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Review

The role of donor-derived exosomes in lung allograft rejection

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ABSTRACT

Lung transplant recipients (LTxRs) with acute or chronic rejection release circulating exosomes that mostly originate from donor lung tissue and express mismatched human leucocyte antigens (HLA) and lung-associated self-antigens (SAgs), Collagen-V and K alpha 1 Tubulin. During lung transplant (LTx), donor lungs often undergo injuries that increase the antigenicity of the transplanted organ. 30% of LTxRs also have pre-transplant antibodies (Abs) to HLA and lung SAgs, which may induce conditions that increase the risk of chronic lung allograft dysfunction (CLAD). Post-transplant, some recipients experience *de novo* development of Abs to mismatched donor HLA (donor-specific antibody [DSA]) and Abs to lung SAgs, which have been implicated in CLAD pathogenesis. Because most LTxRs who develop DSA also develop Abs to SAgs, some have suggested a synergistic relationship between alloimmunity and autoimmunity in CLAD immunopathogenesis. These processes likely occur from stress-induced exosome release. Exosomes carry allo-antigens, lung SAgs, several micro RNAs, proteasome, co-stimulatory molecules, and pro-inflammatory transcription factors—resulting in efficient antigen presentation by direct, semidirect, and indirect pathways, leading to immune responses to both allo-antigens and lung-associated SAgs. This review summarizes recent findings on the role of exosomes, and processes triggering immune responses to allo-antigens and lung SAgs that ultimately culminate in CLAD.

1. Introduction

Exosomes are emerging as important immune mediators of rejection after organ transplantation [1,2]. Exosomes have been identified in the circulation and in bronchoalveolar lavage fluid after lung transplant (LTx), and these exosomes had different RNA profiles in normal and inflammatory states [3]. Our previous study demonstrated the presence of mismatched donor human leukocyte antigen (HLA) and lung-associated tissue-restricted self-antigens (SAgs) on the surfaces of exosomes, suggesting that exosomes may play an important role in inducing immune responses that lead to lung allograft rejection [4,5]. Exosomes derived from a donor's dendritic cells have been demonstrated to promote allograft-targeting immune responses by transferring functional antigen-presenting cells, which activates signals and donor HLA molecules [6].

The main target for rejection is the mismatched donor HLA of the allograft, which is recognized by the recipient's immune surveillance. Many studies have shown that development of antibodies (Abs) to mismatched donor HLA is associated with development of chronic rejection after LTx, which is clinically diagnosed as bronchiolitis obliterans syndrome (BOS). Evidence suggests a mixed anti-donor response due to both *de novo*-developed, donor-specific antibodies (DSA) and cellular immune pathways that contribute to chronic lung allograft dysfunction (CLAD) [7,8]. DSA developed both pre- and post-transplant is associated with antibody-mediated rejection, which increases the risk of CLAD [9–12]. Our previous study demonstrated the development of Abs to lung SAgs after DSA development as well as the clinical onset of BOS after human LTx, suggesting a possible synergistic relationship between immune responses to alloantigens and tissue-restricted SAgs in the pathogenesis of chronic rejection. Analysis of 103 LTxRs

Abbreviations: Ab, antibody; AM, alveolar macrophages; BOS, bronchiolitis obliterans syndrome; CLAD, chronic lung allograft dysfunction; ColV, Collagen V; CF, cystic fibrosis; DC, dendritic cells; DSA, donor-specific antibody; ELISA, enzyme-linked immunosorbent assay; ER, endoplasmic reticulum; EV, extracellular vesicle; HLA, human leukocyte antigen; IPF, idiopathic pulmonary fibrosis; Kα1T, K alpha 1 Tubulin; LTx, lung transplantation; LTxRs, lung transplant recipients; MHC, major histocompatibility complex; mRNA, messenger RNA; miRNA, micro RNA; OAD, obliterative airway disease; PGD, primary graft dysfunction; RVI, respiratory viral infection; SAgs, self-antigens

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demonstrated that 42.7% of LTxRs developed DSA and 30.1% of them also developed Abs to Kα 1 Tubulin (Kα1T) and Collagen V (ColV) supporting that development of DSA often precedes development of Abs to lung SAgs [13]. Studies have also associated preexisting Abs and respiratory viral infections (RVIs), demonstrating loss of peripheral Treg cells in the pathogenesis of CLAD [14]. Several pre- and post-transplant risk factors (e.g., HLA mismatches, *de novo* development of DSA, recurrent/refractory acute rejection, cytomegalovirus, RVIs, ischemia–reperfusion injury, etc.) have been significantly associated with increasing the risk of CLAD development [15,16].

We postulate that these risk factors lead to inflammation, placing stress on the organ and resulting in tissue remodeling. This facilitates SAg exposure and results in the induction of immune responses—not only to alloantigens, but also to SAgs. Furthermore, the immunological mechanism by which immune responses to alloantigens and SAgs occur is most likely due to the release of exosomes from the stressed, inflamed transplanted organ. Although most evidence suggests that circulating exosomes have an important physiological part in biological functions, this review will focus on the role of exosomes in inducing allograft rejection after solid organ transplant, especially after human LTx.

1.1. Exosomes

Extracellular vesicles are shed by cells and are divided into 3 subclasses based on their biogenesis: exosomes, microvesicles, and apoptotic bodies. The diameter of these vesicles varies from 40 to 5000 nm. Exosomes are small extracellular vesicles (small EVs), measuring less than 200 nm in diameter, and are endocytic in origin [17]. The term *exosome* was coined in the 1980s by Pan and Johnstone [18]. Exosomes carry nucleic acids (i.e., DNA or RNA), messenger RNA (mRNAs), microRNA (miRNAs), circular RNA, long noncoding RNA, proteins, and metabolites. Common groups of proteins identified in exosomes originating from different cell types are annexin and rab family proteins, which are involved in membrane fusion and transport. Exosomes also contain cell adhesion molecules, costimulatory molecules, and tetraspanins (i.e., CD9, CD63, and CD81), TSG101, Alix, CD82 antigen-presentation proteins, and major histocompatibility complex (MHC) classes I and II (Fig. 1) [19,20].

1.2. Exosome isolation and characterization

Exosomes can be isolated using different techniques, including exosome isolation kits with manufacturer's isolation protocols and ultracentrifugation. We found that ultracentrifugation followed by sucrose cushion gradient purification is the best method to obtain high-purity exosomes. The size of the exosomes can be analyzed using nanoparticle tracking analysis (NanoSight NTA system) and electron microscopy. The total protein of exosomes can be measured using various colorimetric assays (e.g., Bradford or micro-bicinchoninic acid) or fluorimetric methods. Specific molecules in) exosomes can be quantified using techniques such as enzyme-linked immunosorbent assay (ELISA), bead-based flow cytometry, aptamer- and carbon nanotube-based colorimetric assays, and antibody-coated nanorods [17]. Our lab has used ELISA to quantify donor-derived exosomes by determining donor-derived HLA molecules and/or lung SAgs.

1.3. Exosomes: guidelines for functional studies

According to recently published guidelines [17], functional studies should determine the selective functions of different types of, exosomes in the absence of direct cell-to-cell contact using transwell migration assay. Experiments need to be performed to delineate the functions of other EVs (> 200 nm) in comparison to exosomes (< 200 nm). However, no clear recommendations exist for the use of the normalization strategy to determine the amount of exosomes that should be used for comparative functional studies, especially when exosomes are isolated from bodily fluids such as lavage or urine, and volume normalization may not be appropriate [17]. In the past, the functional significance of exosomes was measured either by stimulation or inhibition assays of exosome secretions. Exosome secretion can be diminished by inhibiting neutral sphingomyelinases and ceramide generation (by drugs such as GW4869 shRNA, genetic editing, of, Rab GTPases [21,22], SRC, or SNARE proteins [23]), or by using drugs (e.g., calcium signaling [ionomycin] or sodium channel blockers [monensin, amiloride]) [24,25]. Although inhibition of neutral sphingomyelinases decreased exosomes, a recent study demonstrated that it enhanced secretion of larger plasma membrane-derived EVs [26]. Rab27a inhibition has also been shown to reduce secretion of some non-EV-bound soluble factors [27], suggesting

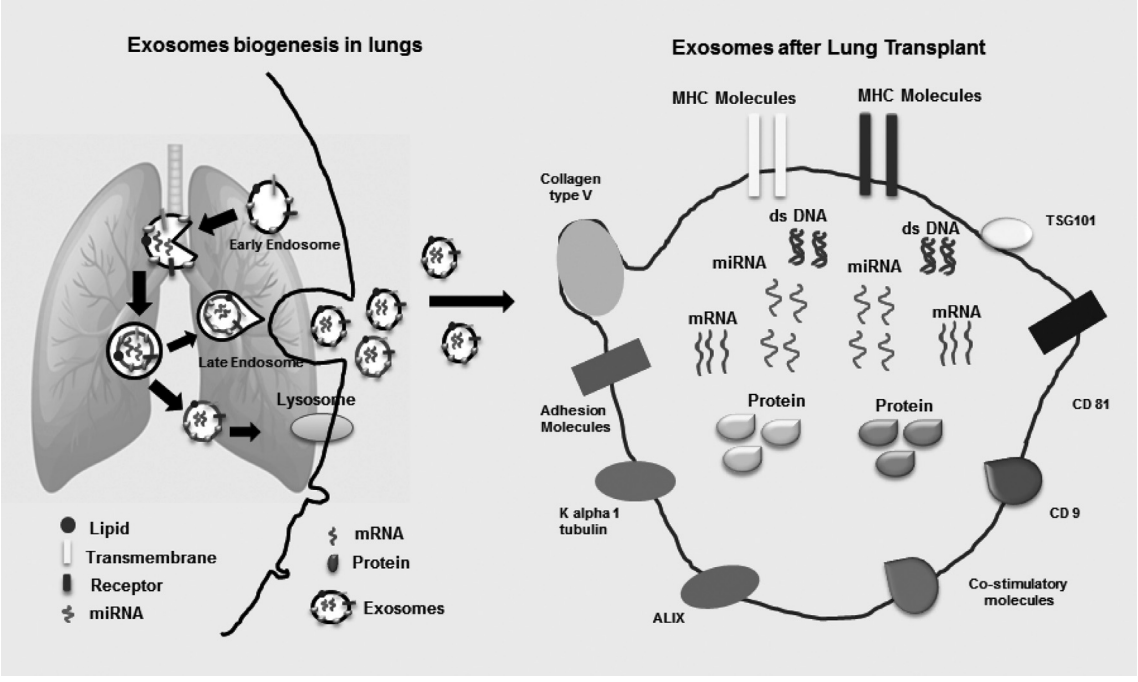

Fig. 1. Exosome biogenesis and exosomal contents after lung transplantation.

that these inhibition methods may modify cell functions and indirectly affect the composition and secretion of EVs.

Exosome composition varies depending on origin, disease state, and pathophysiological conditions of cells (e.g., infections of multiple types, cancers, and organ transplantation). An exosome database organized based on exosome contents, Exocarta (Version 4), indicates that exosomes from various organisms and cell types have been characterized as containing 4563 proteins, 1639 mRNAs, 764 miRNAs, and 194 lipids [28,29]. In the past decade, researchers have determined that exosomes play a critical role in primary tumor growth and metastasis [30]. From a clinical standpoint, exosomes are useful as potential biomarkers for disease progression and for development of novel therapeutic agents targeting cancer and metastases [31]. Exosomes can coordinate multiple pathophysiological processes (e.g., coagulation, vascular leakiness, reprogramming stromal recipient cells) to sustain pre-metastatic niche formation.

Exosome composition changes based on their origin (e.g., cancer, infectious disease, organ transplant, and other clinical conditions) [32]. Donor-derived exosomes with unique signatures have been found to be released after solid organ transplant [33]; however, the study of their role in transplantation biology is in its infancy.

1.4. Lung-derived exosomes and their role in lung allograft immunity

Our group identified the presence of two lung SAgs—ColV and Kα1T—on circulating exosomes that had been released from LTx recipients (LTxRs) with either acute or chronic rejection (clinically diagnosed as BOS) [34]. Recently, we noted that exosomes containing lung SAgs are released from the cells into the circulation *before* development of Abs to ColV and Kα1T (unpublished). Using electron microscopy and immunofluorescence, we found donor HLA and SAgs on the surfaces of donor-derived exosomes in LTxRs experiencing allograft rejection. Donor-derived exosomes from patients with stable lung allografts, on the other hand, had nonsignificant levels of either lung SAg. These results strongly suggest that circulating exosomes from the transplanted organ are released after immune insult or other injury, including primary graft dysfunction (PGD) or RVI. It has also been demonstrated that not only mismatched donor HLA and SAgs are present in exosomes, but exosomes also contain miRNAs known to induce inflammation, endothelial activation, antibody-mediated rejection, and Th17 differentiation, triggering immune responses [4]. Furthermore, mice immunized with exosomes isolated from LTxRs diagnosed with BOS induced development of both humoral and cellular immune responses to ColV and Kα1T, whereas mice immunized with exosomes from stable LTxRs did not develop these responses. This demonstrates significant differences in exosomes between BOS and stable LTxRs with respect to immunogenicity [34]. Exosomes with lung SAgs were detected prior to development of Abs to lung SAgs in LTxRs diagnosed with rejection but not stable LTxRs. We propose that exosomes released during rejection and other stresses to the transplanted organ (DSA, Ischemia reperfusion injury, RVI) differ from exosomes released from stable LTxRs.

We recently showed that circulating exosomes isolated from LTxRs diagnosed with PGD, symptomatic RVI, acute rejection, DSA, and BOS contained not only alloantigens and lung SAgs, but also immunoregulatory molecules, pro-inflammatory transcription factors, proteasome, and stress markers [35]. Furthermore, in most patients, exosomes isolated 1 month post-transplant after PGD diagnosis showed no lung SAgs or donor HLA. This suggests that when a transplanted organ is not under stress, exosomes containing lung SAgs or donor HLA are not released into the circulation. However, in some LTxRs we noted persistence of circulating exosomes for more than 1 month with lung SAgs were induced during PGD grade 3, which correlated with *de novo* development of DSA (unpublished). These LTxRs, therefore, are at risk for CLAD. In addition, circulating exosomes isolated from LTxRs with *de novo* DSA have higher expression of lung SAgs [36], suggesting that the exosomes released following DSA development can augment immune responses, leading to chronic rejection. Immune insult during rejection including that induced by DSA results in release of exosomes with lung SAgs and donor mismatched HLA and, therefore, we propose that stress to the transplanted organ including rejection leads to exosome release with lung SAgs.

In a recent study of LTxRs with RVI so severe it required intervention, we found that circulating exosomes in these patients contained not only lung SAgs and 20S proteasome, but also viral antigens [35]. Furthermore, mice immunized with exosomes from LTxRs with RVI developed both humoral and cellular immune responses to lung SAgs, whereas mice immunized with exosomes from stable LTxRs did not. This suggests that RVI-induced exosomes are immunogenic, and persistence of these exosomes can lead to CLAD. Hence, RVI may increase the risk of CLAD in LTxRs.

1.5. Murine models of obliterative airway disease (OAD) induced by Abs to MHC: role of exosome release in inducing immune responses to lung SAgs and development of OAD

We developed a clinically relevant murine model in which Abs to MHC administered intrabronchially induced OAD, a correlate of chronic lung allograft rejection in humans [37]. In this model, prior to development of OAD, Abs to lung SAgs (ColV and Kα1T) were detected. Passive administration of Abs to interleukin 17 after administration of Abs to MHC significantly reduced Abs to lung SAgs as well as OAD lesions [38]. Using this same model, we have also demonstrated an important role for exosomes in activating the immune responses that lead to Abs to lung SAgs and result in OAD. Anti-MHC class I administered to wildtype mice released circulating exosomes with increased levels of lung SAgs (ColV or Kα1T), CIITA, and 20S proteasome before development of Abs to SAgs (unpublished). We also described that induction of a transcription factor, zinc finger and BTB domain containing protein 7a (Zbtb7a), in alveolar macrophages (AMs) was a critical regulator of the inflammatory circuit associated with the development of Abs to SAgs in an OAD model. In this model, we observed a roughly 75% decrease in the Zbtb7a-deficient donor AM-production of allo-exosome (H-2K^{d+}) following allogeneic transfer of huCD68-K^{d} Tg AMs into C57BL/6 recipients [39]. This suggests that exosomes released from AMs are critical regulators of inflammation, and that they lead to Abs to lung SAgs and ultimately OAD. Details of exosomes released following LTx and their characterizations are provided in Table 1.

1.6. Role of circulating exosomes in a murine model of chronic lung allograft rejection

Recently, we reproduced a chronic rejection model in which the left lung from an F1 mouse is transplanted into a wildtype mouse (B6D2F1 to DBA/2J), a model initially reported by Mimura et al. [40]. In our experiment, approximately 80% of animals developed chronic rejection of the transplanted lung by day 28 post-transplant. Abs to lung SAgs, ColV and Kα1T, were seen by day 14; therefore, immune responses to lung SAgs occurs before histopathological evidence of chronic rejection is available. More importantly, we found that exosomes containing lung SAgs are also demonstrable in the circulation prior to development of Abs to lung SAgs and before development of chronic rejection. The exosomes released into the circulation not only contain MHC and lung SAgs on their surfaces, but also have co-stimulatory molecules, immune-potentiating transcription factors (i.e., NFκβ, HIFα), miRNA, and 20S proteasomes, rendering them highly immunogenic. Taken together, these results strongly suggest that induction and release of circulating exosomes with allo-antigens and lung SAgs (ColV and Kα1T) play an important part in the development of immune responses, as Abs to alloantigens and lung SAgs are evident before histological evidence shows chronic rejection of the transplanted lungs. In this murine model, we

Table 1
Characteristics of exosomes and development of antibodies to self-antigens in lung transplant recipients.

Conditions in lung transplant, Reference	Sample	Species	*de novo* DSA	Antibodies to SAgs	Exosomal profile
BOS/AR [4]	Serum, BAL fluid	Human	+ (Post-transplant)	Col-V (+), Kα1T (+) (post-transplant)	SAgs, Col-V and Kα1T, mismatched donor HLA, miR-92a [endothelial activation], miR-182 [inflammation], miR-142-5p [antibody-mediated chronic rejection], and miR-155 [T cell activation]
BOS [34,65]	Serum	Human	+	Col-V (+), Kα1T (+) MICA (+) (post-transplant)	Col-V and Kα1T, MHC class II molecules, costimulatory molecules CD40, CD80, and CD86, and transcription factors class II MHC *trans*-activator, NF-kB, hypoxia-inducible factor 1-a, IL-1R– associated kinase 1, MyD88, and 20S proteasome were detected in exosomes from BOS
AR [5]	BAL fluid	Human	ND	ND	RNA and miRNA related many immune pathway
Primary graft dysfunction [57]	Serum	Human	+ (Post-transplant)	Col-V (+), Kα1T (+) (Pre-transplant)	Col-V and Kα1T (post-transplant) unpublished data
OAD [37]	Serum	Mice	+	Col-V (+), Kα1T (+)	Col-V and Kα1T, MHC class II molecules, transcription factors class II MHC *trans*-activator, NF-kB, 20S proteasome, unpublished data
OAD/Zbtb7a alveolar macrophage [39]	Serum	Mice	+	Col-V (+), Kα1T (+)	Col-V and Kα1T, less allo-exosome (H-2Kd+) released by Zbtb7a-deficient AMs
Chronic rejection model [66]	Serum	Mice	+	Col-V (+), Kα1T (+) (Post-transplant)	Col-V and Kα1T, co-stimulatory molecules, immune-potentiating transcription factors (NFκβ, HIFα), miRNA and 20S proteasomes, unpublished data

AR – acute rejection; BOS – bronchiolitis obliterans syndrome; Col-V – Collagen V; Kα1T – K alpha 1 Tubulin; OAD – obliterative airway disease; SAgs – self antigens.

also demonstrated increased frequency of lung-SAgs-specific IFN-γ and TNF-α secreting cells as well as a marked reduction of lung-SAgs-specific IL-10-secreting cells following development of chronic rejection, suggesting that loss of peripheral tolerance may also contribute to the development of chronic rejection after transplantation (unpublished).

1.7. Donor-derived exosomes: mechanisms involved in lung allograft immunity

Multiple studies have shown that endoplasmic reticulum (ER) stress can promote inflammation, including delivery of danger signals to antigen-presenting cells after ER-stress-induced apoptosis. Exosomes have recently been found to carry danger signals to antigen-presenting cells [41]. Severe ER stress can lead to release of exosomes associated with upregulation of stress proteins, particularly CBP/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 2 (Cited-2) and phosphorylated JNK [42]. A recent study demonstrated that SRC kinase stimulates the secretion of exosomes by syndecan endocytosis pathway and on syntenin–syndecan endosomal budding, by upstream of ARF6 small GTPase and its effector phospholipase D2, leading to pro-migratory activity on endothelial cells [43]. These findings support that SRC kinase/syntenin acts in cell-to-cell communication by controlling the biogenesis and the activity of exosomes [43]. Exosomes help protect cells against intracellular stress by maintaining cellular homeostasis. Inhibition of neutral sphingomyelinase 2 (an enzyme that generates ceramide from sphingomyelin) by GW4869 has been shown to reduce exosomal release. Recent reports suggest that exosome secretion maintains cellular homeostasis by secreting cytoplasmic DNA and other harmful cellular constituents from cells, and that inhibition of exosome release is not beneficial [44]. Interestingly, preventing the sorting of cargo may be beneficial, and that SUMOylated protein heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1), a sequence motif that controls the loading of miRNAs into exosomes through binding, can be targeted [45]. Naji et al demonstrated that transplant tissue releases specific exosomes with donor HLA, and observed changes in intra-exosomal cargo with respect to rejection [46]. Furthermore, donor exosomes carrying HLA and costimulatory molecules (CD40 and CD80/86) also induced alloimmune responses of T cells by cross-dressing recipient antigen-presenting cells with donor HLA in allo-transplantation [47].

Cellular and humoral immune processes play essential roles in the pathogenesis of rejection. Allorecognition is a process in transplantation wherein the recipient's immune system recognizes mismatched MHC antigens from the donor organ and activates T lymphocytes, initiating allograft rejection. Two main pathways lead to activation of T cells: a direct pathway (i.e., T cell stimulation by donor-antigen presenting cells) and an indirect pathway (i.e., T cell stimulation by SAg presenting cells) [5,6]. However, in an additional pathway, called the *semidirect pathway*, T cell activation occurs via donor-derived exosomes (Fig. 2) [47,48]. Exosomes interact with cells via numerous ligand–receptor interactions. Endocytosed exosomes fuse with the membrane of the endocytic vacuoles and "inject" their intraluminal cargo into the cytosol of the acceptor leukocytes, where the exosome-shuttled miRNAs regulate their target mRNAs [24,49] in recipient cells [50]. Thus, exosomes released by dendritic cells (DCs) bearing donor H2 molecules had high levels of heat shock proteins and mir-155, which are powerful inducers of DC activation [51], and higher donor H2 bearing recipient DCs than the number of donor passenger DCs after transplantation. Cross-dressed recipient DCs presenting donor MHC molecules acquired via donor-derived exosomes effectively activated the allo-reactive T cells through the semidirect pathway [52,53]. Exosome-like EVs have also been shown to induce autoantibodies involved in allograft rejection [54]. We demonstrated that mice immunized with exosomes isolated from LTxRs diagnosed with BOS developed both humoral and cellular immune responses to ColV and Kα1T [34]. Dieude et al also demonstrated that exosome-like EVs enriched in the LG3

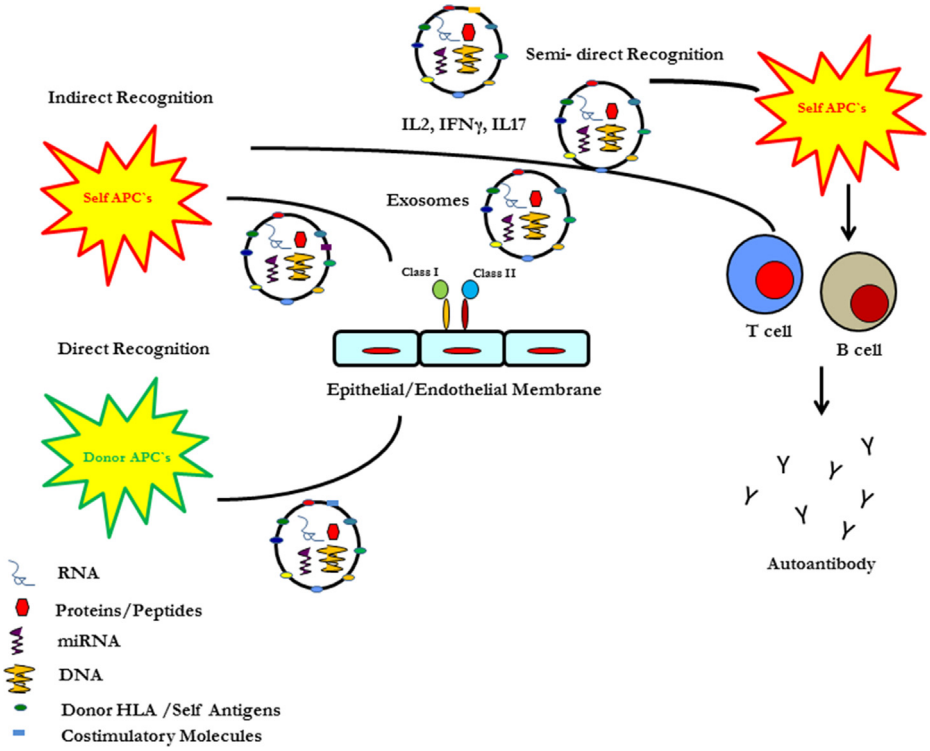

Fig. 2. Donor-derived exosomes via the semidirect pathway stimulate donor antigen-presenting cells and induce production of antibodies to self-antigens.

fragment of the vascular extracellular matrix protein perlecan triggered production of Abs against the auto-antigen LG3 [55].

1.8. Role of SAgs in lung allograft rejection

Ischemia–reperfusion injury during LTx can result in exposure of otherwise-masked collagen residues, which are released in bronchoalveolar lavage fluid leading to development of DSA to HLA [56]. Studies have shown that PGD (a form of ischemia–reperfusion injury) is associated with pre-formed Abs to ColV or Kα1T, which can increase the risk of BOS/chronic rejection after LTx [57]. LTxRs with pre-transplant Abs to SAgs also increases the risk of PGD (odds ratio 3.09, $p = 0.02$).

A retrospective analysis of 317 individuals who underwent LTx between 2000 and 2011 for diseases including chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis (IPF), cystic fibrosis (CF), and other diseases demonstrated that patients with IPF and CF had the highest prevalence of Abs to SAgs, which increased their risk for development of PGD, DSA, and BOS [58]. Tiriveedhi et al demonstrated that epitopes shift in ColV Ab from α1 and α2 to α1 only, occurred at the time of BOS, and these shifts in immunodominant epitopes correlated with decreases in the expression of IL-10 and increased expression of IFN-γ, which demonstrated the role of Th1 and Th2 cells [59]. Studies have also demonstrated that *de novo* development of Abs to ColV was associated with ColV specific Th17 cells, and that monocyte/macrophage accessory cells lead to progressive airway obliteration [60]. *De novo* immunity to ColV and BOS was associated with an HLA-DR15+ donor lung, and may be due to presentation by donor-derived HLA- DR15, of novel self-peptides to recipient T cells [61]. In a clinical study of 142 adult LTxRs, we demonstrated that the presence of Abs to Kα1T pre-transplant leads to increase in pro-inflammatory cytokines IL-1 (2.1-fold increase), IL-2 (3.0-fold increase), IL-12 (2.5-fold increase), IL-15 (3.0-fold increase), and chemokines IP-10 (3.9-fold increase) and MCP-1 (3.1-fold increase), and increases the risk of PGD and BOS in the long term [57].

2. Conclusions

Several clinical and pre-clinical studies have shown a strong association between HLA and non-HLA antigens in eliciting immune responses that lead to allograft rejection; however, certain questions remain unanswered. Periodic testing for DSA post-transplant is being performed in many transplant centers. Testing of Abs to SAgs are now being developed with the goal to institute clinically. Current therapies to treat DSA include plasma exchange, intravenous immunoglobulin, rituximab, bortezomib, etc. However, the efficacy of these therapies to completely remove DSA and its influence in the development of chronic rejection still remains unclear. To better understand the synergistic relationship between alloimmunity and the development of Abs to SAgs, as well as the mechanisms of this relationship, we sought to define the role of exosome release after transplantation and the consequences of exosome release. We, and others, have demonstrated that circulating exosomes released from the transplanted organ indeed play an important part in eliciting immune responses leading to rejection [6,34,46,47]. The presence of mismatched donor HLA and lung SAgs, ColV and Kα1T, was noted primarily in exosomes isolated from LTxRs diagnosed with rejection or other clinical conditions thought to increase the risk for rejection, which demonstrates that the exosomes are indeed originating from the transplanted organ.

Donor-derived exosomes possess immunoregulatory molecules and also carry cell-derived antigens and donor HLA molecules. Recent studies have demonstrated that exosomes can induce tolerance in the transplant setting [62,63]. Further studies should investigate the role of exosomes in a tolerant *in vivo* model and should define their profiles. This may help us understand the role of circulating exosomes in induction and in maintaining tolerance post-transplant. Exosome secretion maintains cellular homeostasis; therefore, complete inhibition of exosome release will not be beneficial clinically. However, emerging technology such as ex vivo lung perfusion has been shown to reduce lung injury, and successful transplantation can be achieved even from the lungs of marginal donors [64]. Blocking exosome formation and release by pharmacological agents during ex vivo lung perfusion is now

feasible.

Another potential value of analyzing circulating exosomes with donor HLA and tissue-restricted SAgs will be development of circulating exosomes with specific characteristics as potential biomarkers to identify patients at risk for rejection. The murine rejection models detailed above and the data from human LTxRs strongly suggest that circulating exosomes with lung SAgs can be detected prior to Ab development and before rejection is histologically evident. These exciting results require further validation via multicenter analysis. It has been a longstanding clinical observation that PGD and RVI increase the risk of chronic rejection after LTx. The release and persistence of exosomes in the circulation have the potential to augment immune responses, leading to an increased risk of chronic rejection in the above-mentioned scenarios; however, further studies are needed to define the mechanisms by which PGD or RVI increases the risk of CLAD. Finally, our analysis is focused on LTx as a model for understanding the role of exosomes in allograft rejection. The study of circulating exosomes is also important in recipients of other solid organ transplantations for defining mechanisms of rejection and tolerance, and to determine their utility as potential biomarkers.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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