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Nanoparticle delivery of donor antigens for transplant tolerance in allogeneic islet transplantation

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ABSTRACT

Human islet cell transplantation is a promising treatment for type 1 diabetes; however, long-term donor-specific tolerance to islet allografts remains a clinically unmet goal. We have previously shown that recipient infusions of apoptotic donor splenocytes chemically treated with 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide (donor ECDI-SP) can mediate long-term acceptance of full major histocompatibility complex (MHC)-mismatched murine islet allografts without the use of immunosuppression. In this report, we investigated the use of poly(lactide-co-glycolide) (PLG) particles in lieu of donor ECDI-SP as a synthetic, cell-free carrier for delivery of donor antigens for the induction of transplant tolerance in full MHC-mismatched murine allogeneic islet transplantation. Infusions of donor antigen-coupled PLG particles (PLG-dAg) mediated tolerance in ~20% of recipient mice, and the distribution of cellular uptake of PLG-dAg within the spleen was similar to that of donor ECDI-SP. PLG-dAg mediated the contraction of indirectly activated T cells but did not modulate the direct pathway of allorecognition. Combination of PLG-dAg with a short course of low dose immunosuppressant rapamycin at the time of transplant significantly improved the tolerance efficacy to ~60%. Furthermore, altering the timing of PLG-dAg administration to a schedule that is more feasible for clinical transplantation resulted in equal tolerance efficacy. Thus, the combination therapy of PLG-dAg infusions with peritransplant rapamycin represents a clinically attractive, biomaterials-based and cell-free method for inducing long-term donor-specific tolerance for allogeneic cell transplantation, such as for allogeneic islet transplantation.

1. Introduction

Cell therapies are currently a billion dollar market with significant growth expected [1,2]. Applications that are being developed commercially include cell immunotherapies, hematopoietic progenitor cells, chondrocytes for cartilage repair, and keratinocytes for wound healing [1,3]. While autologous cells are ideal to avoid immune rejection, allogeneic sources are attractive for diseases in which autologous cells are not readily available, such as type 1 diabetes (T1D) or trauma (e.g., spinal cord injury) [4]. Allogeneic islet cell transplantation is a promising treatment for type 1 diabetes and exemplifies the potential of the approach [4]. However, life-long immunosuppressive drugs are currently used for allogeneic islet cell transplantation to prevent rejection, which can be harmful to the transplanted islets and lead to the non-specific suppression of the entire host immune system [5]. The non-specific effects of these drugs result in enhanced patient susceptibility to opportunistic infections and/or an increased risk of neoplasia.

An alternative to systemic immunosuppression is the induction of donor-specific tolerance, for which successful regimens have been

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developed in small and large animal models, as well as in human clinical trials [6–8]. One type of tolerance approaches involves conditioning the recipient pre- and post-transplant with infusions of donor cells administered in combination with co-stimulation blockade [9] or apoptotic donor cells in the absence of additional treatment [10]. We have previously demonstrated that treatment of donor splenocytes (SP) with the chemical crosslinker 1-ethyl-3-(3′-dimethylaminopropyl)-carbodiimide (ECDI) induces apoptosis, and infusions of such donor ECDI-SP mediate long-term donor-specific allograft acceptance in full MHC-mismatched allogeneic islet transplantation models [10,11]. Mechanistic studies revealed that in the treated recipients, apoptotic ECDI-SP regulate both directly- and indirectly-activated T cells through anergy and deletion, respectively, accompanied by an induction of regulatory T cells (Treg) [11]. Despite the success of this method, several limitations challenge its direct translation to clinical applications. Inherent difficulties in adapting cell-based therapies to humans include complex *ex vivo* human cell manipulation, donor-to-donor variability, and stringent safety considerations for clinical applications.

A promising alternative to donor splenocytes for recipient preconditioning is to employ synthetic particles as the carrier of soluble donor antigens obtained from processed donor cells. This approach would allow for a storable form of donor antigens for the manufacturing of therapeutic products for tolerance induction, hence significantly expanding the applicability of this approach to include deceased donor organ transplantation and facilitate repetitive tolerance boosters post-transplant if needed. Synthetic particles can also be manufactured reproducibly, further providing a platform upon which modifications can be made to enhance the therapeutic efficacy of this tolerance approach. Recent work by our lab and collaborators has demonstrated that biodegradable poly(lactide-co-glycolide) (PLG) particles, with an approximate diameter of 500 nm, are capable of effectively delivering peptide antigens to mediate tolerance to autoimmunity in both preventative and therapeutic models of relapsing-remitting experimental autoimmune encephalomyelitis (R-EAE) [12]. PLG has been shown to be a safe delivery system in preclinical models [13–15], and has also been approved by the FDA for a number of therapeutic applications [16–18].

In this report, we investigated the ability of PLG particles carrying allogeneic donor antigens for transplant tolerance induction in full MHC-mismatched allogeneic islet transplantation models. Based on our prior work showing the efficacy of donor ECDI-SP in inducing transplant tolerance in the same models, the current study investigated the efficacy of replacing splenocytes with soluble donor antigens coupled to PLG particles in the establishment and maintenance of long-term tolerance in allogeneic islet transplantation. Initial studies focused on optimizing donor antigen (dAg) coupling to the PLG particles (PLG-dAg). We subsequently investigated the long-term protection of transplanted islet allografts provided by the PLG-dAg and characterized the potential mechanisms of their protection. Our studies thus provide the basis for the future development of synthetic particles for transplant tolerance induction that will likely have a broader impact on cell therapies beyond that for allogeneic islet cell transplantation.

2. Materials and methods

2.1. Mice

Eight to ten week old male BALB/c (H-2^d), C57BL/6 (B6, H-2^b), and SJL/J (H-2^s) mice were purchased from the Jackson Laboratory and Harlan. 4C mice were provided by Dr. Qizhi Tang from the University of California at San Francisco. TCR75 mice were provided by Dr. Anita Chong from the University of Chicago. All mice were housed under specific pathogen free conditions at Northwestern University and protocols were approved by Northwestern University IACUC.

2.2. Islet transplantation

Mice were treated with streptozotocin (Sigma–Aldrich) at 170 mg/kg. Two consecutive glucose readings (1 day apart) greater than 250 mg/dl were used to determine diabetes. The protocol for islet isolation and transplantation has been described previously [19]. Approximately 500 islets were implanted under the kidney capsule of recipient mice. Graft rejection was determined by two consecutive blood glucose readings greater than 250 mg/dL.

2.3. Antibodies and FACS analysis

Cell phenotype was measured by flow cytometry. Isolated cells were stained with fluorochrome-conjugated antibodies for 30 min on ice, washed, read on the FACSCanto II (BD) and analysed using FlowJo v6.4.7 (TreeStar). The following antibodies (clones) were used: CD11c-APC (HL3), I-A^b-PE and CD90.1-PerCPCy5.5 (OX-70) from BD Biosciences, and CD11b-eFluor780 (M1/70), F4/80-PerCPCy5.5 (BM8), B220-PECy7 (RA3-6B2) and CD8α-eFluor450 (53-6.7) from eBiosciences.

2.4. Preparation of donor antigens from donor cells

Donor BALB/c splenocytes were processed into single cell suspensions and eyrthrocytes lysed. Up to 1×10^9 cells were sonicated twice in PBS at an amplitude of 30% for 20 s, followed by 30 s at 60% amplitude (Cole–Parmer). Total protein was quantified by the Coomassie Plus (Bradford) Protein assay (Thermo Fisher Scientific Inc.) prior to coupling to PLG particles.

2.5. PLG particle synthesis

Single emulsion poly(lactide-co-glycolide) (PLG) particles were synthesized with poly(ethylene-alt-maleic acid) (PEMA) as a surfactant as described in Ref. [20]. Briefly, PLG (50% D,L-lactide/50% glycolide) (Lactel Absorbable Polymers) was dissolved in dichloromethane to make a 20% (w/v) solution. This solution was sonicated (Cole–Parmer) at 16 W in 1% w/v PEMA (Polysciences, Inc.) to create particles. After overnight stirring, particles were collected by centrifugation, washed 3 times with 1 M Sodium Bicarbonate buffer, and lyophilized overnight with 4% w/v sucrose and 3% w/v D-mannitol.

2.6. Particle characterization

Particles were imaged with a scanning transmission electron microscope (Hitachi HD2300 Field Emission STEM) operating at 200 kV. Particles were drop casted on 400 mesh Cu/Rh grids containing a carbon membrane and negatively stained with 1% UA in ddH_2O. Particle size and surface ζ-potential distributions were obtained using dynamic light scattering on a Zetasizer Nano ZSP (Malvern Instruments Ltd).

2.7. Preparation of donor antigen-coupled particles (PLG-dAg) and ECDI-SP

PLG particles, 3.0 mg, were washed 3 times to remove sugars from lyophilization and incubated for 1 h with stirring with 30 mg/ml 1-Ethyl-3-(3′ dimethylaminopropyl) carbodiimide, HCl (ECDI) (EMD Millipore Chemicals, Inc.) and 1200 μg lysate (from 2 donor spleens) per dose. Coupled particles were washed twice to remove excess ECDI and filtered through a 40 μm cell strainer (BD Falcon). Lysate coupling efficiency was determined by quantifying remaining protein in supernatants after the coupling reaction using the Coomassie Plus (Bradford) Protein assay (Thermo Fisher Scientific Inc.). Donor ECDI-SP were prepared as previously described [10]. Briefly, splenocytes were incubated with ECDI (Calbiochem, every 3.2×10^8 cells in 1 ml of DPBS (Life Technologies, Grand Island, NY) with a final concentration of 30 mg/ml of ECDI) on ice for 1 h with agitation on a shaker (Labline Instruments Inc., Melrose Park, IL) followed by washing.

2.8. Tolerance induction by PLG-dAg

PLG-dAg (3.0 mg) or control blank PLG particles (3.0 mg) were injected i.v. into recipient B6 mice on day −7 and day +1 with reference to islet transplantation (on day 0). Rapamycin (rapa) (Enzo Life Sciences, Inc.) was dissolved in 0.2% carboxymethyl cellulose solution and sonicated prior to each intraperitoneal injection of 0.1 mg/kg on days −1, 0, +1, and +2.

2.9. PKH67 labeling of ECDI-SP and PLG-dAg

For tracking studies, donor (BALB/c) ECDI-SP were labeled with 2 μM PKH67 (Sigma–Aldrich) according to manufacturer's instructions. Briefly, 2×10^7 cells were resuspended in 1 ml Diluent C and mixed with 1 ml Diluent C containing 4 μl PKH67 dye before washing 3 times in RPMI 1640 (Gibco) supplemented with 10% fetal calf serum (Gibco). To track PLG-dAg, donor SP were labeled with PKH67 as above, sonicated, and then coupled to PLG particles with ECDI as above. 1×10^8 donor ECDI-SP^{PKH67+} or 3.0 mg PLG-dAg^{PKH67+} were injected i.v. to B6 mice. After 18, 48 or 96 h, mice were sacrificed and organs were isolated, processed to single cell suspensions, and $PKH67^+$ cells were analysed by flow cytometry (BD FACSCanto II).