studies have only confirmed the presence of cellular PrP (PrPC) on exosomes of ovine cerebrospinal fluid. The identification of PrP in main body fluid systems remains elusive and controversial. Here, we show that PrPC is unequivocally associated with hamster blood exosomes in vivo. Western blot analysis of purified plasma exosome preparation revealed the presence of aggregates of high molecular weight. They were detected on plasma exosomes but not in vesicles derived from other sources such as cell-conditioned medium. Interestingly, the use of stringent conditions progressively disaggregates large molecular weight bands while increases the intensity of monomeric PrP bands. Immunoprecipitation showed that these aggregates include exosomal markers. These results suggest that PrP in plasma is associated with exosomes, but other proteins could mask PrP, possibly also explaining why the detection of prions in body fluids is elusive.

Funded by: Istituto Superiore della Sanità, Rome, Italy

Reference

1. Gould SJ, Booth AM, Hildreth JE. Sci USA. 2003 Sep 16;100(19)

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47. Small RNA sequencing of neuronal exosomes identifies a distinct miRNA signature associated with prion disease

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Scientific Program 2012 ISEV meeting
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The City of Gothenburg (**www.goteborg.se**) is kindly inviting all ISEV delegates to the welcome reception at the congress venue. Enjoy! Interact!

All Posters in Session 1

1. Production, Secretion and Function

1. Characterization of syndecan-syntenin-1 endosomal trafficking routes

Rania Ghossoub[1], Frédérique Lembo[1], Rudra Kashyap[1] and Pascale Zimmermann[1,2]
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Syndecans constitute a family of heparan sulfate proteoglycans and are implicated in the transport of different cargos such as growth factors, chemokines and morphogens (1). Syndecans contain a conserved cytoplasmic domain that interacts with the PDZ protein syntenin-1. Syntenin-1 supports the plasma membrane recycling (2) and the exosomal sorting of syndecans and their heparan sulfate cargo (3). Yet, how these two pathways are regulated and possibly interconnected is unknown. Addressing this question is providing some insight on the molecular mechanisms controlling cis versus trans cellular signaling.

Funded by: Fondation Santé Sport Développement Durable Institut Paoli-Calmettes Inserm-U1068

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3. Baietti M-F, et al. Syndecan-syntenin-Alix regulate the biogenesis of exosomes. Nat Cell Biol. In press.

2. Poster 2 is withdrawn

3. Selecting markers to analyze plasma vesicles: lack of correlation between antigens expressed on vesicles and their parent cells

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To identify the cellular origin of plasma microvesicles and exosomes, specific markers are required. In vitro-derived vesicles provide the ideal platform to determine whether surface antigens specific for a particular cell type are also present on vesicles derived from them. We have used flow cytometry and nanoparticle tracking analysis (NTA) in parallel to rapidly size, quantitate and phenotype in vitro-derived vesicles from platelets, red blood cells (RBCs), endothelial cells, lymphocytes, monocytes and granulocytes. Using a side-scatter threshold, we determined that our standard BD LSRII flow cytometer could analyze vesicles $\geq$ 290 nm but nothing smaller, whereas NTA could measure cellular vesicles down to approximately 50 nm in size. NTA of platelet, RBC and endothelial-derived vesicles revealed that their size distribution differed, ranging 50–900 nm, 50–400 nm and 50–650 nm, respectively. The modal size of vesicles from each preparation was ~200 nm. Vesicle counts as determined by NTA vs. flow cytometry were elevated by 75-fold for platelet vesicles, 2855-fold for RBC vesicles and >10,000 fold for endothelial vesicles. Our flow cytometry data showed that: 1) not all markers are cell-specific; e.g., the typically used platelet marker CD61 was not only expressed on platelets (99%) but also on endothelial cells (99%), monocytes (>90%) and a subset of lymphocytes (~40%) and granulocytes (~15%); (2) antigens expressed on the parent cell are not necessarily expressed on the vesicles derived from them; e.g., 99% of endothelial

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adipocytes and osteoblasts grown in medium containing adipocyte MV. *Results*: Here, we show that MSC-derived adipocytes are able to release MV containing adipocyte-specific RNA. RNA expression level in osteoblasts cultured in medium containing adipocyte MV is indeed directly proportional to the amount of RNA inside MV. As a result, MVs seem to be one of the way by which adipocytes can influence osteoblast phenotype. They could be a new intercellular communication mode identified inside bone marrow and open new leads in understanding of osteoporosis physiopathology and discovery of new therapeutic ways.

Funded by: Université du Littoral Côte d'Opale

37. Characterization of osteoblast-secreted vesicles: a new mode of intercellular communication

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Osteoblasts are in close contact with neighboring cells in their bone marrow microenvironment, and proper communication systems are crucial for the regulation of healthy bone turnover as well as pathogenesis. Recently, a novel mode of communication via extracellular vesicles has attracted much attention in the scientific community. During bone formation, osteoblasts secrete matrix vesicles (MVs) involved in the mineralization of the extracellular matrix; however, information about other secreted vesicles is still lacking. In this study, we focus on the characterization of human osteoblast-secreted vesicles and investigate their potential as mediators of communication with bone marrow cells and other cells in the vicinity, e.g., metastatic cancer cells. We used a human preosteoblast-based in vitro bone formation model (svHFOs) to isolate vesicles by ultracentrifugation at various timepoints during osteoblast differentiation. We characterized the vesicles by transmission electron microscopy (TEM) and mass spectrometry-based proteomics and studied their interaction with other cells by fluorescent labeling and FACS analysis. Our TEM images showed structural heterogeneity among osteoblast-secreted vesicles, and proteomics analyses showed that the vesicles contained proteins not primarily linked to mineralization, suggesting the presence of vesicles other than MVs. Confocal analyses of fluorescently labeled vesicles showed that osteoblast-secreted vesicles were taken up by HEK 293 cells, and most interestingly by prostate cancer cells and hematopoietic stem cells, in a dose-dependent manner, proposing a novel mechanism of intercellular communication.

Funded by: Erasmus stem cell institute for regenerative medicine

4. Inflammation and Infection

38. Reticulocyte-derived exosomes from malaria infections are involved in antigen presentation and modulation of immune responses

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Reticulocyte-derived exosomes have a unique role in the removal of proteins during the maturation of reticulocytes to erythrocytes. Noticeably, there are malaria parasites with a unique tropism for reticulocytes, including *Plasmodium vivax*, the most widely distributed human malaria parasite. Here, we describe the isolation and characterization of exosomes from peripheral blood of human and mice infected with reticulocyte-prone malaria parasites. Importantly, proteomic analysis revealed the presence of parasite proteins in these vesicles. Moreover, immunization of mice with purified exosomes from peripheral blood elicited IgG antibodies capable of recognizing malaria-infected red blood cells. Furthermore, lethal challenge of immunized mice caused attenuation in the course of parasitemia and increased survival time. These results were obtained also when exosomes were isolated from in vitro cultures of infected reticulocytes, demonstrating that reticulocyte-derived exosomes are involved in antigen presentation and immune modulation in reticulocyte-prone malaria infections. Moreover, inclusion of CpG ODN 1826 in exosome immunizations elicited IgG2a and IgG2b antibodies and promoted survival and subsequent sterile protection of 83% of the animals lethally challenged. Present experiments are being conducted to determine the proteomics composition of reticulocyte-derived exosomes from human patients and to compare the adjuvanticity of flagellin vs. CpG. To our knowledge, this is the first report of immune responses elicited by exosomes derived from reticulocytes, opening new avenues for the exploration of these non-inflammatory nanovesicles in modulation of immune responses.

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39. Microvesicles of *Dictyostelium discoideum* as a model of eukaryotic extracellular vesicles

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We found that the secretion of extracellular vesicles is a constitutive process of *Dictyostelium discoideum* cells, occurring both during cell growth and early development. Moreover, this process was found to be associated to a detoxification mechanism of different structurally-unrelated drugs. The secreted vesicles, acting as "Trojan horses," are capable of both transporting the drug and transferring it into human cells. This is why *Dictyostelium* microvesicles have been suggested as a new biological drug delivery tool (1). Constitutive *Dictyostelium* microvesicles secreted during starvation-induced development do also play a role in intercellular communication, by inhibiting normal cell aggregation and inducing an apoptotic process. Considering that *Dictyostelium* cells are much easier to manipulate than human cells, we suggest that they can be used to unravel the biological functions of extracellular vesicles. However, a better knowledge of these vesicles is an important prerequisite. Therefore, we studied *Dictyostelium* extracellular vesicles by cryoelectron microscopy, near-infrared Raman and spectrofluorimetry. We also report tentative endogeneous cell labeling of *Dictyostelium* extracellular vesicles by using fluorescent nanoparticles or a lipid-specific dye.

Reference

1. Tatischeff et al. Patent European priority No. 03 291 752 07/15/2003 (DRITT-UPMC) European Patent (Danemark, Deutschland, France, Great Britain, Italy, Netherland and Spain), US Patent and Pending Canadian Patent.

40. Interaction of *Trypanosoma cruzi* infective forms and monocytes produces microvesicles that increase parasite infection

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Trypanosoma cruzi, the causative agent of Chagas disease, has a complex life cycle and presents several mechanisms to subvert vertebrate host recognition and clearance. We have seen that, when in contact with monocytes, metacyclic trypomastigotes (infective form from the insect vector) and cell derived trypomastigotes

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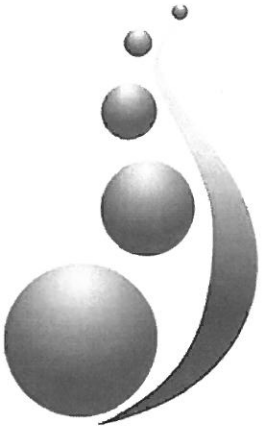

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