Published in final edited form as:

Curr Opin Organ Transplant. 2018 February ; 23(1): 22–27. doi:10.1097/MOT.0000000000000489.

Emerging role of exosomes in allorecognition and allograft rejection

Bruno Gonzalez-Nolasco, Mengchuan Wang, Aurore Prunevieuille, and Gilles Benichou

Department of Surgery, Transplant Research Center, Center for Transplantation Sciences, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA

Abstract

Purpose of review—This article reviews recent literature on the nature of extracellular vesicles released by allogeneic transplants and examine their role in T-cell alloimmunity involved in rejection and tolerance of these grafts.

Recent findings—Donor cells release extracellular vesicles, including exosomes, after transplantation of allogeneic organs and tissues. Consequently, recipient APCs take up these exosomes and present donor MHC antigens on their surface (allo-MHC cross-dressing) thus, activating some alloreactive T cells via a mechanism called semi-direct pathway of allorecognition. In addition, one study shows that exosomes carrying noninherited maternal antigens are associated with maternal microchimerism and tolerance in offspring. Finally, a few studies describe potential utilization of exosomes as modulators of alloimmunity and biomarkers of rejection in allotransplantation.

Summary—Extracellular vesicles, including exosomes, released by allografts contribute to recognition of donor antigens by T cells after allotransplantation. This occurs through cross-dressing of recipient APCs with donor MHC antigens and subsequent activation of T cells, a process called semi-direct alloreactivity. The relevance of this phenomenon in rejection and tolerance of allografts and the potential utilization of exosomes as biomarkers in transplantation are discussed.

Keywords

allorecognition; antigen cross-dressing; exosomes; immune tolerance; transplantation; transplant rejection

INTRODUCTION

Allograft rejection is initiated in the recipient's secondary lymphoid organs by T lymphocytes recognizing donor major histocompatibility (MHC) antigens (allorecognition). During this phase, activation of alloreactive T cells occurs via two distinct pathways: the

Correspondence to Gilles Benichou, Department of Surgery, Transplant Research Center, Center for Transplantation Sciences, Massachusetts General Hospital and Harvard Medical School, 55 Fruit Street, Thier 807, Boston, MA 02114, USA. Tel: +1 617 724 4206; fax: +1 617 724 3901; gbenichou@mgh.harvard.edu.

Conflicts of interest

There are no conflicts of interest.

direct pathway in which T cells recognize intact allogeneic MHC proteins on donor MHC class II⁺ leukocytes (passenger leukocytes) and the indirect pathway whereby T cells recognize donor peptides processed and presented by recipient antigen-presenting cells (APCs) [1,2,3■]. Direct allorecognition by T cells triggers a massive polyclonal inflammatory response, which leads to acute rejection of allografts [4]. In contrast, indirect alloreactivity involves a limited set of T-cell clones specific to a few dominant determinants on donor MHC [5,6]. The role of indirect alloresponses in acute allograft rejection is still unclear. On the other hand, there is strong circumstantial evidence supporting the view that indirectly activated alloreactive T cells play a key role in alloantibody production and chronic graft rejection, a condition characterized by graft tissue fibrosis and blood vessel obstruction [7–10].

Two recent studies demonstrate that, in addition to passenger leukocytes, allografts release extracellular vesicles, which migrate out of the graft and carry donor MHC molecules to the recipient's lymphoid organs [11■,12■]. These donor-derived vesicles, including exosomes, are regularly taken up by recipient APCs, which then present on their surface donor MHC molecules, a phenomenon that we refer to as allo-MHC cross-dressing [11■]. Previous studies by Lechler's laboratory had shown that recipient cells displaying allogeneic MHC molecules could activate allospecific T cells. Therefore, it is plausible that this type of alloantigen recognition called semi-direct pathway could be involved in allograft rejection [13■,14]. Alternatively, a few reports suggest that certain extracellular vesicles can promote allograft tolerance [15,16■,17,18]. This article reviews recent studies evaluating the role of extracellular vesicles released after allotransplantation and their involvement in immunity associated with allograft rejection or tolerance.

EXTRACELLULAR VESICLES IN REJECTION OF SKIN ALLOGRAFTS

Conventional, nonprimarily vascularized, skin allografts induce potent direct and indirect alloresponses by CD4⁺ and CD8⁺ T cells. The traditional view is that direct priming of T cells in draining lymph nodes occurs via presentation of allogeneic MHC antigens by passenger leukocytes having migrated out of the skin graft (dendritic cells) [19–21]. However, the presence of donor dendritic cells in lymph nodes draining skin allografts has never been formally proven. Recently, our laboratory showed that, after skin grafting in mice, donor MHC molecules are actually transported to the host's lymphoid organs by extracellular vesicles [3■]. Recipient dendritic cells and B cells then acquire these vesicles and present intact donor MHC class I and II molecules to allospecific T cells. Furthermore, we showed that injection of naïve mice with allogeneic extracellular vesicles (purified exosomes) or with recipient cells cross-dressed *in vitro* with donor exosomes was sufficient on its own to elicit a direct inflammatory alloresponse *in vivo* and sensitize mice to donor antigens [11■]. This corroborates former studies by R Lechler's laboratory showing the ability of allo-MHC cross-dressed cells to elicit allospecific T-cell responses *in vitro* (mixed lymphocyte reaction) and *in vivo* in mice [11■]. In a subsequent study, Smyth *et al.* [22■] showed continuous acquisition of MHC–peptide complexes by recipient cells and its role in the generation of anti-graft CD8⁺ T-cell immunity in skin-grafted mice. Altogether, these studies suggest that the presentation of donor MHC by recipient APCs rather than by donor passenger leukocytes represents the driving force behind direct alloreactivity and acute

rejection in skin transplantation. However, it is important to note that this conclusion may not apply to primarily vascularized skin allografts, whose passenger leukocytes can migrate out of the graft through blood vessels right after their placement and activate T cells in the recipient's spleen, as observed with heart transplants [4]. Finally, it is still unknown whether donor exosomes are involved in the initiation of indirect T-cell alloresponse, which is an essential component of skin allograft rejection. Further studies designed to inhibit the production or secretion of extracellular vesicles by donor cells or their uptake by recipient cells will be required to evaluate the actual role of exosomes and donor antigen cross-dressing in skin allograft rejection.

EXTRACELLULAR VESICLES IN REJECTION OF VASCULARIZED SOLID ORGAN TRANSPLANTS

Cardiac allografts are connected to the host's blood vessels through surgical anastomosis during their placement. This facilitates immediate trafficking of donor passenger leukocytes out of the graft, presumably through reverse transendothelial migration. Indeed, seminal studies by Larsen [23] have documented the presence of donor dendritic cells in the spleen of mice within a day after transplantation of an allogeneic heart. Recently, we readdressed this question using imaging flow cytometry and confirmed the presence of donor MHC class II⁺ cells in the spleen of heart-transplanted mice. However, less than 100 donor cells per million spleen cells were detected at day 1 posttransplantation and their number declined rapidly thereafter [11■]. On the other hand, one day after transplantation, we found 30 000 recipient spleen cells cross-dressed with donor MHC molecules. Strikingly, by day 7, nearly 100 000 cross-dressed cells were detected [11■]. Such high frequencies of allo-MHC cross-dressed cells in lymphoid organs of heart-transplanted mice were also observed in a concurrent study in Morelli's laboratory [12■]. In both Marino *et al.*'s [11■] and Liu *et al.*'s [12■] reports, transfer of allogeneic MHC proteins was shown to involve extracellular vesicles displaying characteristic features of exosomes. This corroborates former studies by the Wong's group showing extensive transfer of MHC molecules *in vivo* after cardiac transplantation [24,25]. Importantly, Morelli's studies showed that recipient cDCs having acquired exosomes became activated and stimulated alloreactive T cells. Furthermore, depletion of recipient cDCs after cardiac transplantation markedly decreased presentation of donor MHC antigens to alloreactive T cells and delayed graft rejection in mice [12■]. In further support of this view, Harper *et al.* [26] showed the key role of acquired alloantigens in CD8⁺ T-cell responses during acute rejection of heart allografts. These studies support the view that the same recipient APC can present allopeptides indirectly to CD4⁺ T cells and donor MHC class I directly to CD8⁺ T cells. Such a 'three cell model' would explain how CD4⁺ T cells help the differentiation of cytotoxic CD8⁺ T cells in a MHC class I-only disparate transplant setting (depicted in Fig. 1). Taken together, these studies support the view that release of exosomes by heart transplants and subsequent presentation of donor MHC molecules by recipient APCs in the recipient's spleen triggers T-cell alloresponses involved in acute rejection of these allografts.

EXTRACELLULAR VESICLES IN BONE MARROW TRANSPLANTATION

Transplantation of allogeneic bone marrow (BMT) or hematopoietic stem cells along with recipient's conditioning is a treatment of choice for hematological malignancy. In addition, induction of allogeneic hematopoietic chimerism has been used in animal models and patients to mediate tolerance of allografts [27,28]. However, graft-versus-host disease (GvHD) caused by donor T cells reacting to recipient MHC and minor histocompatibility antigens is a major complication associated with this therapy. Apparently, both direct and indirect recognition of recipient MHC and minor histocompatibility antigens by donor T cells are involved in GvHD [29–31]. An elegant study by Markey *et al.* [32] demonstrated that many donor cells become cross-dressed with recipient MHC class I and II molecules following BMT. Interestingly, the presence of recipient MHC proteins on donor dendritic cells (cross-dressing) was transient in the setting of myeloablative conditioning but durable after nonmyeloablative conditioning, in which recipient hematopoietic cells remain in high numbers [32]. This suggested that only recipient professional APCs can transfer their MHC antigens to donor dendritic cells. Next, using TCR transgenic mice (TEa) whose T cells recognize a single MHC–peptide complex (A^b MHC class II bound to Ea 52–68 peptide), they observed that cross-dressed cells could not activate TEa T cells through semi-direct pathway. In turn, such cross-dressed cells could enhance indirect alloresponse by T cells recognizing the same MHC–peptide complex, presumably by increasing the level of interaction between APCs and CD4⁺ T cells [32]. These results suggest that MHC cross-dressing of donor cells with recipient MHC antigens occurs regularly after BMT. Further studies will be needed to determine the contribution of exosomes in this process and the role of semi-direct alloresponses in GvHD.

EXTRACELLULAR VESICLES AS BIOMARKERS IN TRANSPLANTATION

Reliable and early diagnosis of rejection is critical in clinical transplantation. Indeed, current methods often detect deleterious immunity whenever it is too late to revert its course and/or the organ transplant is irreversibly damaged. Most important, there is a need for noninvasive methods to detect chronic allograft rejection, a process characterized by graft tissue fibrosis and vasculopathy affecting a large proportion of organ transplants within 1–5 years after their placement [33,34]. Finally, current histological examination of small sample biopsies does not necessarily reflect the entire allograft's disease status.

It is well documented that the presence of selected extracellular vesicles, including exosomes, in bodily fluids reflects histological and physiological features of organs and tissues. For instance, the presence of exosomes carrying defined proteins and RNA are found in urine and blood of patients with various kidney diseases [35–37]. Similar findings have been reported in patients with different types of cancer and autoimmune diseases [38–41]. This suggests that, in transplanted patients, the release of certain exosomes in the blood or urine might correlate with the type and stage of rejection of their allografts. In support of this view, a recent article from Mohanakumar's laboratory showed that donor-derived exosomes carrying lung tissue-specific antigens (collagen V) are regularly found in the serum of lung-transplanted patients with bronchiolitis obliterans syndrome (BOS), which is characteristic of chronic rejection [42■]. Interestingly, detection of graft-derived exosomes

preceded clinical diagnosis suggesting that it could serve as a method to predict the onset of chronic rejection and adjust patients' treatment accordingly. In another study, Tower *et al.* [43■] reported that the number of plasma C4d⁺ endothelial microvesicles correlated with antibody-mediated rejection and its severity in patients, recipient of a renal allograft. Furthermore, Sigdel *et al.* [44] and Alvarez *et al.* [45] reported altered protein contents in urinary exosomes from patients undergoing acute rejection of kidney allografts. Finally, a recent article from Naji and co-workers showed the presence of donor-derived exosomes displaying the endocrine markers, insulin, glucagon and somatostatin, in blood samples of recipients after islet transplantation [46■]. Therefore, monitoring exosomes and their cargoes in blood or urine of patients might represent a promising noninvasive method to evaluate the status of allografts and the nature and severity of their rejection.

EXOSOMES AND ALLOGRAFT TOLERANCE

Immune tolerance of allografts is often associated with alloantigen presentation in a tolerogenic fashion, that is, by selected APCs in a context favoring protective rather than destructive immunity. This suggests that, under certain circumstances, exosomes carrying donor antigens could be involved in tolerance rather than rejection of allografts.

In support of this view, a recent study by Burlingham's laboratory shows the key role of donor exosomes in the amplification of maternal microchimerism and regulation of immunity to noninherited maternal antigens in adults [16■]. In addition, some studies show that allogeneic exosomes given in a defined fashion and Treg-derived exosomes can be used to promote tolerance to allografts [15,17,18,47■].

CONCLUSION

It is now clear that allogeneic cells release extracellular vesicles such as exosomes, after organ and tissue transplantation. Subsequently, recipient professional APCs (dendritic cells and B cells) take up these vesicles and present donor MHC antigens on their surface (MHC cross-dressing). These allo-MHC cross-dressed cells can activate recipient alloreactive *in vitro* and *in vivo* (semi-direct pathway). Also, donor MHC and minor antigens carried by donor exosomes might represent a major source of allodeterminants for processing and indirect presentation by recipient APCs. On the other hand, no or very few donor passenger leukocytes migrate to recipient lymphoid organs after transplantation and they are eliminated rapidly by the host's immune system. This suggests that donor exosomes rather than passenger leukocytes are the main source of antigens for allorecognition by T cells after transplantation. However, this still remains to be demonstrated as well as the contribution of exosomes and antigen cross-dressing in rejection and tolerance of allografts.

Acknowledgments

None.

Financial support and sponsorship

This study was supported to G.B. by NIH grant NIH AI 124096. A.P. was supported by a fellowship from Université Sorbonne Paris Cité (USPC).

References

Papers of particular interest, published within the annual period of review, have been highlighted as:

■ of special interest

■ of outstanding interest

1. Benichou G, Valujskikh A, Heeger PS. Contributions of direct and indirect T cell alloreactivity during allograft rejection in mice. *J Immunol.* 1999; 162:352–358. [PubMed: 9886406]
2. Gould DS, Auchincloss H Jr. Direct and indirect recognition: the role of MHC antigens in graft rejection. *Immunol Today.* 1999; 20:77–82. [PubMed: 10098326]
- 3■. Marino J, Paster J, Benichou G. Alloreognition by T lymphocytes and allograft rejection. *Front Immunol.* 2016; 7:582. This review provides an overview of current knowledge regarding different pathways of alloreognition by T cells and their contribution to the alloresponse and allograft rejection. [PubMed: 28018349]
4. Kant CD, Akiyama Y, Tanaka K, et al. Primary vascularization of allografts governs their immunogenicity and susceptibility to tolerogenesis. *J Immunol.* 2013; 191:1948–1956. [PubMed: 23833234]
5. Benichou G, Fedoseyeva E, Lehmann PV, et al. Limited T cell response to donor MHC peptides during allograft rejection. Implications for selective immune therapy in transplantation. *J Immunol.* 1994; 153:938–945. [PubMed: 7517977]
6. Liu Z, Sun YK, Xi XYP, et al. Limited usage of T cell receptor V beta genes by allopeptide-specific T cells. *J Immunol.* 1993; 150(Pt 1):3180–3186. [PubMed: 8468463]
7. Lee RS, Yamada K, Houser SL, et al. Indirect recognition of allopeptides promotes the development of cardiac allograft vasculopathy. *Proc Natl Acad Sci U S A.* 2001; 98:3276–3281. [PubMed: 11248069]
8. Baker RJ, Hernandez-Fuentes MP, Brookes PA, et al. Loss of direct and maintenance of indirect alloresponses in renal allograft recipients: implications for the pathogenesis of chronic allograft nephropathy. *J Immunol.* 2001; 167:7199–7206. [PubMed: 11739543]
9. Reznik SI, Jaramillo A, SivaSai KS, et al. Indirect alloreognition of mismatched donor HLA class II peptides in lung transplant recipients with bronchiolitis obliterans syndrome. *Am J Transplant.* 2001; 1:228–235. [PubMed: 12102256]
10. Ciubotariu R, Liu Z, Colovai AI, et al. Persistent allopeptide reactivity and epitope spreading in chronic rejection of organ allografts. *J Clin Invest.* 1998; 101:398–405. [PubMed: 9435312]
- 11■. Marino J, Babiker-Mohamed MH, Crosby-Bertorini P, et al. Donor exosomes rather than passenger leukocytes initiate alloreactive T cell responses after transplantation. *Sci Immunol.* 2016; 1:aaf8759. This article describes the migration of donor passenger leukocytes and extracellular vesicles out of skin, heart and pancreatic islet allografts after transplantation. It shows the frequencies and nature of cross-dressed and donor vesicles and their ability to elicit pro-inflammatory alloimmune responses by T cells *in vitro* and *in vivo*.
- 12■. Liu Q, Rojas-Canales DM, Divito SJ, et al. Donor dendritic cell-derived exosomes promote allograft-targeting immune response. *J Clin Invest.* 2016; 126:2805–2820. This article describes the contribution of exosomes derived from donor dendritic cells and their contribution to the alloimmune response by T cells involved in rejection of heart allografts in mice. [PubMed: 27348586]
- 13■. Morelli AE, Bracamonte-Baran W, Burlingham WJ. Donor-derived exosomes: the trick behind the semidirect pathway of alloreognition. *Curr Opin Organ Transplant.* 2017; 22:46–54. This article discusses some recent knowledge regarding the role of exosomes in allotransplantation and microchimerism associated with tolerance of maternal antigens. [PubMed: 27898464]
14. Smyth LA, Herrera OB, Golshayan D, et al. A novel pathway of antigen presentation by dendritic and endothelial cells: implications for alloreognition and infectious diseases. *Transplantation.* 2006; 82(1 Suppl):S15–S18.

15. Peche H, Renaudin K, Beriou G, et al. Induction of tolerance by exosomes and short-term immunosuppression in a fully MHC-mismatched rat cardiac allograft model. *Am J Transplant.* 2006; 6:1541–1550. [PubMed: 16827854]
- 16■ Bracamonte-Baran W, Florentin J, Zhou Y, et al. Modification of host dendritic cells by microchimerism-derived extracellular vesicles generates split tolerance. *Proc Natl Acad Sci U S A.* 2017; 114:1099–1104. This article describes the role of exosomes and cross-dressing in the alloresponse to noninherited maternal antigens and their ability to influence direct and indirect alloresponses and sensitization or tolerance to alloantigens depending upon the nature of presented antigens and costimulation or co-inhibitory receptors. [PubMed: 28096390]
17. Yu X, Huang C, Song B, et al. CD4+CD25+ regulatory T cells-derived exosomes prolonged kidney allograft survival in a rat model. *Cell Immunol.* 2013; 285:62–68. [PubMed: 24095986]
18. Agarwal A, Fanelli G, Letizia M, et al. Regulatory T cell-derived exosomes: possible therapeutic and diagnostic tools in transplantation. *Front Immunol.* 2014; 5:555. [PubMed: 25414702]
19. Billingham RE. The passenger cell concept in transplantation immunology. *Cell Immunol.* 1971; 2:1–12. [PubMed: 4399153]
20. Larsen CP, Austyn JM, Morris PJ. The role of graft-derived dendritic leukocytes in the rejection of vascularized organ allografts. Recent findings on the migration and function of dendritic leukocytes after transplantation. *Ann Surg.* 1990; 212:308–315. [PubMed: 2204317]
21. Wachtel SS, Silvers WK. The role of passenger leukocytes in the anomalous survival of neonatal skin grafts in mice. *J Exp Med.* 1972; 135:388–404. [PubMed: 4551219]
- 22■ Smyth LA, Lechler RI, Lombardi G. Continuous acquisition of MHC:peptide complexes by recipient cells contributes to the generation of anti-graft CD8+ T cell immunity. *Am J Transplant.* 2017; 17:60–68. This article shows the contribution of donor MHC cross-dressing of recipient antigen-presenting cells on the development of cytotoxic CD8+ T-cell responses generated after allotransplantation. [PubMed: 27495898]
23. Larsen CP, Morris PJ, Austin JM. Migration of dendritic leukocytes from cardiac allografts into host spleens. A novel pathway for initiation of rejection. *J Exp Med.* 1990; 171:307–314. [PubMed: 2404081]
24. Brown K, Sacks SH, Wong W. Extensive and bidirectional transfer of major histocompatibility complex class II molecules between donor and recipient cells in vivo following solid organ transplantation. *FASEB J.* 2008; 22:3776–3784. [PubMed: 18632850]
25. Brown K, Sacks SH, Wong W. Coexpression of donor peptide/recipient MHC complex and intact donor MHC: evidence for a link between the direct and indirect pathways. *Am J Transplant.* 2011; 11:826–831. [PubMed: 21401861]
26. Harper SJ, Ali JM, Wlodek E, et al. CD8 T-cell recognition of acquired alloantigen promotes acute allograft rejection. *Proc Natl Acad Sci U S A.* 2015; 112:12788–12793. [PubMed: 26420874]
27. Sykes M, Sachs DH. Bone marrow transplantation as a means of inducing tolerance. *Semin Immunol.* 1990; 2:401–417. [PubMed: 2104278]
28. Slavin S, Strober S. Mechanisms of transplantation tolerance to allogeneic bone marrow cells following total lymphoid irradiation (TLI). *Haematol Blood Transfus.* 1980; 25:323–331. [PubMed: 7021350]
29. Koyama M, Kuns RD, Olver SD, et al. Recipient nonhematopoietic antigen-presenting cells are sufficient to induce lethal acute graft-versus-host disease. *Nat Med.* 2011; 18:135–142. [PubMed: 22127134]
30. Markey KA, Banovic T, Kuns RD, et al. Conventional dendritic cells are the critical donor APC presenting alloantigen after experimental bone marrow transplantation. *Blood.* 2009; 113:5644–5649. [PubMed: 19336758]
31. Matte CC, Liu J, Cormier J, et al. Donor APCs are required for maximal GVHD but not for GVL. *Nat Med.* 2004; 10:987–992. [PubMed: 15286785]
32. Markey KA, Koyama M, Gartlan KH, et al. Cross-dressing by donor dendritic cells after allogeneic bone marrow transplantation contributes to formation of the immunological synapse and maximizes responses to indirectly presented antigen. *J Immunol.* 2014; 192:5426–5433. [PubMed: 24790149]

33. Hayry P. Molecular pathology of acute and chronic rejection. *Transplantat Proc.* 1994; 26:3280–3284.
34. Tejani A, Emmett L. Acute and chronic rejection. *Semin Nephrol.* 2001; 21:498–507. [PubMed: 11559891]
35. Miranda KC, Bond DT, Levin JZ, et al. Massively parallel sequencing of human urinary exosome/microvesicle RNA reveals a predominance of noncoding RNA. *PLoS One.* 2014; 9:e96094. [PubMed: 24816817]
36. Miranda KC, Bond DT, McKee M, et al. Nucleic acids within urinary exosomes/microvesicles are potential biomarkers for renal disease. *Kidney Int.* 2010; 78:191–199. [PubMed: 20428099]
37. Gonzales PA, Pisitkun T, Hoffert JD, et al. Large-scale proteomics and phosphoproteomics of urinary exosomes. *J Am Soc Nephrol.* 2009; 20:363–379. [PubMed: 19056867]
38. Andaloussi ELS, Mager I, Breakefield XO, Wood MJ. Extracellular vesicles: biology and emerging therapeutic opportunities. *Nat Rev Drug Discov.* 2013; 12:347–357. [PubMed: 23584393]
39. Thery C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. *Nat Rev Immunol.* 2002; 2:569–579. [PubMed: 12154376]
40. Staals RH, Pruijn GJ. The human exosome and disease. *Adv Exp Med Biol.* 2011; 702:132–142. [PubMed: 21713683]
41. Sheng H, Hassanali S, Nugent C, et al. Insulinoma-released exosomes or microparticles are immunostimulatory and can activate autoreactive T cells spontaneously developed in nonobese diabetic mice. *J Immunol.* 2011; 187:1591–1600. [PubMed: 21734072]
- 42■. Gunasekaran M, Xu Z, Nayak DK, et al. Donor-derived exosomes with lung self-antigens in human lung allograft rejection. *Am J Transplant.* 2017; 17:474–484. This article describes the presence of donor-derived exosomes carrying lung tissue specific antigens involved in rejection of lung allografts and bronchiolitis obliterans syndrome (BOS). Remarkably, exosomes expressing Collagen V were isolated from sera from recipients 3 months before allograft rejection and 6 months before BOS diagnosis, suggesting that exosomes displaying this antigen may be a noninvasive rejection biomarker. [PubMed: 27278097]
- 43■. Tower CM, Reyes M, Nelson K, et al. Plasma C4d+ endothelial microvesicles increase in acute antibody-mediated rejection. *Transplantation.* 2017; 101:2235–2243. The authors studied microvesicle concentration in the plasma of 95 kidney transplant patients with allograft dysfunction and compared with 23 healthy volunteers. Quantification of plasma C4d+ microvesicles provided information about presence of AMR, its severity and response to treatment in transplant patients. [PubMed: 27846156]
44. Sigdel TK, Ng YW, Lee S, et al. Perturbations in the urinary exosome in transplant rejection. *Front Med (Lausanne).* 2014; 1:57. Perturbed proteins in the exosomal compartment of urine collected from kidney transplant patients were specific to inflammatory responses, and were not observed in the urine exosomal fraction from normal healthy individuals. [PubMed: 25593928]
45. Alvarez S, Suazo C, Boltansky A, et al. Urinary exosomes as a source of kidney dysfunction biomarker in renal transplantation. *Transplant Proc.* 2013; 45:3719–3723. [PubMed: 24315007]
- 46■. Vallabhajosyula P, Korutla L, Habertheuer A, et al. Tissue-specific exosome biomarkers for noninvasively monitoring immunologic rejection of transplanted tissue. *J Clin Invest.* 2017; 127:1375–1391. Transplant islet exosomes were purified using anti-HLA antibody-conjugated beads, and their cargoes contained the islet endocrine hormone markers: insulin, glucagon, and somatostatin. In the clinical settings of islet and renal transplantation, donor exosomes with respective tissue specificity for islet beta cells and renal epithelial cells were characterized in recipient plasma over follow-up periods of up to 5 years. This study suggests the biomarker potential of transplant exosome characterization for providing a noninvasive window into the state of transplant tissue. [PubMed: 28319051]
- 47■. Aiello S, Rocchetta F, Longaretti L, et al. Extracellular vesicles derived from T regulatory cells suppress T cell proliferation and prolong allograft survival. *Sci Rep.* 2017; 7:11518. This article shows that extracellular vesicles produced by regulatory T cells (Treg-EVs) contain a unique molecular cargo of specific miRNAs and iNOS, which, once delivered into target cells, blocked cell cycle progression and induced apoptosis. Treg-EV-exposed T cells were in turn converted into regulatory cells and their in-vivo administration prolonged kidney allograft survival in rats. [PubMed: 28912528]

KEY POINTS

- Extracellular vesicles, including exosomes, carrying donor MHC antigens are released by allografts after transplantation.
- Graft-derived exosomes are captured by recipient antigen-presenting cells, which then present intact donor MHC molecules on their surface, a phenomenon referred to as allo-MHC cross-dressing.
- Recipient T cells are activated *in vitro* and *in vivo* following recognition of donor MHC molecules presented on recipient APCs, a process called semidirect alloreactivity.
- Selected exosomes can be used to prolong allograft survival.
- Graft-derived exosomes can serve as biomarkers in acute and chronic rejection or just chronic rejection of solid organ allografts.

FIGURE 1.

MHC cross-dressing can promote cooperation between alloreactive CD4⁺ and CD8⁺ T cells. Panels (a) and (b) depicts two models describing how indirectly activated CD4⁺ T cells can provide help to CD8⁺ T cells after placement of a MHC class I-mismatched allograft. Panel (a) shows a classical four-cell cluster whereby CD4⁺ and CD8⁺ T cells recognize donor MHC indirectly (donor MHC peptide) and directly (intact donor MHC), respectively on distinct APCs. Panel (b) shows how donor MHC class I cross-dressing can promote CD4⁺-CD8⁺ T-cell cooperation by having self-MHC class II and donor peptide (indirect presentation) and donor MHC class I (direct presentation) presented on the same antigen-presenting cell (three-cell cluster).