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Exosome-Allorecognition in Lung Transplantation Rejection

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ABSTRACT

Purpose of review: Innate and adaptive immunity both contribute to allorecognition mechanisms that drive rejection after lung transplantation. Classic allorecognition pathways have been extensively described, but there continues to be several unanswered questions. Exosome research appears to be a novel and potentially significant area of allorecognition research, and could be the missing link that answers some existing questions. This article reviews literature that is associated with allorecognition pathways and the role of exosomes in alloreactivity.

ABBREVIATIONS

CLAD: Chronic Allograft Dysfunction; AR: Acute Rejection; HLA: Human Leukocyte Antigen; APC: Antigen Presenting Cell; DC: Dendritic Cell; MHC: Major Histocompatibility Complex; SAg: Self-Antigen; OB: Bronchiolitis Obliterans

INTRODUCTION

For end-stage lung disease, lung transplantation is the sole therapeutic option [1]. Despite advances in the field, patients continue to face the worst post-transplant survival statistics. The half-life of a lung transplant is still limited to 5 years due to several complications including acute and chronic allograft dysfunction (CLAD) [1-3]. Classically, immune mechanisms that drive the development of acute rejection (AR) and CLAD have been explained by mismatched donor human leukocyte antigens (HLA) and HLA-independent lung associated antigens. These immune responses are primarily dictated through T cell: alloantigen responses, resulting in a series of immune cell signaling cascades that elicit activation of naïve cells after transplantation [3]. In the area of alloimmunity, there are several implicated pathways describing T cell: alloantigen interactions. These pathways include direct, indirect, and semi-direct allorecognition [4,5]. It has been classically accepted that at an early post-transplant stage, donor leukocytes migrate to recipient secondary lymphoid organs and activate naïve T cells in the direct pathway, generating alloreactive T cells which are mostly responsible for the development of AR. As donor leukocytes diminish overtime, the indirect pathway plays a major role in chronic rejection [3,4]. Recipient antigen presenting cells (APC) prime CD4+ T cells by internalizing and presenting allopeptides locally or in circulation. Later, it became evident that directly alloreactive

T cells also recognize donor MHC molecules acquired intact by recipient APC through a third mechanism, the semi-direct pathway. The semi-direct pathway is also used to explain the cross-regulation between CD4 and CD8 T cells activated by distinct pathways [4]. In the semi-direct pathway, the mechanism by which recipient APC acquire and retain intact and functional donor MHC molecules is unclear. New evidence in the field of extracellular vesicles, especially exosomes, is currently providing new answers to these long-standing questions in the transplantation and allorecognition.

Exosomes are 30-100 nm extracellular vesicles released from the plasma membrane from an immune or non-immune parent cell and can be isolated from various biological fluids including plasma, bronchioalveolar lavage (BAL), and urine [4-7]. Exosome composition is dependent on the parent cell lineage, activation/differentiation states, and stages of infection. Exosomes are able to mediate immune responses for both innate and adaptive immunity through exosome: cell interactions [8-10]. Characterization of exosomes through identifying surface markers and internal content, and correlating to functional capabilities are imperative to understanding their roles in transplant rejection [11-14]. Most critical is to elucidate their roles in allorecognition pathways that drive acute and chronic rejection, and potentially redefine alloresponse paradigms at an exosome: cell level. In this review, we describe the classic allorecognition pathways that are involved in lung transplant rejection. In addition, we describe the potential roles of exosomesin allorecognition pathways or "allo-exo-responses," which provides a new and intriguing dynamic to the conventional thinking.

APC PRODUCTION OF EXOSOMES OR "ALLO-EXO-ANTIGEN"

Exosomes have been previously described to be secreted by various APC, including mast cells [8], natural killer cells [9], DCs, macrophages, T cells, and B cells [15]. Exosomes generated by APC are highly enriched for surface MHC-peptide complexes suggesting that they could function as Ag-presenting vesicles or as vehicles to disseminate allogeneic antigens for priming of anti-donor T cells [15,16]. Exosomes generated by DCs have been observed in primary DCs and DC cell lines and have been the focus of many studies in the field of exosome-mediated allorecognition [15-18]. Exosomes internally carry a wide range of cargo including proteins, mRNA, miRNA, and DNA from the parent cells and are able to shuttle these components between cells. Exosomes released by mature DCs (mDCs) are enriched for MHC, adhesion and T cell costimulatory molecules, home to lymphoid organs and prime T cells [17-19]. mDCs also encounter secreted exosomes from various other cell subsets, and present exo-allo-antigen to stimulate or inhibit cells. Monteclavo et al. [17] described a short-range mechanism of alloantigen distribution through exosome delivery mechanisms to DCs, resulting in elicitation of anti-donor T response observed in transplantation. It has also been elucidated that exosomes are more important than soluble allopeptides in transferring antigen to DCs because antigens that are internalized and secreted in the form of phagosomes are presented 10^3-10^4 fold more efficiently by MHC class II [17].

Macrophages are known to play important roles in both innate immunity and adaptive immunity by phagocytosing pathogens, shaping innate responses, eliciting communication with adaptive immunity, and facilitating DC in antigen presentation. Exosomes isolated from macrophages have been found to enhance CD4+ and CD8+ T cell responses particularly in the presence of DCs [20]. In a mouse model of *M. tuberculosis* infection, mice with Rab27a-deficiency were found to be less efficient at stimulating T cells as a result of compromised capacity to release exosomes [21]. Additionally, human lung microvascular endothelial and small airway epithelial cells can be induced to express MHC class II upon stimulation [22]. Cultured intestinal epithelial cells released MHC class II-carrying EVs could efficiently activate CD4 T cells, when cross-dressed onto DCs [23].

ALLO-EXO-RECOGNITION PATHWAYS

Exogenous antigens including exosomes, can be acquired by APC residing within the lung and lymphoid tissues (monocytes, macrophages, DCs, B cells, epithelial cells) through various mechanisms, including phagocytosis of dead cells, endocytosis, or micropinocytosis [24-26]. Exo-alloantigens are released by passenger leukocytes and have the potential to activate surrounding resident and infiltrating cells, as well as stimulate DC presentation within the lymphoid tissues. This process is facilitated by recipient DCs cross-dressed with donor exosomes displaying intact donor MHC-peptide complexes. Recently, exosomes in circulation containing donor HLA and lung-

associated self-antigen (SAg) have been detected and characterized post-lung transplantation. Levels of these SAg exo-allo-antigens were associated with increased incidence of acute and chronic rejection [27,28]. Interestingly, acute rejection episodes have also been observed in non-vascularized allografts where donor leukocytes are unable to migrate out of the transplanted tissue. Previous studies show that graft-derived exosomes can travel across lymphatic endothelium *in vitro*, and can be transported within lymphatic vessels *in vivo* [29]. It appears that donor-derived exosomes "leak out" of the graft and travel through extravasation or severed openings of recipient lymphatic capillaries towards the graft-draining lymphoid organs [30].

Direct Allo-exo-recognition

The direct allorecognition pathway explains how recipient T cells in the lymphoid organs recognize the intact donor peptide-major histocompatibility complexes (p-MHC) displayed on the surface of donor APCs. Passenger donor APCs travel to recipient secondary lymphoid organs, resulting in the development of alloreactive T cells with a wide range of receptor specificities. The persistence of these alloresponse signaling cascades ultimately leads to the development of acute and chronic rejection in patients. In a kidney rat transplant model, prolonged allograft survival was achieved by the depletion of donor DCs, and tolerance of the allograft was prevented by repletion of donor DCs [31], demonstrating the importance of direct allorecognition in the development of tolerance or rejection.

Though several studies demonstrate the importance of donor DCs, many groups have reported that donor-derived DCs are undetectable in graft-draining lymphoid organs during the first week after transplantation of non-vascularized or vascularized allografts [25,32], suggesting there may be other mechanisms in facilitating the direct allorecognition. Recent evidence indicated that exosomes may enhance the efficiency of allo-sensitization of T cells against donor MHC molecules in the direct pathway. Exosomes maintain the topology of the APC of origin, exposing the extracellular domain of MHC molecules at the vesicle surface. Exosomes released by APCs carry surface MHC class I and MHC class II of parent cells, and potentially have capacities to directly stimulate CD8 and CD4 T cells [31].

Indirect Allo-exo-recognition

By the indirect pathway, alloantigens are internalized by recipient APCs, processed and presented as peptide by self-MHC class II molecules to CD4+ T cells [32,33]. This mechanism has been described to be persistent throughout the duration of the allograft and is considered to be the major contributor of chronic graft rejection, which is dominated by CD4+ T cells and the generation of alloreactive antibodies. Patients with bronchiolitis obliterans (OB) show significantly higher frequencies of indirect donor alloreactivity compared to patients without OB [34]. Assessment of a direct alloresponse in T cells, showed less reactivity and a presence in both patients with or without OB. These data, along with several other groups, demonstrates that indirect allorecognition pathways are associated more with the development of chronic rejection. While free exosomes have limited ability to induce alloreactive T cells directly, the p-MHC complexes carried by them can be used as a source of peptide to indirectly prime T cells when internalized by APCs through phagocytosis or micropinocytosis [35]. The Morelli group demonstrated that exosomes carrying allopeptide could trigger specific CD4 T cell responses in wild-type mice, but not in MHC class II deficient hosts [6].

Semi-direct Allo-exo-recognition

The semi-direct pathway occurs when intact donor MHC molecules are transferred from donor to recipient dendritic cells, and subsequently presented by recipient dendritic cells to T cells [36]. This mechanism enables the cross-regulation between directly and indirectly activated T cellsthrough the same APC. Lechler's lab demonstrated that recipient-derived, conventional DCs cross-dressed with allogeneic donor MHC molecules could activate allospecific T cells, setting the basis of the semi-direct pathway of T-cell allorecognition in transplantation [25]. Later, the presence of donor MHC molecules on recipient APCs was confirmed in both solid organ and allogeneic bone marrow transplantation [37,38].

A recent study suggests the involvement of exosomes in the semi-direct pathway based on super structural observations [31]. Post-skin and heart allograft transplantation, recipient-derived DCs and B cells in the graft-draining lymphoid organs were cross-dressed with donor-derived exosomes, carrying intact donor MHC molecules [30,31]. Interestingly, rather than being internalized by recipient DCs as previously described, exosomes adhered to the surface of recipient DCs in small

clusters, retaining intact and functional donor MHC molecules and APC-activating signals. The depletion of these DCs drastically impaired T cell priming against donor MHC and delayed cardiac allograft rejection [31]. Interestingly, the semi-direct allo-exo-recognition pathway can give rise to a form of "split tolerance" [39-42]. This process is observed during chronic allo-exo exposure due to indirect alloreactive T helper cells and direct alloreactive T effectors differentially impacted by host DCs after exposure to "cross-dressed" exosomes [43].

Innate Allo-exo-recognition

Traditionally, innate immunity has been described as a short-lived, rapid, nonspecific immune response to pathogens by macrophages, NK cells, and neutrophils. Most recently, this concept has been challenged, more specifically describing a potential role in determining non-self in tissues and cells in a non-microbial setting through an innate allorecognition pathway [41]. Recent studies have shown allospecific responses in macrophages contribute to the process of rejection [44,45]. In a model of T cell specific allorecognition restricted to a single foreign antigen, rejection was only observed when allogeneic non-self signals were recognized by host innate immunity [46]. Additionally, mice deficient in T cells, B cells, and innate lymphoid cells transplanted with allogeneic grafts were still able to persistently differentiate monocytes into mature DCs [44,45]. This could explain the role of innate immunity in triggering or enhancing graft rejection through maturation of host monocyte-derived DC function to activate T cells towards an effector phenotype and inducing allotoxicity activity in macrophages [44]. Innate allorecognition could be the missing link in the development of chronic rejection due to the persistence of macrophages residing in allografts long after transplant.

The role of exosomes in innate allo-exo-recognition has yet to be fully investigated and there are several areas that exosomes could play a critical role. These include macrophage phagocytosis and antigen presentation, maturation of infiltrating monocytes towards an effector DC phenotype within the graft, and secretion of alloreactive, donor specific exosomes that migrate to host lymphoid tissues for further antigen processing [44,45]. Recently, studies have revealed a potential mechanism based on a polymorphic molecule, signal regulatory protein α (SIRPα) that exists on donor monocytes and secreted exosomes, and appears to contribute to donor: recipient alloreactivity [28].

CONCLUSION

Exosome-mediated allorecognition involves both innate and adaptive immunity. There have been numerous studies investigating the roles of direct, indirect, and semi-direct pathways in allorecognition driving transplant rejection. Further studies investigating donor or host-derived exosomes locally or in the secondary lymphoid organs in exosome-mediated allorecognition may provide new perspectives in addressing outstanding questions in the area of transplantation. The addition of potential exo-allo-recognition pathways to the conventional theories, provide significant insight into other mechanisms contributing to donor and recipient alloreactivity. The clearance of donor passenger leukocytes, or inability to migrateto recipient lymphoid tissues, suggests that donor graft-derived exosomes play a significant role in alloantigen recognition pathways. Though mechanisms in which exosomes mediate acute and chronic rejection is still unclear, further studies investigating these pathways are pivotal to understanding the development of these immune processes, and will provide insight for future therapeutics and identification of biomarkers for better patient outcomes.

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