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Exosomes: from cell debris to potential biomarkers in transplantation

Adrian E. Morelli¹

¹T.E. Starzl Transplantation Institute, and Departments of Surgery and Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

It has been classically assumed that the graft and the leukocytes of the transplant recipient exchange information through direct cell-to-cell interaction or via release of soluble mediators. During the past 2 decades, however, accumulated evidence has revealed that eukaryotic cells also communicate by exchanging different types of extracellular vesicles (EVs), including microvesicles, exosomes, and apoptotic cell-derived vesicles, among others. EVs differ in their biogenesis, size, and composition. Microvesicles –also termed microparticles or ectosomes- range between 0.2–1 μ m in size and are shed from the plasma membrane of living cells. Exosomes are smaller vesicles, between 70–120 nm in diameter, derived from the endocytic compartment of the cell. Exosomes are generated by reverse budding of the limiting membrane of early endosomes -termed multivesicular bodies-, and secreted by fusion of the multivesicular bodies with the cell membrane ¹. Nearly all mammalian cell lineages examined so far have been shown to release exosomes, which are found in vivo in the extracellular milieu, attached to the extracellular matrix, or as free-floating EVs in bodily fluids (eg, plasma, urine, breast milk, saliva, semen, and sputum) ^{1,2}.

Interestingly, the protein cargo and intraluminal content of exosomes depend to some extent to the lineage, and state of activation, infection, or transformation of the parent cell. As example, exosomes released by activated antigen (Ag)-presenting cells (APCs) are enriched in functional MHC class-I and II molecules, CD86, and the adhesion molecule CD54 (ICAM-1) on the vesicle membrane. EVs also carry in their lumen functional mRNAs and microRNAs, which amount and composition represent to a degree the RNA content of the parent cell ¹.

Although originally considered membrane debris with no biological relevance, accumulated evidence has shown that EVs, and in particular exosomes, play multiple functions in vivo ². Exosomes are a mechanism of release of unnecessary molecules in those cells with few or no lysosomes, such as reticulocytes. Cells use exosomes as platforms to transfer proteins, mRNAs and microRNAs -cellular and from pathogens- to other cells. Exosomes released by APCs carry Ag-presenting, costimulatory and adhesion molecules on the vesicle surface and -when bound to the surface of acceptor cells-, function as Ag-presenting vesicles for T cells. Cytotoxic T cells release the cytolytic molecule Fas-ligand on the surface of exosomes

Corresponding author: Adrian E. Morelli. W1556 Biomedical Science Tower. 200 Lothrop St. Pittsburgh, PA 15261, USA. Tel. # 412-624-2193. morelli@pitt.edu.

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secreted at the site of contact with the target cells. Exosomes generated by tumors participate in initiation, invasion, angiogenesis, premetastatic niche formation, and drug-resistance of neoplastic cells ³. Following transplantation of heart or skin allografts in mice, donor-derived exosomes -bearing donor MHC Ag, T cell costimulatory molecules, and still not well identified APC-activation signals-, cross-dress a relative high number of recipient APCs in the graft-draining lymphoid organs ^{4,5}. The recipient APCs cross-dressed with donor-derived exosomes stimulate proliferation and terminal differentiation of directly allo-reactive T cells through the semi-direct pathway of allo-recognition ^{4,5}. Transfer via exosomes of noninherited maternal MHC allo-Ag (NIMAs) from maternal microchimeric cells to host APCs is behind the T cell split tolerance against NIMAs ⁶.

Due to the ability of exosomes to carry native Ags and promote or suppress innate and adaptive immunity, these nanovesicles have been tested as Ag-delivery platforms for positive or negative immunization in animal models and clinical trials ^{1,2}. Interestingly, the protein and RNA cargo of exosomes represent to some extent a snap-shot of the parent cell, and exosomes can be isolated from bodily fluids. Therefore, there has been increasing interest during the past 5 years in the potential use of exosomes as protein or RNA diagnostic or predictive biomarkers that could replace the need for a biopsy of the graft in transplantation ^{7,8}. However, until recently, purification of exosomes from bodily fluids was a very laborious task that required the combination of multiple steps of ultra-filtration, ultra-centrifugation, precipitation, immuno-isolation, and size exclusion chromatography. The advent of commercially available kits for isolation of exosomes or its contents more easily, from small volumes of bodily fluids, and in quantities that warrant further analysis of exosomal proteins and RNAs, has rekindled the field of exosomes as biomarkers. There are still, nevertheless, problems that need to be addressed. Isolation of exosomes from certain bodily fluids as urine is still challenging ⁹. In most cases, the new commercially available methodologies do not discriminate exosomes from other subpopulations of EVs, or separate exosomes based on the cell of origin, the latter important for the analysis of bodily fluids that contain a mix of EVs secreted by different cell types. These concerns, which are critical for basic research on the biology of exosomes, may be less relevant when the aim is to simply purify EVs for its analysis as biomarkers.

In this issue of *Transplantation*, H. Zhang and colleagues reported that the mRNA cargo of exosomes isolated from plasma of HLA-sensitized kidney transplant patients could be used as a predictive biomarkers of development of antibody (Ab)-mediated rejection (ABMR) ¹⁰. The authors conducted a retrospective study on EVs isolated from EDTA-plasma samples collected from kidney recipients within 1 month before diagnosis by biopsy of ABMR or cell-mediated rejection (CMR), or without rejection. The 21 candidate mRNAs selected for analysis were transcripts of genes encoding chemokines, cytokines, surface receptors, and other molecules previously shown to play a role in Ab-dependent cellular cytotoxicity, ABMR of kidney grafts, or inflammation. Analysis of the exosome-shuttle mRNAs by reversed transcription followed by quantitative PCR, revealed that plasma exosomes from patients with ABMR carry higher content of 6 of the mRNAs analyzed. However, only the amount of transcript for IL-6R beta chain (gp130) was significantly higher in plasma exosomes of ABMR patients than in EVs from recipients with CMR or without rejection. By contrast, gene score combining 4 of the mRNAs assessed in the plasma exosomes was a

much better predictor of future development of ABMR compared to risk for CMR or to absence of rejection.

Considering that ABMR is 1 of the major hurdles to the long-term survival of kidney allografts, the use of exosomes isolated from plasma of kidney transplant recipients as predictive biomarkers of ABMR represents a potential alternative or complement to renal biopsy, which remains as the gold standard for diagnosis of kidney transplant rejection. In this scenario, future multicenter studies are required to define and validate the optimal molecular signatures of the EVs (ie, RNAs, proteins, lipids, carbohydrates), the best method(s) of EV purification, the bodily fluids from where the EVs should be isolated (plasma vs urine), the cell source of the EVs (entire mix of plasma or urine EVs, EVs released by a subset of parent cells, donor- vs recipient-derived EVs). More importantly, side-by-side comparisons of the specificity and sensitivity of EVs isolated from bodily fluids with those of the gold-standard markers currently in use will tell future of EVs in the competitive field of biomarkers in transplantation.

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ABBREVIATIONS

APCs	Activated antigen-presenting cells
ABMR	Antibody-mediated rejection
Ag	Antigen
CMR	Cell-mediated rejection
EVs	Extracellular vesicles
NIMAs	Noninherited maternal MHC allo-Ag

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