Commentary

Donor-derived exosomes: key in lung allograft rejection?

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In a recent publication, Gunasekaran and colleagues conducted a case-control observational study in patients who underwent bilateral lung transplantation (LTx), determining whether exosomes are generated during allograft rejection and define their origin and antigenic phenotype (1). LTx is the sole effective treatment to save the life for patients with end-stage lung disorders (2). One of the major drawbacks for the clinical outcome of LTx is allograft rejection by acute rejection (AR) or bronchiolitis obliterans syndrome (BOS) (2). A link between autoimmunity and immune responses to tissue restricted self-antigens (SAgs) such as K-alpha-1-tubulin (K-α1T) and Collagen V (Col-V) with acute or chronic lung allograft rejection has been proposed (3,4). However, the mechanisms underlying the evolution of acute or chronic lung rejection are poorly understood.

Previous studies have analyzed the intercellular communication profiles in allograft rejection by studying exosomes, the smallest membranous extracellular vesicles (40–100 nm) secreted into body fluids. Donor-derived exosomes induce specific regulatory T cells to suppress immune inflammation in the allograft heart (5) and urinary exosome-specific protein alterations provide a potential unique panel of biomarkers for monitoring AR in kidney transplant (6). In LTx, exosomes have been identified in bronchoalveolar lavage (BAL) fluid and were different in normal and inflammatory states (7,8). A recent study described a differential exosomal shuttle RNA in BAL and suggested this exosome RNA like a biomarker of AR (9). However, before Gunasekaran's study the presence of mismatched donor HLA and SAgs on exosome surfaces such as sensitive biomarkers for monitoring allograft rejection in LTx, serum exosome miRNA profile and their targeted pathways involved in lung transplant rejection have never been settle.

The strong adaptative immune response against organ allografts was attributed to migration of donor-derived professional antigen-presenting cells (APCs) to recipient lymphoid tissues, where donor APCs trigger activation of allospecific T cells against donor MHC molecules (10). Nevertheless, there is indirect evidence that donor DCs mobilized from organ allografts into graft-draining lymphoid tissues in relatively low numbers; although, these low donor DCs are able to elicit the potent anti-donor response that acutely rejects the graft (10,11). These apparently contradictory findings could be explained by the transference of clusters of extracellular vesicles bearing functional donor MHC molecules and APC-activating signals to a higher number of recipient cells (12). In this sense, Gunasekaran *et al*., suggested exosome-mediated immune responses due to the presence of SAgs and donor HLA on exosome surfaces originated from the transplanted lung. Furthermore, these exosomes containing SAgs are detectable in sera before clinical diagnosis of AR and chronic rejection. Many investigators have attempted to identify diagnostic and predictive cellular and biochemical biomarkers, especially in BAL fluid, of acute

and chronic rejection in lung transplantation. Gunasekaran and colleagues performed serial analysis of exosomes in circulation and have demonstrated that exosomes containing Col-V were detectable 1 month after LTx, 2 and 6 months before clinical diagnosis of AR and BOS, respectively. Thus, the quantification of these exosomes could be considered as a noninvasive biomarker of impending rejection.

Moreover, molecular (genetic and epigenetic) alterations by which a normal and abnormal process can be recognized or monitored are also considered like biomarkers. Therefore, identification of single nucleotide polymorphisms, gene expression and miRNA arrays analysis in BAL or serum fluids rise as potential marker candidates of lung allograft rejection in the last years (9,13). The results presented by Gunasekaran's group showed that serum miRNAs were differently expressed in exosomes from BOS and AR LTxRs compared with stable LTxRs, including lower and higher miRNA signals. Microarray analysis is particularly well suited for discovering complex changes in miRNA expression profiles under different conditions. Inherent within these changes is that knowledge of the dynamics of a single miRNA or even a small group of miRNAs may be insufficient to understand the process occurring due to the change in specific conditions. Therefore, the comparative pathway analysis performed in this study by authors suggests that exosomal miRNAs are involved in the B cell, T cell and TGF-β signaling pathways, regulating significant cellular processes involved in auto- and alloimmune responses in pathogenesis of AR and BOS. Consequently, this report points out exosomes such as a key in mediating immune responses that could contribute to acute and chronic pathogenesis, ultimately leading to allograft rejection.

To reach these conclusions, Gunasekaran *et al*. performed western blotting and transmission electron microscopy techniques to determine the presence of SAgs (Col-V) on exosome surfaces in sera and BAL in LTxRs with AR and BOS. Furthermore, flow cytometry analysis have allowed them to demonstrate donor origin (HLA-A2 antigen positive staining, but not recipient HLA-A3) followed by alloimmune responses from sera of LTxRs with BOS. These selected methods have been widely approved and tested to phenotype exosomes in different biofluids such as serum, urine, breast milk and BAL (14,15). Moreover, in this report the global miRNA profile from pooled sera was performed using a GeneChip miRNA array and miRNA-mediated targeted pathways were also generated. However, the small RNA sequencing of the samples instead of miRNA array could be a better approach to identify miRNAs involved in these processes. Since deep sequencing (miRNA-seq) generates millions of reads from a given sample, it allows to establish individual profile miRNAs and discover novel miRNAs that may have eluded by traditional screening and profiling methods (16). Furthermore, after the amount of miRNAs are quantified for each sample, their expression levels can be compared between samples. Perhaps, miRNA sequencing of the individual serum samples from each AR and BOS patient could have provided an important additional information. But, miRNA profiling analysis approached by authors is enough to show a differential expression in LTxRs with AR and BOS and stable LTxRs.

On the other hand, the study have two methodological flaws that undermine their conclusions. One limitation is the relative small sample size analyzed for LTxRs with AR and BOS and stable LTxRs. The number of patients and patient samples in this work needs to be increased greatly to improve statistical validity. This will require the establishment of collaborative study groups that share biological samples from hundreds of subjects. Another potential shortcoming is they do not address the biological role of exosomes in transplanted lungs. The mechanism by which exosomes may participate in augmenting immune responses that lead to rejection is not yet defined.

Overall, although the mechanisms that lead to lung allograft rejection are mixed, well conducted investigation as the one of Gunasekaran and colleagues provides a light that rose in the darkness of the AR and BOS pathogenesis. However, a lot of questions remain unanswered, with the most relevant being the functional role of exosomes and the cross-talk between them and their recipient cells involved in the lung allograft rejection.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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