Extracellular vesicles (EVs) are membrane-contained vesicles released by most cell types, have attracted a large amount of research interest over the past decade. Because of their ability to transfer cargo via regulated processes, causing functional impacts on recipient cells, these structures may play important roles in cell-cell communication and have implications in the physiology of numerous organ systems. In addition, EVs have been described in most human biofluids and have wide potential as relatively noninvasive biomarkers of various pathologic conditions. Specifically, EVs produced by the pancreatic β-cell have been demonstrated to regulate physiologic and pathologic responses to β-cell stress, including β-cell proliferation and apoptosis. β-Cell EVs are also capable of interacting with immune cells and may contribute to the activation of autoimmune processes that trigger or propagate β-cell inflammation and destruction during the development of diabetes. EVs from adipose tissue have been shown to contribute to the development of the chronic inflammation and insulin resistance associated with obesity and metabolic syndrome via interactions with other adipose, liver, and muscle cells. Circulating EVs may also serve as biomarkers for metabolic derangements and complications associated with diabetes. This minireview describes the properties of EVs in general, followed by a more focused review of the literature describing EVs affecting the β-cell, β-cell autoimmunity, and the development of insulin resistance, which all have the potential to affect development of type 1 or type 2 diabetes. (Molecular Endocrinology 29: 1535–1548, 2015)
for comparison of future results between different groups (20). The commonly used nomenclature incorporates the vesicle source and includes 3 main groups: (1) exosomes, (2) microvesicles, and (3) apoptotic bodies. Exosomes are released extracellularly by fusion of an endosomal multi-vesicular body with the plasma membrane (4, 21). Microvesicles form via direct blebbing off the plasma membrane (21). Although apoptotic bodies are also formed by blebbing of the plasma membrane, these are often larger and arise from apoptotic cells (22). Table 1 lists the features commonly used to differentiate EV subtypes, although considerable overlap limits these markers from truly being “subtype specific.”

**EV Formation and Release**

Several important contributions suggest that EV formation and release occur via carefully orchestrated processes. At the levels of both the plasma membrane and multivesicular body, membrane curvature causes sorting of membrane proteins and lipids to microdomains with the most favorable membrane free energy profiles (1, 23). Endosomal-sorting complex required for transport (ESCRT) machinery has been shown to regulate budding and segregation of cargo into EVs (24). Alternatively, EV release may occur in an ESCRT-independent manner. In such cases, ceramide-rich intraluminal vesicles bud from endosomal microdomains associated with sphingolipid-rich lipid rafts. This process requires neural sphingomyelinase 2, the enzyme responsible for ceramide synthesis from sphingolipids (25).

Several other proteins have been identified as regulating this process. Rab small GTPases have been shown to selectively inhibit exosome secretion, with specific effects on exosome size and multivesicular endosome docking at the plasma membrane (26). Radiation-induced DNA damage increased EV secretion from lung carcinoma cell lines. This increase was absent in p53 knockouts but was recapitulated by expression of a p53 gene product, tumor suppressor activated pathway 6, suggesting a role for p53 and this downstream multipass transmembrane protein in stress-induced EV release (27).

**EV Interaction with Target Cells**

Figure 2 summarizes the current understanding of EV formation and interaction with target cells. EVs can interact with target cells in several ways. Intracellular signaling can be activated through interaction of surface molecules or molecules released from EVs and target cells (28, 29). Alternatively, EVs can affect target cells by transfer of cargo. Multiple groups have demonstrated membrane fusion or EV internalization by target cells using real-time imaging (30–33). Uptake can occur via multiple subtypes of endocytosis, macropinocytosis, or phagocytosis (30, 33–36). This energy-dependent internalization is regulated by multiple factors, including the presence of a functioning cytoskeleton and physiologic conditions (30, 31, 34, 36, 37).

Specific parent cell and target cell characteristics can affect the specificity of EV internalization. EV and recip-

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**Table 1. Commonly Cited Features of Extracellular Vesicle Subtypes**

| Vesicle Subtype         | Origin                                      | Size, mm | Density, g/mL | Commonly Used Markers                                              |
|-------------------------|---------------------------------------------|----------|---------------|-------------------------------------------------------------------|
| Exosomes                | Multivesicular endosome fusion with plasma membrane | 40–100   | 1.13–1.19     | CD63, CD9, CD81, Alix, flotillin-1, ESCRT-3, TSG101              |
| Microvesicles           | Plasma membrane budding                     | 100–1000 | 1.221–1.198   | Caveolin, CD40 ligand, selectin, flotillin-2, annexin V, phosphatidyl-serine |
| Apoptotic Bodies        | Plasma membrane budding of apoptotic cells  | 1000–5000| 1.16 to 1.28  | Annexin V, DNA, histones, phosphatidylerine                      |

Data from Refs. 1, 20, 138, 139.
ient cell surface proteoglycans and glycoproteins, as well as transmembrane tetraspanins and integrins, can be important in EV endocytosis (37–39). Phosphatidylserine on the EV surface probably plays a role in phagocytosis (40). Inhibition of each of these proteins on EVs and/or recipient cells has been demonstrated to reduce EV uptake (34, 36, 37, 39).

Variations in the presence or distribution of EV and recipient cell surface molecules may allow specificity of EV uptake or trafficking to recipient cells. Intercellular adhesion molecule 1 (ICAM-1) acts as a recipient cell surface ligand for tetraspanin 8–cluster of differentiation (CD) 49 complexes on EVs (41, 42). EVs expressing the tetraspanin 8–CD49 complex were selectively internalized by endothelial and pancreatic cells, based on the presence of ICAM-1 on the target cell surface (42). Similarly, the interaction between glycoproteins on the surface of breast milk EVs and lectin receptors on dendritic cells allows the preferential uptake of milk EVs compared to plasma-derived EVs (39).

Changes in physiologic conditions may alter the interaction between EVs and target cells via changes in surface molecules, EV affinity, or EV uptake. Treatment with epidermal growth factor and chemokine receptor ligands induces large-scale stimulation of macropinocytosis (35). Activation of T cells induces a conformational change in the integrin, leukocyte function-associated antigen-1, allowing for increased affinity with ICAM-1 and, therefore, with dendritic cell EVs. This interaction allows transfer of major histocompatibility complex (MHC) class II molecules from the EVs to the T cells (43). Although more research is indicated to comprehensively understand the mechanisms of EV trafficking in different cell types, these results suggest a complex and coordinated regulatory system that can respond to different physiologic and pathologic microenvironments.

**EV Cargo and Functional Significance**

Because functional RNAs were discovered in EVs, RNAs have been the most commonly studied form of EV cargo (6). Although EVs containing variable amounts of fragmented and intact mRNAs, long noncoding RNAs, small noncoding RNAs, rRNAs, tRNAs, and vault RNAs have been described, depending on the parent cell and isolation methods, most EV RNAs are <700 nucleotides in length (44–49).
EV RNAs transferred into recipient cells function in classic or nonclassic manners (5). Transferred mRNAs can be translated using recipient cell machinery (5). EVs can also contain large quantities of 3′-untranslated region mRNA fragments. Once transferred, these fragments could competitively bind to recipient cell RNA binding proteins or microRNAs (miRNAs), affecting regulation of target cell translation (44). EV miRNAs, which may or may not be associated with RNA-induced silencing complexes, can inhibit translation of target cell mRNAs (50–53). Alternatively, EV miRNAs may function through nonclassic mechanisms, including regulated miRNA disposal or direct activation of Toll-like receptors (TLRs) (51, 54). Different physiologic conditions, such as hypoxia or oxidative stress, alter EV RNA content and functional effects in target cells. These differences suggest a role for EV RNAs in cellular responses to physiologic and pathologic stress (55, 56).

Array and sequencing data reveal that EV RNA composition differs significantly from that of parent cells, implying that RNAs are selectively packaged into EVs (5). Preferential packaging of certain RNA subsets depends on the 3′ nucleotide sequence, specific miRNA binding sites, parent cells, and physiologic circumstances (49, 57–59). Extracellular export of some miRNAs may be also be regulated by Argonaute 2 or heterogeneous nuclear ribonucleoproteins (60–62).

Proteins are also frequently described as EV cargo. As mentioned, this can include ligands to membrane receptors that are able to trigger intracellular signaling or proteins that are transferred to recipient cells (18, 28, 29, 63). For example, antigen presentation by MHC molecules may occur directly or by transfer of the EV complex to other immune cells (18, 63). Associations of EVs with soluble mediators without signal sequences, including numerous cytokines, have also been identified (64–67).

EVs containing DNA, including double- and single-stranded genomic DNA as well as mitochondrial DNA, from multiple parent cells have been described and may differ depending on the EV subtype (68–71). Tumor cell EV DNA harbors the same mutations or amplifications as the parent cells, providing potential as a biomarker (68, 70). Similar to other cargo, EV DNA also may be transferred and have functional impacts in target cells (72, 73).

Lipids are another important EV component. Lipidomic studies of EVs from multiple cell types reveal wide variability but some consistent components in lipid composition. Compared with parent cells, most EVs are enriched for the following: sphingomyelin; cholesterol and ceramides; phosphatidylserine, phosphatidylinositol, and phosphatidic acids; and glycosphingolipids and hexosylceramides. These components allow a rigid membrane that is resistant to degradation in biologic fluids (1, 74). EVs can also carry lipids as cargo, often along with lipid-related enzymes, allowing transfer or release of bioactive lipids (75, 76).

In 2014, a report using nanoparticle tracking analysis quantified copies of miRNA per exosome in samples of plasma, seminal fluid, and several cell culture supernatants and found that <1 copy of miRNA is present per exosome (77). Given that most research on EV function is performed with supraphysiologic EV concentrations, these findings call into question the biologic relevance of transferred EV miRNA for cell-cell communication at physiologic concentrations. These results may merely reflect the importance of different EV subtypes or nonclassic mechanisms for miRNA function. However, they shed light on the importance of defining the significance of observed EV effects in relevant physiologic systems.

### Applications of EVs

Because cargo can reflect parent cell characteristics, EVs have been proposed as biomarkers of various pathologic conditions. These “liquid biopsies” provide a relatively non-invasive snapshot of the parent cell of interest. For example, cargo of circulating EVs in oncology patients yields information regarding tumor presence, mutations, and aggressiveness and can even predict response to treatment (78–80). Recently, microfluidic biochips have been developed that can simultaneously assay multiple EV markers (80). These early results hold great promise for the development of biofluid EVs as high-throughput disease biomarkers.

The remarkable stability of EVs, their ability to protect their cargo from degradation, and their ability to target specific cells has given rise to research involving use of EVs as delivery tools for therapeutic cargo (81). This can involve loading of antigens into EVs to induce an endogenous immune response. For example, “vaccination” with antigen presenting cell EVs loaded with tumor antigens induces T cell activation and targets tumors for destruction (82). Alternatively, small interfering polynucleotides or therapeutic compounds could be loaded into isolated EVs or artificially generated liposomes for delivery to target cells (81, 83, 84). Conjugation of targeting moieties to therapeutic agents to improve their delivery or modulate clearance are a subject of intense research, particularly in the field of cancer. Alternatively, in vitro generated or synthetic EVs may offer a superior modality for targeted delivery because of their cargo protective capability and target cell type specificity (85, 86).

Despite such intriguing potential, several challenges need to be overcome before the clinical application of EVs
becomes feasible. For example, strict characterization criteria, production methodologies that will result in specific and homogeneous populations of engineered EVs, and safety assessments will have to be developed (87). In addition, optimized purification techniques to provide consistent yield and purity of specific EVs are yet to be established (88). Furthermore, overall safety is an important concern. EVs have been shown to modulate the immune system, and in light of their potentially high bioavailability, screening for undesirable immune-reactive components will have to be incorporated into production protocols. Another potential concern is the ability of EVs to serve as a transfection system for viral RNA, capable of spreading infection even in the absence of fully formed enveloped virions (86, 89, 90). Although this area needs more development, several clinical trials using EVs are under way or planned (registration numbers NCT02138331, NCT01668849, and NCT01294072 at clinicaltrials.gov), and further work could yield major effects in drug/gene therapy delivery.

**Role of EVs in Diabetes and Related Metabolic Diseases**

Given the ubiquitous nature of EV production by most cells in the human body, and the roles EVs appear to play in cell-cell communication, it is not surprising that EVs could contribute to the complex multiorgan regulation of insulin release and glucose homeostasis. Diabetes, the most common metabolic disorder worldwide, results from inadequate insulin release. This can be caused by autoimmune destruction of the pancreatic β-cell (type 1 diabetes [T1D]) or intrinsic β-cell dysfunction and death exacerbated by systemic insulin resistance (type 2 diabetes [T2D]) (91–93). As detailed in Table 2, multiple studies have explored the role of EVs in organ systems coordinating the regulation and dysregulation of glucose metabolism, potentially affecting development of either form of diabetes.

**EVs and the Islet**

A handful of articles describing EVs released by the insulin-producing pancreatic β-cell have been published. Electron microscopy of EVs secreted by NIT-1 insulinoma cells under baseline conditions demonstrated that these β-cell EVs were homogeneous, and mostly in the typical exosome size range (30–100 nm size). Proteomic analysis revealed typical EV proteins, including endocytosis and exocytosis proteins, metabolic modulators, and cytoskeletal components. In addition, less common EV proteins were present, particularly those with functional relationships to ubiquitination and protein degradation, and ribosome and RNA-related proteins. Interestingly, increased β-cell EV release was induced by treatment with glucose or calcium (94).

Isolation and characterization of EVs released from human islets revealed that islet-derived EVs were also composed mostly of smaller vesicles. Examination of EV contents revealed a large population of RNAs and proteomic contents consistent with those of islet cells. In particular, the presence of insulin transcripts and insulin and C-peptide proteins, with low levels of glucagon and endothelial nitric oxide synthase, suggested that these EVs were mostly of β-cell origin, rather than α cells or endothelial cells. However, islet EVs also displayed significant content differences from β-cells, with enrichment for a subset of miRNAs in islet EVs (95).

Importantly, β-cell EV production and content is altered by exposure to inflammatory cytokines. In 1990, electron microscope analysis of rat pancreata perfused with IL-1β revealed “blebs” from the cytosol of β-cells consistent with the morphologic appearance of EVs (96). Microarray of the mouse insulinoma MIN6 cell line EVs revealed EV-specific changes in the miRNA profile compared to those in cytokine-treated cells, including several miRNAs known to affect cell survival (97). Later proteomic studies uncovered significant differences between EV contents of cytokine-stimulated and unstimulated rat 6HI 6F Tu28 β-cells, in which microvesicles from cytokine-treated cells were shown to contain increased levels of multiple proteins from the TNFα signaling pathway. Both microvesicles and exosomes derived from cytokine-treated cells harbored increased N-linked sialylated glycopeptides and sialylated CD82, as well as ICAM-1. The authors could not quantify whether these differences were due to increased cargo per EV vs increased total exosome concentration (98). In aggregate, the cytokine-induced changes in EV profiles and cargo suggest that β-cell EVs could play a role in physiologic or dysfunctional responses to the inflammatory milieu occurring in the diabetic microenvironment.

Exploration of β-cell–derived EV physiologic effects has revealed functional impacts on the surrounding β-cells. Rat insulinoma (INS-1) cells with an inducible inactivating mutation in the monogenic diabetes transcription factor hepatocyte nuclear factor 1A, undergo apoptosis. These apoptotic cells release EVs that stimulate proliferation in naive INS-1 cells, through increased expression of pancreatic stone protein/regenerating protein. Filtering the cell supernatant or pretreating parent cells with a caspase inhibitor blocked the effect on prolif-
| Parent Cell/Tissue | Target Cell/Tissue | Findings | Ref. |
|-------------------|-------------------|----------|------|
| INS-1 cells       | INS-1 cells       | Exposure of naive β-cells to β-cell apoptotic bodies increased proliferation and insulin expression by induction of pancreatic stone protein/regenerating protein. | Bonner et al., 2010 (99) |
| INS-1 cells       | INS-1 cells       | EVs from INS-1 cells treated with low-dose inflammatory cytokines protected other INS-1 cells against high-dose cytokine-induced apoptosis via transfer of neutral ceramidase. | Zhu et al., 2014 (100) |
| MIN6 and INS-1 cells | MIN6 and INS-1 cells | EV transfer of miRNAs originating from high-dose cytokine-conditioned β-cells induced apoptosis in untreated recipient β-cells. | Guay et al., 2015 (97) |
| MIN6 and NIT-1 cells | Mouse splenocytes, CD4+ and CD8+ T cells | β-Cell–derived EVs activate autoreactive T cells and induce proinflammatory cytokine production by splenocytes. | Sheng et al., 2011 (105) |
| MIN6 and NIT-1 cells | Mouse splenocytes, B cells | β-Cell EVs activate marginal zone–like B cells in NOD mice, an effect associated with onset of diabetes. | Bashratyan et al., 2013 (106) |
| iMSCs              | Mouse splenocytes | iMSCs from NOD mice released EVs that stimulated splenocytes to release inflammatory cytokines and induced splenocyte antigen presentation. | Rahman et al., 2014 (107) |
| Human islets      | Human islet endothelial cells | Human islet EV treatment of islet endothelial cells led to transfer of multiple RNAs as well as antiapoptotic and proangiogenic effects. | Figliolini et al., 2014 (95) |
| Endothelial progenitor cells | Human islets | Endothelial progenitor cell EVs increased islet viability and insulin production. Islet transplant grafts exhibited enhanced graft vascularization. This effect was mediated by EV miR-126 and miR-296 | Cantaluppi et al., 2012 (102) |
| Pancreatic cancer cells | INS-1 cells and human islets | Pancreatic cancer cell EVs deliver adrenomedullin to β-cells, inducing endoplasmic reticulum stress, reactive oxygen species production, and apoptosis, while decreasing glucose-stimulated insulin secretion. | Javeed et al., 2015 (103) |
| Mouse VAT         | Peripheral blood monocytes, bone-marrow derived macrophages | VAT EVs from high-fat diet or ob/ob mice induced macrophage differentiation and promoted secretion of TNF-α and IL-6 from bone marrow–derived macrophages in culture. Intravenous injection of obese VAT EVs caused insulin resistance in C57BL/6J mice. | Deng et al., 2009 (121) |
| Large rat adipocytes | Small rat adipocytes | Horizontal transfer of RNA species from large adipocytes facilitated transcriptional reprogramming in small adipocytes to induce differentiation and lipogenesis. | Müller et al., 2011 (115) |
| Rat adipocytes    | Small adipocytes | Large adipocytes up-regulate the lipogenesis of small adipocytes by EV-driven signaling in response to fatty acids, reactive oxygen species, or antidiabetic medication. | Muller et al., 2011 (140) |
| 3T3-L1 adipocytes | 3T3-L1 preadipocytes | Hypoxia promotes mature hypertrophic adipocytes to secrete EVs that carry a defined cargo of lipogenic enzymes. These EVs induce differentiation and lipogenesis when internalized into preadipocytes. | Sano et al., 2014 (116) |
| Human adipose tissue | HepG2 hepatocellular carcinoma cells, C2C12 myoblasts | Adipose EVs from obese patients modulated insulin responses in hepatocytes and muscle cells. The number of circulating adipose EVs correlated to HOMA-IR and elevated systemic liver function tests. | Kranendonk et al., 2014 (117) |
| Differentiated SGBS adipocytes, human adipose tissue | Human peripheral monocytes | Adipocyte EVs contained multiple immunomodulatory adipokines. When internalized into monocytes both SAT and VAT induced differentiation of monocytes into macrophages with ATM phenotype. Medium conditioned by these macrophages inhibited insulin signaling in adipocytes. | Kranendonk et al., 2014 (122) |

Continued
against apoptosis to other inflammatory cytokines actually conferred protection derived from INS-1 cells and islets treated with low doses of miRNA-gated effect (97). In contrast to these results, EVs delivered inhibitory RNA for Argonaute 2, suggesting a miRNA-mediated effect. This ultimately inhibits glucose-stimulated insulin secretion and induces apoptosis (103). Based on these findings, EVs from VAT of obese and nonobese human subjects integrated into hepatic cell lines, but only those from the obese patients caused transcription-driven hyperstimulation of the TGFβ pathway. These processes were dependent on small miRNAs, including insulin mRNA, miR-375, miR-200c, and miR-21. Intriguingly, uptake of islet EVs conferred protection effects of the EPC EVs (102).

Other work has also been described to affect β-cells. Pancreatic cancer frequently causes diabetes, yet the definitive cause of this effect has not been established. One clue to the mechanism stems from a recent discovery of pancreatic cancer cell EVs delivering adrenomedullin into distal pancreatic β-cells. Here, adrenomedullin binds to receptors within the β-cell, activating endoplasmic reticulum stress and production of reactive oxygen species. This ultimately inhibits glucose-stimulated insulin secretion and induces apoptosis (103). Based on these findings, HOMA-IR, homeostatic model assessment-insulin resistance; NFκB, nuclear factor κB; SGBS, Simpson-Golabi-Behmel syndrome; VCAM-1, vascular cell adhesion molecule-1.

### Table 2. Continued

| Parent Cell/Tissue | Target Cell/Tissue | Findings | Ref. |
|--------------------|--------------------|----------|------|
| Human THP-1 monocyctic cell line | Human SAT adipocytes | Compared with EVs from alternatively activated/M2 macrophages, EVs from proinflammatory/M1 polarized macrophages activated NFκB signaling and induced insulin resistance in treated adipocytes. | Zhang et al., 2015 (123) |
| Human visceral adipose tissue | HepG2 cells, HHStec hepatic stellate cells | EVs from VAT of obese and nonobese human subjects integrated into hepatic cell lines, but only those from the obese patients caused transcription-driven hyperstimulation of the TGFβ pathway. | Koeck et al., 2014 (112) |
| Human adipose tissue | A549 lung epithelial cells | Comparison of miRNA content of adipose tissue-derived exosomes from obese and nonobese adolescents revealed that EVs originating from obese adipocytes stimulate up-regulation of canonical TGFβ and Wnt signaling in target cells. | Ferrante et al., 2015 (120) |
| Mouse skeletal muscle, C2C12 myoblasts | C2C12 myoblasts | Muscle EVs from C57BL6/J mice fed a high palmitate diet or cells treated with palmitate increased target cell palmitate levels, induced proliferation and differentiation, but did not induce insulin resistance in muscle cells. Intravenously injected muscle EVs were internalized by multiple tissues including pancreas and liver. | Aswad et al., 2014 (124) |
| Rat plasma | Rat cardiac endothelial cells | Endothelial cells treated with plasma EVs from rats fed a high-fat diet showed increased VCAM-1 expression and production of reactive oxygen species. | Heinrich et al., 2015 (125) |

Cytokine treatment of β-cells confers several important functional properties to β-cell EVs. Exposure of naïve MIN6 cells to EVs originating from cytokine-conditioned β-cells induced recipient cell apoptosis, thus confirming the ability of β-cell-derived EVs to induce phenotypical changes in recipient cells. This effect was abrogated by pretreatment of recipient cells with a small inhibitory RNA for Argonaute 2, suggesting a miRNA-mediated effect (97). In contrast to these results, EVs derived from INS-1 cells and islets treated with low doses of inflammatory cytokines actually conferred protection against apoptosis to other β-cells. This effect was mediated via EV delivery of the protective protein, neutral ceramidase. EV neutral ceramidase activity was only increased when β-cells were treated with low doses of cytokines but not high doses (100). These seemingly opposite responses are consistent with a known divergent response of the β-cell to different doses of cytokines and could provide insight into the underlying mechanism behind this effect (101).

Other work has demonstrated functional contributions of β-cell EVs on other target cells. Islet-derived EVs have the capacity to effect the surrounding endothelial cells, which are able to internalize islet EVs in a dose-dependent manner. This process is inhibited by treatment with blocking antibodies for ICAM-1 and CD44. Internalization of islet EVs resulted in transfer of multiple RNAs, including insulin mRNA, miR-375, miR-200c, and mir-21. Intriguingly, uptake of islet EVs conferred endothelial cell resistance to apoptosis and up-regulated expression of numerous proangiogenic factors (95). Conversely, endothelial progenitor cell (EPC) EVs may have an impact on islets. EPC EVs cocultured with human islets were internalized by β, α, and islet endothelial cells. Internalization resulted in improved glucose-stimulated insulin secretion and islet viability, as well as islet endothelial cell proliferation and angiogenesis. When co-transplanted with islets, EPC EVs improved graft vascularization. These processes were dependent on small miRNAs, as treatment with RNase or EPC Dicer knockout blocked the protective effects of the EPC EVs (102).
pancreatic cancer-induced diabetes has been proposed to be an exosomopathy (104).

Clearly, EVs originating from or homing to the pancreatic islet have the ability to affect physiologic control of β-cell homeostasis as well as physiologic or pathologic responses to stress. Such profound effects underscore the importance of further studies into paracrine and systemic effects of islet-associated EVs and other affected organ systems.

**Islet EV Contributions to Autoimmunity**

Investigations into the role of EVs in activation of islet autoimmunity have uncovered several interesting mechanisms of EV-driven immunomodulation. For example, MIN6-derived EVs were shown to carry glutamic acid decarboxylase 65, confirming the ability of β-cell EVs to harbor islet autoantigens. Furthermore, β-cell EVs were capable of directly stimulating immune cells: EV treatment of nonobese diabetic (NOD) mouse splenocytes induced proliferation and secretion of inflammatory cytokines. In addition, antigen-presenting cells were activated via up-regulation of MHC II and costimulatory molecules. When spleen preparations were depleted for T cells, EVs did not induce proliferation. Interestingly, the effects of EVs were most pronounced on splenocytes from female NOD mice, which are known to be at the highest risk for spontaneous diabetes development. Compared with controls, female NOD lymph nodes cocultured with MIN6 EVs were associated with a 10-fold increase in Th1 cells compared with that of lymph nodes from diabetes-resistant strains. However, intravenous injection of these EVs was also able to increase insulitis in diabetes-resistant NOR/Ltj mice (105).

Further efforts focused on β-cell EV effects on autoreactive B cells. In this work, purified B cells from NOD spleen isolations were treated with MIN6 EVs. Results revealed a B-cell subpopulation that increased proliferation in response to EV treatment, even in the absence of T helper cells. Most of these B cells were B220+ marginal zone B cells, and one fifth were positive for CD27, consistent with previous activation. β-Cell EV immunization in B6 mice doubled the number of EV-responsive B cells, but did not increase marginal zone B cells. The percentage of female NOD EV-reactive B cells was nearly twice that of male animals, and peak levels of EV-reactive B cells occurred at 10 weeks, before diabetes development (106). Using an ELISA for CD81, the authors quantified total serum EVs. NOD serum EVs increased prediabetes development (8 weeks) and remained elevated until the time of diabetes onset (106, 107).

Fibroblast-like islet cells that proliferated upon ex vivo islet culture also demonstrate immunomodulatory capabilities. These CD105+CD45− cells were coined islet mesenchymal stem cell–like cells (iMSCs), because they expressed the mesenchymal stem cell marker stem cell antigen-1. Similar to MIN6 EVs, NOD spleen cells treated with EVs isolated from NOD iMSCs increased inflammatory cytokine release and activated antigen presentation. Here, iMSC EVs only activated B-cell proliferation in spleen cells from older NOD animals. The sensitivity to EV treatment correlated with total serum EVs. In contrast to NOD iMSCs, iMSCs isolated from C57BL6/J mice did not induce immune activation. To verify these results in vivo, iMSC EVs were injected into NOD mice that were <3 weeks old, followed by transplant of effector T cells from BDC2.5 mice. EV pretreatment increased the memory T-cell and B-cell responses, as well as susceptibility to insulitis (107).

Cumulatively, these works identify islet EVs as conceivable sources of β-cell autoantigens. In addition, islet-derived EVs have the ability to activate primed autoreactive B and T cells, suggesting potential contributions to exacerbation of islet autoimmunity in diabetes. These findings were absent in spleen cells from mice lacking myeloid differentiation factor 88 (105–107). Because this protein is needed for TLR family signal transduction, these results suggest a role for EV activation of TLRs in EV effects (108–110). Further experimentation is indicated to identify the exact mechanisms of this impact on memory B and T cells and whether injection of these islet-derived EVs can cause or accelerate diabetes onset in vivo.

**EVs in Adipose Homeostasis and Insulin Resistance**

Regulation by EVs has been widely implicated in adipose tissue development and homeostasis (111–116). Protein analysis of adipose-derived EVs has identified adipokines also present in adipose tissue itself: including adiponectin, IL-6, monocyte chemoattractant protein-1, macrophage migration inhibitory factor, retinol binding protein 4 (RBP-4), and resistin (117).

Multiple studies have explored the role of EVs in the development of insulin resistance and metabolic syndrome. Elevated blood levels of adipocyte protein 2 have been strongly associated with the development of type 2 diabetes and metabolic syndrome. Upon lipolysis, adipose cells package adipocyte protein 2 into EVs, where it could potentially be transferred to other cells (118). Adiponectin, an adipokine typically linked to insulin sensi-

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*Lakhter and Sims EVs in Diabetes and Related Disorders Mol Endocrinol, November 2015, 29(11):1535–1548*
tivity, has been described in circulating EVs, and EV adiponectin content is decreased in mice fed high-fat diets compared with control mice (119).

Comparative study of the RNA content of visceral adipose tissue (VAT)–derived EVs from obese and nonobese human subjects identified no mRNA, but abundant differentially expressed miRNA species. Follow-up pathway analysis revealed down-regulation of the miRNA species that suppress TGFβ and Wnt signaling in obese subjects. The association of TGFβ and Wnt signaling with inflammation has been well described, and these findings identify VAT EVs as a potential up-regulators of abnormal TGFβ and Wnt signaling (120). In aggregate, these results raise the possibility that adipose EVs act as effectors in the systemic response to obesity-related comorbidities.

This proposition is strongly supported by the fact that VAT EVs have the ability to cause systemic inflammation and insulin resistance. Analysis of EV isolates from VAT of leptin-deficient ob/ob and high-fat diet mouse models of obesity and insulin resistance revealed that the obese-origin VAT released increased EVs compared with controls. Furthermore, intravenous injection of obese VAT EVs into wild-type mice increased serum IL-6 and TNFα levels and induced glucose intolerance and insulin resistance (121).

Further study demonstrated that within 24 hours of intravenous injection, fluorescently labeled VAT-derived EVs were internalized by a subpopulation of CD11bF4/80+ monocytes. These monocytes had increased expression of ICAM receptors and MHC II molecules compared with that of monocytes that did not internalize obese VAT EVs. In vitro treatment of bone marrow precursor cells with obese VAT EVs increased macrophage differentiation and secretion of inflammatory cytokines. Because injection of recombinant RBP-4 protein recapitulated the obese VAT EV-induced phenotypes, these effects were thought to be partially due to serum RBP-4 protein contained in obese VAT EVs. All effects were reduced in TLR-4 and Toll-IL-1 receptor domain-containing adaptor protein inducing interferon-β knockout tissues, pointing to TLR-4 and Toll-IL-1 receptor domain-containing adaptor protein inducing interferon-β as key regulators of these processes (121).

Comparison of EVs from human samples of subcutaneous adipose tissue (SAT) and VAT demonstrated that both SAT and VAT EVs induce differentiation of macrophages with gene expression profiles consistent with proinflammatory and antiinflammatory adipose tissue macrophages (ATMs). Treatment of adipocytes with macrophages induced by either SAT or VAT-derived EVs increased insulin resistance (117, 122). ATM EVs also probably contribute to this process; EVs released by proinflammatory ATMs are internalized by adipocytes and induce insulin resistance via activation of nuclear factor-κ B signaling (123).

Adipose EVs may also have an impact on other organs affected in metabolic syndrome. In a small cohort, treatment of liver cells and muscle cells with either human SAT or VAT EVs tended to induce insulin resistance although neither effect was statistically significant. Interestingly, in some cases, EVs from the same subject had opposite effects on liver vs muscle tissue (117). Total EVs generated by VAT correlated with measurements of homeostasis model assessment-insulin resistance and elevations in liver function tests, reflecting a relationship with whole-body insulin resistance and hepatic dysfunction (113, 117). PCR performed on obese VAT-treated hepatocyte and hepatocyte stellate cell lines revealed alterations in multiple TGFβ pathway genes (112). These results point to EVs as a direct link between VAT and the insulin resistance, systemic inflammation, and TGFβ dysfunction characteristic of metabolic syndrome and nonalcoholic fatty liver disease.

The role of skeletal muscle–derived EVs in obesity and insulin resistance has also been investigated. Compared with that of controls, the skeletal muscle of mice treated with a high-palmitate diet released increased total EVs. When cocultured with muscle cells, these EVs were internalized and increased recipient cell proliferation and Akt levels but had no effect on insulin resistance. Lipidomic analysis of muscle cell lines incubated with EVs from palmitate-treated cells revealed an increase in recipient cell palmitate. Microarray showed that treated cells had an increase in cell cycle, mitosis, and DNA metabolic processing gene expression, but decreased expression of genes regulating cell adhesion, differentiation, development, and protein metabolic processing (124). This work suggests that although EVs derived from lipotoxic skeletal muscle are able to transfer lipids and affect gene regulation of recipient muscle cells, they are not directly responsible for induction of insulin resistance.

Work to date has demonstrated that adipose-derived EVs can activate ATMs, inducing local and systemic inflammation, as well as directly induce insulin resistance in target tissues. Given the wide circulation of EVs and the multifaceted cross talk between TGFβ and Wnt signaling and other cardinal biochemical pathways, future studies of great interest include (1) elucidating wider effects on target tissues in vitro and in vivo and their role in the pathophysiology of obesity-related disorders and (2) identifying roles of EVs from other affected tissues. In addition, studies using greater numbers of human samples will be necessary to decrease and understand the
causes of intersubject variability in the functional effects of adipose tissue EVs.

**Circulating EVs in Obesity, Metabolic Syndrome, and Diabetes**

Increased circulating EVs have been described in models of obesity and insulin resistance. In rats, high-fat diet treatment increased plasma phosphatidylserine-positive EVs of leukocyte, endothelial, and platelet origin. Interestingly, treatment of cardiac endothelial cells with obese circulating EVs increased endothelial vascular cell adhesion molecule 1 expression and reactive oxygen species production, suggesting that circulating EVs may contribute to the cardiovascular complications associated with obesity (125).

Several studies have examined circulating EVs in humans with obesity, metabolic syndrome, and/or diabetes (126, 127). Severe obesity increases circulating EVs independent of metabolic syndrome (128). Circulating EV miRNA profiles of 219 participants with either metabolic syndrome, T2D, hypercholesterolemia, or hypertension, showed that each disorder had its own specific EV miRNA profile (126).

Increased total circulating EVs, including those of leukocyte, platelet, and endothelial origins, have been described in T1D and T2D patients. Changes in platelet-derived EVs occurred independently of obesity, were associated with dyslipidemia, and decreased with antiplatelet therapy (129–133). Likewise, significant differences exist in circulating EV surface marker profiles between T1D and T2D patients: EVs of endothelial origin, platelet origin, and apoptotic bodies were shown to be elevated in T1D, whereas only apoptotic bodies were increased in T2D. In T1D patients, increased procoagulant activity of apoptotic vesicles was present and correlated with hemoglobin A1c and the presence of albuminuria; this was not found in T2D patients (134).

In contrast, other studies have described elevated levels of leukocyte- and platelet-derived, tissue factor–positive EVs in T2D subjects. These tissue factor–positive EVs did not increase coagulation but correlated with numerous functional and inflammatory markers of the metabolic syndrome (135). miRNA profiling of plasma exosomes in eighteen patients with T2D, revealed a significant dysregulation of 25 miRNAs, which was independent of body mass index, age, or sex. Specifically, EV miR-326 inversely correlated with circulating adiponectin, a known target of miR-326 (127).

Circulating EVs have also been linked to macrovascular and microvascular dysfunction in human metabolic syndrome and diabetes. Plasma EV markers associated with vascular disease correlate with a diagnosis of metabolic syndrome or increased C-reactive protein levels (113). Increased circulating endothelial cell–derived EVs have been linked to clinical measures of arterial stiffness in T1D and T2D patients (136, 137). T1D patients with microvascular complications also had increased levels of endothelial cell–derived EVs (134).

Therapeutic interventions have the ability to reverse some disease-induced changes in circulating EVs. Along with reductions in body mass index and hemoglobin A1c, T2D patients undergoing bariatric surgery showed significantly reduced circulating monocyte-, endothelial cell-, and platelet-derived EVs. Improvement in T2D-related hyperglycemia with oral diabetes medications normalized the circulating EV miRNAs let-7a and let-7f (127). It is unclear whether these changes are merely reflective of improvement in underlying diseases, have implications in terms of circulating EV contributions to disease pathophysiology, or, most likely, represent a combination of the 2.

The above findings contain some discrepancies, probably due to variations in subject characteristics, techniques for EV isolation/characterization, and identification of EV parent cells. However, these studies share a common finding: metabolic syndrome and diabetes lead to alterations in circulating EVs that have the ability to affect target organs on a systemic level. Further investigation into the functional effects and other sources of circulating EVs in subjects with diabetes or metabolic syndrome might provide insight into their physiologic significance.

**Conclusions**

Despite its status as a relatively young field, EV research has already yielded enormous insights into the normal physiology and pathophysiology of many organ systems. Clearly, the β-cell and maintenance of glucose homeostasis are affected. Further investigations into the mechanisms behind EV release, recipient cell targeting, and EV-cell interactions will lead to a better understanding of EV contributions to normal β-cell physiology, β-cell dysfunction, and systemic insulin resistance. Identification of parent cells and target cells of systemic EVs will improve specificity as biomarkers and yield insights into functional effects. One can also envision potential therapeutic applications of EV-mediated delivery of tolerizing agents to immune cells or antiapoptotic agents to β-cells. All of these possibilities point toward EVs as a new direction in the world of diabetes research.
Acknowledgments

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This work was supported by the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health (Grant K08DK103983 and Pilot and Feasibility Award P30 DK097512 to E.K.S.) and the Pediatric Endocrine Society (Clinical Scholar Award to E.K.S., and Grant T32DK064466 to A.J.L.).

Disclosure Summary: The authors have nothing to disclose.

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