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PATHOGENESIS OF TYPE 1 DIABETES (A PUGLIESE AND SJ RICHARDSON, SECTION EDITORS)

Extracellular Vesicles in Type 1 Diabetes: Messengers and Regulators

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Abstract

Purpose of Review Theories about the pathogenesis of type 1 diabetes (T1D) refer to the potential of primary islet inflammatory signaling as a trigger for the loss of self-tolerance leading to disease onset. Emerging evidence suggests that extracellular vesicles (EV) may represent the missing link between inflammation and autoimmunity. Here, we review the evidence for a role of EV in the pathogenesis of T1D, as well as discuss their potential value in the clinical sphere, as biomarkers and therapeutic agents.

Recent Findings EV derived from β cells are enriched in diabetogenic autoantigens and miRNAs that are selectively sorted and packaged. These EV play a pivotal role in antigen presentation and cell to cell communication leading to activation of autoimmune responses. Furthermore, recent evidence suggests the potential of EV as novel tools in clinical diagnostics and therapeutic interventions.

Summary In-depth analysis of EV cargo using modern multi-parametric technologies may be useful in enhancing our understanding of EV-mediated immune mechanisms and in identifying robust biomarkers and therapeutic strategies for T1D.

Keywords Type 1 diabetes · Extracellular vesicles · Islets of Langerhans · β-cell injury · Autoimmunity · Biomarkers

Introduction

Type 1 diabetes (T1D) is a chronic autoimmune disease that causes severe loss of the pancreatic beta (β) cells. Islet autoimmunity is typically marked by the presence of circulating autoantibodies against glutamic acid decarboxylase 65 (GAD65), insulin (IAA), insulinoma antigen-2 (IA-2), and zinc transporter 8 (ZnT8) [1–4]. The appearance of one or more of these autoantibodies often precedes the clinical onset of T1D by months or years [5]. At least one of these four autoantibodies is present in > 95% of de novo patients with T1D [6]. Genetic susceptibility, environmental triggers, and epigenetic changes have been postulated to contribute to disease susceptibility, but the identification of specific triggers has never progressed beyond the level of associations [7]. Extensively studied and potential environmental triggers include viral and bacterial pathogens, toxins, and nutrients [8–10]. While the genetic predisposition of individuals with T1D has been extensively studied, with several HLA and non-HLA loci contributing to disease susceptibility [11–15], the connection between heredity and environment, which ultimately determines disease phenotype, remains unclear.

Strong evidence supports the implication of islet inflammation in T1D, including reports of lymphocytic infiltration of both T and B cells in the islets of deceased donors with T1D and evidence of innate immune activation, with monocyte, macrophage, and polymorphonuclear leukocyte infiltration in patients with recently diagnosed T1D [5, 16–19]. Emerging evidence suggests that β cells play a central role in triggering autoimmune responses by releasing aberrant antigens under inflammatory conditions. β cells are particularly sensitive to endoplasmic reticulum and oxidative stress that are known to introduce alternative splicing, misfolding of proteins, and various post-translational modifications leading to aberrant expression of modified proteins or neoantigens [20]. Studies have reported that modified proteins or peptides may escape immune tolerance and trigger activation of highly

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immunogenic autoreactive T and B cells leading to destruction of β cells in T1D [21–24].

Over the last decade, the concept of extracellular vesicles (EV) as critical mediators of cell to cell communication has garnered great interest from the scientific community. Accumulating evidence suggests that EV may play an important role in the establishment, progression, and modulation of autoimmune processes, and may play a role in the presentation of autoantigens to the immune system. Here, we provide an overview of the current state of knowledge of the role of EV in the context of T1D (Fig. 1).

Extracellular Vesicles

EV are small, membrane bound structures that are released by most, if not all, cell types under physiological and pathological conditions. They have been detected in a wide range of culture supernatants and biological fluids including plasma, urine, saliva, synovial fluid, cerebrospinal fluid, and tears [25]. Due to their ability to co-transport multiple cellular contents, they are uniquely able to communicate a "snapshot" of their cell of origin.

EV can be broadly classified into three groups based on their mode of biogenesis: exosomes, microvesicles, and apoptotic bodies, although isolating and characterizing individual subtypes represents an ongoing challenge in the field [26]. Exosomes represent the smallest vesicles, ranging from 30 to 120 nm in size and are released upon fusion of multivesicular bodies with the plasma membrane. Microvesicles are up to 1000 nm in size and are known to be released via direct blebbing from the plasma membrane. Finally, apoptotic bodies are the largest EV, with a diameter ranging from 1000 to 5000 nm. They are released by cells undergoing active apoptosis and may contain large cellular organelles including nuclear fragments [27]. While the reports summarized in this review have variably used the above terms, we will, for simplicity, refer to all vesicle types as EV.

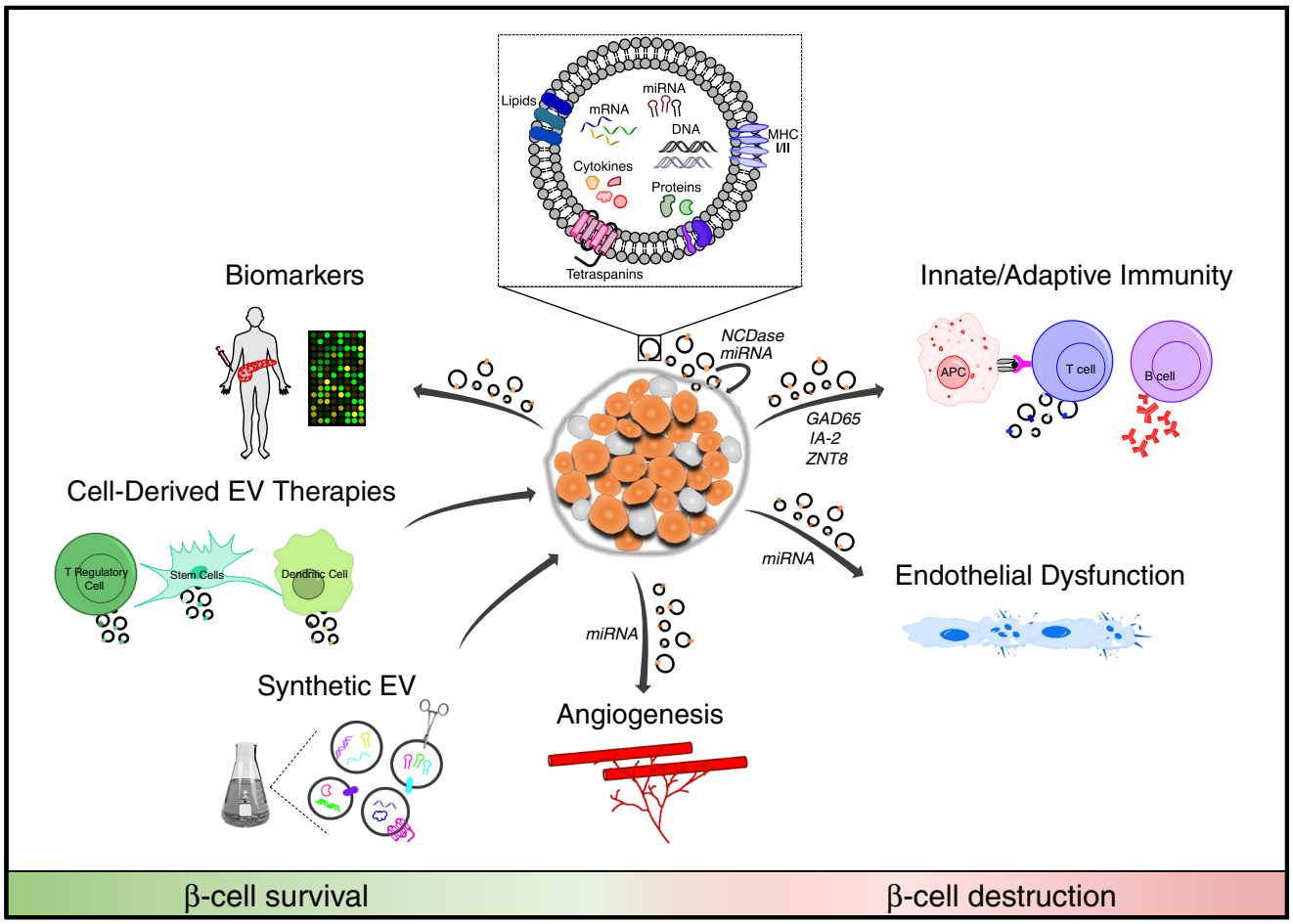

Fig. 1 Schematic representation of the implications of extracellular vesicles in type 1 diabetes (T1D). β cells release extracellular vesicles (EV) containing antigens, proteins, miRNA, etc. that are involved in islet cell cross-talk, innate and adaptive immune mechanisms, endothelial dysfunction, and angiogenesis. Moreover, EV derived from T regulatory cells, mesenchymal stem cells, or dendritic cells, as well as synthetic EV, may serve as potential therapeutic agents in T1D. Importantly, the molecular content of EV represents potential biomarkers for diagnostic and prognostic applications

Independently of their biogenesis, the composition of EV includes specific proteins, lipids, nucleic acids (including DNA, RNA, and miRNA), and active metabolites from their cell or tissue of origin [28]. This cargo content has been shown to vary depending on specific disease status or stimuli during formation. Once released, EV cargo can enter the cytoplasm or nucleus of recipient cells and modify their function and phenotype, thereby influencing their biological behavior.

Composition of β Cell-Derived EV

Christagau et al. first demonstrated in 1992, by electron microscopy, that the GAD65 autoantigen is released by rat pancreatic β cells in "small vesicles" that resemble neuronal synaptic vesicles [29]. To our knowledge, this represents the first evidence suggesting that pancreatic β cells release EV harboring β cell-specific antigens. Since then, EV release by β cells has been characterized in insulinoma cell lines, rat, mouse, and human islets [30–34]. EV derived from NIT-1 and INS-1 cells have been characterized, and specific proteins including those involved in metabolic pathways, endocytosis/exocytosis, membrane transport, and signaling have been identified [30]. Furthermore, it has been shown that insulinoma cell-derived EV contain β cell-specific markers including GAD65, insulin, and IA-2 [31]. Purification and characterization of EV released from islets has revealed the presence of common EV markers such as CD9, CD63, CD81, and flotillin 1 [32, 34]. Additionally, these EV are enriched in specific proteins such as GAD65, IA-2, ZnT8, insulin, C-peptide, and GLUT-2 as well as islet-specific transcription factors [32, 33, 35]. It is noteworthy that EV released from cultured human islets have lower levels of glucagon (GCG), CD31, and endothelial-nitric oxide synthase at both protein and RNA levels, indicating that they are mainly derived from β cells, rather than alpha (α) cells or other phenotypes, reflecting the physiological architecture of the islets [36]. Sorting of these EV will be promising to characterize all islet cell types in depth, including α and β cells, in order to monitor changes throughout T1D progression. In addition to protein cargo, islet EV have been shown to contain specific small RNAs and miRNAs implicated in β-cell function, insulin secretion, and angiogenesis [36, 37].

Proinflammatory cytokines have been shown to be implicated in the destruction of pancreatic β cells in T1D [38, 39]. In vitro treatment with proinflammatory cytokines to mimic the inflammatory milieu of early T1D can trigger modifications in EV phenotype. In the early 1990s, electron microscopy of rat pancreata perfused with IL-1β revealed "bleb-like cytoplasmic protrusions," consistent with what would later be identified as EV [40]. Upon exposure to proinflammatory cytokines (IL-1β, IFN-γ), rat insulinoma cells released EV with increased levels of proteins involved in the TNF-α and ICAM-1 signaling cascades. Furthermore, cytokine-treated cells expressed specifically higher levels of N-linked sialylated glycopeptides, such as CD82 and ICAM-1 [41]. Inflammatory cytokines are known to induce multiple post-translational modifications of signaling molecules associated with β-cell function and cell-cell interaction [42–45]. Later studies demonstrated that cytokine stimulation with a low concentration of proinflammatory cytokines, which triggers ER stress, augmented EV expression of highly neutral ceramidase (NCDase) and immunogenic chaperones including GP96, calreticulin, and ORP150; in contrast, common EV markers such as CD9 and CD81 remained constant [32, 46]. Interestingly, no increase in concentrations of GAD65, IA-2, and insulin was detectable, suggesting that ER stress causes trafficking of selective immunostimulatory chaperones into β cell-derived EV [32]. It would be of interest to investigate whether cytokine treatment leads to aberrant expression and post-translational modifications of autoantigens within EV, which may serve as vehicles to deliver neoantigens to trigger autoreactive responses. Furthermore, cytokine-mediated inflammatory stress can also modify the miRNA profile of EV. Exposure of MIN6 cells to a high dose of proinflammatory cytokines did not affect the total number of EV released but upregulated miRNA cargo associated with cell death [37]. Cytokine treatment of islets resulted in an increase in EV miR-375-p and miR-21-5p, miRNAs known to be associated with T1D [34, 47, 48••]. In summary, it is evident that environmental signals result in changes in the molecular profile of EV. Unique EV phenotypes may reprogram target cells and mediate diverse physiological functions, thereby influencing their survival.

EV as Immune Modulators

EV have gained considerable interest as mediators in the pathophysiology of autoimmune diseases. Depending on the nature of the parent cell and environmental stimuli, EV package and transport selective molecular content and display a range of functions, resulting in activation or suppression of immune responses. It is evident that islet EV express several immunomodulatory signals along with T1D autoantigens and diverse stimuli can modify both EV release and content. Here, we discuss how islet EV may modulate immune responses and participate in the establishment of autoimmunity in T1D.

Role in Antigen Presentation

The hallmark of T1D is the production of an autoreactive cellular and humoral response, the presence of a destructive insulitis, and the appearance of antibody and T cell specificities to β-cell autoantigens. In this process, the presentation of autoantigens to autoreactive T cells is central [24, 49]. A study of six adult patients with recent onset T1D showed that intra-islet dendritic cells (iDC) are enriched in insulitic lesions [50].

Several murine models have confirmed that iDC contain high levels of insulin-like granules, islet antigens, and/or antigen-MHC complexes [23, 51–53] and that they can activate autoreactive T cells and trigger insulitis [54, 55]. β cells have been shown to transfer insulin-containing vesicles to phagocytes in their vicinity [56]. Generally, dendritic cells (DC) or macrophages acquire donor cell antigens via phagocytosis of apoptotic cells; however, iDC retain islet peptides even in steady-state conditions; β-cell death or inflammation is not required for acquisition of islet peptides [52, 53]. This suggests that other modes of antigen delivery alternative to phagocytosis of apoptotic cells are likely implicated in disease initiation and progression. EV seem to be an ideal candidate to transport unique signals which can be recognized by immune surveillance systems to initiate environment-dependant autoreactive or regulatory immune responses.

Several recent studies have established the role of EV in antigen presentation and in activation of immune responses in T1D. EV released from cultured human islets, expressing autoantigens, are internalized by monocytes, leading to activation of DC, T cells, and B cells supporting the role of EV in conveying islet antigens to DC and eliciting immune responses in vitro [32, 57••]. We recently demonstrated that in vitro human islet-derived EV are capable of activating memory T and B cells and inducing the production of GAD65 antibodies by T1D PBMC, indicating the natural specificity of islet EV and possible role in autoimmune propagation [57••]. Similarly, murine models have revealed that immunization with insulinoma derived EV increases the percentage of EV-reactive B cells and induces insulitis in mice [31, 58]. Taken together, these studies suggest the potential of EV in mediating delivery of T1D antigens to DC and triggering autoreactive T and B cell responses.

Furthermore, EV released by DC and B cells express HLA and costimulatory molecules and can elicit immune responses via direct antigen presentation [59–61]. Hyperexpression of HLA class I molecules and aberrant expression of HLA class II molecules on β cells have been reported in both recent onset and long-standing T1D pancreata [62–65]. Moreover, inflammatory cytokines induce the expression of HLA class I and HLA class II molecules on β cells in vitro [66–69]. HLA class I molecules have been detected on EV isolated from human islets [70]; however, expression of HLA class II molecules on islet EV remains to be investigated. Since EV are highly stable in the circulation, it is plausible that islet EV transport antigen/HLA complexes to the regional lymph nodes, directly interacting with autoreactive T and B cells.

Role in Cellular Communication

Emerging evidences suggests that EV play an important role as mediators of intercellular communication. This is thought to be due to their contribution in both autocrine and paracrine signaling via horizontal transfer of their cargo. Depending on the physiological state of the donor cell, EV may exert activating or inhibitory effects on target cells. Specific EV cargo can act as signaling molecules and can induce phenotypic and functional changes in the recipient cells [28, 71–73]. The role of EV in inducing immune responses has been explored in other autoimmune contexts which has been shown to be mediated through different EV components such as immune complexes, proteasomes, or miRNA [74–76].

In particular, intercellular communication between β cells could be critical for a coordinated response of β cells to metabolic stressors, or other environmental conditions. A dominant-negative HNF1A mutation induced apoptosis of INS-1 cells and release of annexin V-positive EV. Exposure of these EV to wild-type cells induced genes associated with regenerative pathways and proliferation [77]. In another study, EV-mediated miRNA transfer between β cells was shown to trigger phenotypic changes in the recipient cell. EV isolated from cytokine-treated MIN6 cells induced apoptosis in recipient β cells that could be prevented by silencing of Argonaute 2, a gene known to be essential for miRNA function [37]. In addition to RNA, other components of EV have also been implicated in cell to cell communication; one study has highlighted the role of ceramidase, critical regulators of stress-induced apoptosis, in EV-mediated cross-talk. Low dose cytokine treatment stimulated the release of neutral ceramidase (NCDase) via EV whereas high dose of cytokines inhibited their secretion in INS-1 cells. Moreover, NCDase-carrying EV exerted a protective effect on apoptosis induced by a high dose of inflammatory cytokines in INS-1 cells [46]. Elevated levels of cytokines have been shown to induce β-cell apoptosis whereas the lower levels improve insulin biosynthesis and promote proliferation [78–80]. It is noteworthy that the divergent responses of β cells to different doses of cytokines are sustained by their EV, resulting in counterregulatory effects in the recipient cells.

Furthermore, EV-mediated intercellular communication may also promote angiogenesis and influence stem cell differentiation. The conditioned medium from cultured rat islets has been demonstrated to induce proliferation and migration of liver and islet endothelium [81]. Another study showed that human islet-derived EV shuttle their contents including several mRNAs and miRNAs to intra-islet endothelial cells (iEC), triggering proangiogenic and antiapoptotic pathways in iEC [36]. EV released from cultured islets are also capable of affecting the stem cell differentiation process. EV derived from both mouse β cells and human islets promote differentiation of therapeutic β cells suggesting their significance in stem cell technology [35, 82].

A recent study explored the contribution of EV in paracrine signaling: activated mouse and human T lymphocytes released miRNA-containing EV which can be delivered to MIN6 cells or human islets. These miRNAs remain

functionally active, upregulate genes associated with cytokine and chemokine signaling, and trigger β-cell apoptosis. [83]. Thus, EV-mediated miRNA transfer from islet infiltrating lymphocytes to β cells during the early phase of autoimmune diabetes appears to play a role in disease progression. Likewise, EV derived from islet mesenchymal stem cell (MSC)-like cells from NOD mice activate autoreactive T and B cells and accelerate insulitis, suggesting the presence of innate stimuli capable of inducing inflammation [84].

Few studies have shown that circulating EV in T1D are inflammatory in nature. EV isolated from T1D patient plasma have been shown to impair islet function and reduce their insulin secretion [85]. Plasma derived EV from patients with diabetes were shown to be enriched in cytokines and other bioactive molecules and induce inflammatory response in endothelial cells [86, 87]. Similarly, circulating EV from diabetic rats were shown in many studies to be proinflammatory and to induce endothelial dysfunction [88–91]. The intercellular communication between β cells and endothelial cells is of interest as intra-islet microvasculature is critical for balancing islet pathophysiology [92, 93]. Taken together, these findings indicate that EV-mediated activation is bidirectional and, depending on the inflammatory environment, may initiate both pro- and anti-inflammatory responses.

EV as Clinical Tools in T1D

EV have recently emerged as potential diagnostic tools that may lead to the identification of novel biomarkers and therapeutic targets. Furthermore, EV have remarkable ability to deliver functional molecules to specific targets, and therefore hold strong therapeutic potential. Here, we summarize the current knowledge of EV in diagnosis and therapeutics applications, including emerging EV-based therapies.

Diagnostic Potential of EV as Biomarkers

Currently, autoantibodies are the most widely accepted predictor of T1D; however, they do not necessarily represent a method of universal monitoring. Screening autoantibodies has identified individuals at risk for T1D among patients' relatives; those with one autoantibody have low risk, while 40–80% of relatives with more than one autoantibodies develop T1D within 5–10 years [5]. Autoantibody titers are not necessarily predictive of disease progression which may vary from months to decades [94–96]. As well, autoantibody patterns are dynamic; up to 60% of persons with a single autoantibody may revert to seronegativity [94]. Moreover, some individuals develop T1D with no detectable autoantibodies [97]. Thus, the development of novel biomarkers would be a valuable tool to predict, treat, and monitor T1D progression and its associated complications. The development of EV-based blood biomarker tools for tissue-specific disease is particularly attractive because of their relative ease of detection and the potential to develop a single assay multi-marker panel.

Relative quantities and phenotype of EV have been compared between T1D and control subjects. Patients with established T1D have higher levels of total circulating EV and endothelial- and platelet-derived EV and have variations in their procoagulant activity indicating the existence of differential EV profiles in T1D [98]. EV miRNA represent a more specific tool for biomarker detection as they are enclosed by lipid membranes, and therefore stable in circulation, whereas circulating miRNA may be subjected to degradation by RNase [99, 100]. As recently reviewed, circulating serum levels of 11 miRNAs are consistently dysregulated in T1D, including miR-21-5p, miR-24-3p, miR-100-5p, miR-146a-5p, miR-148a-3p, miR-150-5p, miR-181a-5p, miR-210-5p, miR-342-3p, miR-375, and miR-1275 [101]. The Ricordi group recently reported that in long-standing T1D, plasma EV samples showed upregulation of one miRNA (miR-25-3p), while six others were downregulated (miR-16, miR-302d-3p, miR-378e, miR-570–3p, miR-574-5p, miR-579) as compared to controls [85]. Interestingly, high levels of miR-25-3p have been shown in new-onset T1D patient samples and its overexpression has been shown to inhibit insulin synthesis [102]. Furthermore, an increase in miR-21-5p was detected in circulating EV of newly diagnosed children with T1D as compared to non-diabetic controls and in the serum EV of prediabetic NOD mice [48]. In contrast, total serum miR-21-5p levels were lower in the same patients, suggesting that certain miRNA could be selectively packaged in EV. Furthermore, miR-375-3p was shown to be elevated in serum EV of STZ treated mice prior to hyperglycemia and in new-onset T1D patients [34]. These two studies indicate the translational relevance of the identified miRNA and their potential to serve as predictive markers. Several other studies have compared serum or urine EV miRNA and protein profiles in T1D and its associated complications and have shown that miR-145 and Wilm's tumor-1 may serve as markers to differentiate and predict diabetes-related complications [103, 104]. In another study, patients with T1D were found to have higher levels of total plasma EV as well as miR-21-3p and miR-30b-5p within EV which were further elevated in diabetic patients with retinopathy [105]. Collectively, these studies reveal strong evidence of altered EV cargo in the pathogenesis of T1D and its complications and the possibility of EV miRNA as clinically applicable biomarkers. Clearly, there is considerable variability in the findings in the reported studies. Reasons for these discrepancies may include variations in study populations and diverse methods of sample collection, storage, and processing [106, 107]. Large-scale studies should be conducted in newly diagnosed patients with T1D and their first degree relatives as well as autoantibody-positive and genetically susceptible individuals in order to find actionable EV-based

serum biomarkers using advanced technologies. Snowhite et al. have shown that differences exist in the miRNA profile of autoantibody-positive and negative siblings which correlate with disease progression, with limited predictive value. This strongly suggests the importance of conducting further longitudinal studies on T1D-susceptible individuals for biomarker development and in assessing the predictive accuracy of serum-EV biomarkers as compared to serum-based biomarkers [108].

In the context of islet transplantation, EV monitoring has been reported as a potential biomarker for graft function and loss. Toti et al. first showed that in the case of islet transplant rejection, recipient plasma EV increased which correlated with a decrease in C-peptide levels. A peak in EV was detected at the time of rejection. Furthermore, treatment of graft loss with a second islet infusion returned EV and C-peptide levels back to baseline, suggesting the possibility of EV as an indicator for graft rejection [109]. Another study explored donor-specific EV in islet transplantation. In all transplant recipients, donor islet EV are quantifiable at all time-points post-transplant using donor-specific HLA. In the case of rejection, a decrease in donor-specific signal preceded the increase in GAD65 autoantibody titers, while the C-peptide to glucose ratio remained constant, suggesting the potential for EV as an early graft rejection marker [70]. Further studies by this group showed a decrease in donor derived islet EV quantities in one patient immediately prior to graft rejection, specifically in insulin-containing EV. Additionally, they reported an increase in GAD65 containing donor EV which preceded the appearance of circulating GAD65 autoantibody levels [110]. These data suggest the potential of EV in routine, non-invasive graft monitoring in islet transplantation which could serve as useful markers in early diagnosis of graft injury, and thus provides opportunity for therapeutic intervention.

A deeper understanding of EV cargo using high-throughput methods will be useful for the screening of novel diagnostic proteins or miRNA biomarkers for T1D diagnosis, progression, and treatment. Methods of EV sample collection, isolation, and phenotyping must be further developed and standardized before the true potential of EV as biomarkers could be realized. Once further developed, we can envision the potential of EV and their cargo as liquid biopsies which could be analyzed through disease-specific microfluidic chips as promising non-invasive tools for diagnostic applications.

EV as Therapeutics Agents

Strategies to replace or regenerate β cells while inhibiting destructive autoimmune responses may allow for durable and robust treatment of T1D. MSCs are considered ideal candidates for cell-based therapies as they possess potential for regeneration, immunomodulation, and tissue repair [111, 112]. Generally, the benefits of MSC therapy are associated with attenuation of DC maturation and improvement in regulatory T cell function. Some preclinical and clinical studies using mesenchymal stem cells (MSC), regulatory T cells (Treg), or dendritic cells (DC) to treat T1D have led to promising results demonstrating safety and efficacy [113–117]. Likewise, EV from MSC, Treg, and DC have been shown to have regenerative and anti-inflammatory effects.

MSC-derived EV were shown to induce an anti-inflammatory milieu in T1D patients PBMC as they inhibit GAD65 antigen-driven T cell activation and IL-17 expression while inducing Treg activation [118–120]. Additionally, numerous studies have described the active release of immunosuppressive EV from Treg and DC [121–123] that may show similar beneficial effects in T1D. MSC-derived EV have displayed the potential to ameliorate various diabetic complications such as nephropathy, retinopathy, and cognitive impairment in animal models [124–126]. Furthermore, EV derived from endothelial progenitor cells (EPC) can enhance both insulin secretion and islet viability [127]. Several clinical trials are in progress or underway in order to investigate the safety and efficacy of EV as therapeutic agents. A registered clinical trial, NCT02138331, exploring the potential of MSC-EV for the treatment of T1D is currently in progress. Taken together, these data suggest that MSC/Treg/DC-derived EV exhibit immunomodulatory actions corresponding to their parent cell, and may represent an attractive therapy to protect or restore β-cell mass and prevent diabetes associated complications, while reducing risks associated with cell therapy.

Therapeutic activity of EV can be enhanced as they are amenable to modifications by several methods. By manipulating parent cells, EV can be loaded with desired bioactive molecules including protein, DNA, or RNA. Overexpression of siFas and anti-miR-375 in MSC was able to deliver functional copies of these RNA via EV to human islets thereby silencing expression of Fas and miR-375. Fas and miR-375 have been associated with islet cell death and blocking their expression has been shown to improve the viability and function of human islets [128]. A significant challenge in developing synthetic EV lies in the fact that, generally, many mammalian cells secrete very small amounts of EV. Recently, a novel strategy has been developed whereby large amounts of EV-mimetic nanovesicles (NV) can be prepared from cells by serial extrusion. These EV-mimetic NV have similar functional characteristics as natural EV and can deliver their cargo to target cells. EV-mimetic NV from TNF-α treated MIN6 cells were shown to induce differentiation of bone marrow cells into therapeutic insulin-producing cells [82]. Moreover, cells could be engineered to produce EV-mimetic NV carrying desired bioactive molecules for targeted delivery [129]. Several challenges must be resolved before EV can be used as effective therapeutic agents. EV secreted by cells are heterogenous in nature in terms of both size and content; it is likely that these EV subpopulations also differ in their therapeutic potential.

Currently, identification and purification of EV subpopulations that may be crucial for their function remains challenging. More complex technologies capable of separating EV phenotypically such as small particle cell sorting will significantly advance the field and potential therapeutic applications. Additionally, another important challenge in the field includes targeted delivery of EV to specific cells or tissues rather than systemic delivery. Few recent studies have demonstrated that EV surfaces can be modified to enhance their targeting ability [130–132].

Fully synthetic EV directly loaded with bioactive molecules or drugs could be fabricated in large scale using bionanotechnological tools and could provide a novel platform for drug delivery [133–135]. Lewis et al. were able to engineer synthetic microparticles carrying either vitamin D3, insulin peptide, TGF-β1, or GM-CSF and their administration prevented development of diabetes in NOD mice [136]. Synthetic EV carrying the essential components required for targeted delivery and therapeutic action may prove to be an important modality for targeted delivery of these molecules. Further research to improve the synthesis, delivery, targeting, and clearance of EV are required for their development as powerful tools for therapeutic interventions.

Conclusions

The discovery and growing interest in EV may have a profound effect on experimental and clinical medicine and could pave the way for new strategies to both diagnose and treat various conditions. Thus far, EV research has provided important insight into the pathophysiology of T1D. Emerging evidence suggests a role for EV in β-cell homeostasis and function. The interplay between EV, their environment, and their target cells can disrupt the balance between regulatory and inflammatory signals that can perpetuate autoimmune processes leading T1D development. The importance of EV in T1D pathogenesis provides exciting future opportunities for multiple novel EV-based therapeutic strategies for treating T1D and other conditions.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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