Immune Rejection after Pancreatic Islet Cell Transplantation: In Vivo Dual Contrast-enhanced MR Imaging in a Mouse Model¹

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Purpose:

To detect adoptively transferred immune attack in a mouse model of islet cell transplantation by using a long-circulating paramagnetic T1 contrast agent, a protected graft copolymer (PGC) that is covalently linked to gadolinium-diethylenetriaminepentaacetic acid with fluorescein isothiocyanate (Gd-DTPA-F), which accumulates in the sites of inflammation that are characterized by vascular disruption.

Materials and Methods:

All animal experiments were performed in compliance with institutional guidelines and approved by the subcommittee on research animal care. Six nonobese diabetic severe combined immunodeficiency mice received transplanted human islet cells under the kidney capsule and adoptively transferred 5×10^6 splenocytes from 6-week-old nonobese diabetic mice. These mice also served as control subjects for comparison of pre- and postadoptive transfer MR imaging results. Mice that received phosphate-buffered saline solution only were included as nonadoptive-transfer control subjects ($n = 2$). In vivo magnetic resonance (MR) imaging was performed before and 17 hours after intravenous injections of PGC-Gd-DTPA-F, followed by histologic examination. Statistical differences were analyzed by means of a paired Student *t* test and repeated two-way analysis of variance.

Results:

MR imaging results showed significantly greater accumulation of PGC-Gd-DTPA-F in the graft area after immune attack initiated by adoptive transfer of splenocytes compared with that of the same area before the transfer (T1, $137.2 \text{ msec} \pm 39.3$ and $239.5 \text{ msec} \pm 17.6$, respectively; $P < .001$). These results were confirmed at histologic examination, which showed considerable leakage of the contrast agent into the islet cell interstitium.

Conclusion:

PGC-Gd-DTPA-F-enhanced MR imaging allows for the in vivo assessment of vascular damage of the graft T cell challenge.

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Islet cell transplantation has emerged as a promising therapeutic approach for type 1 diabetes mellitus with the advent of the Edmonton Protocol (1). However, the long-term survival and function of islet grafts may be compromised by a number of factors. Early losses of islet cell function and mass are primarily driven by the damage sustained during the isolation procedure, ischemia reperfusion–like injury, and other nonspecific inflammatory phenomena (2). Other causes of loss of function include allogeneic rejection, recurrence of autoimmunity, and immunosuppressive drug toxicity (3–5).

Current tools for the immune monitoring of islet cell grafts usually allow identification of graft dysfunction when it is too late to take action, and therefore, cannot be used to guide clinical intervention (2,6). A reliable, noninvasive, real-time monitoring tool is needed to allow for earlier and more accurate detection of harmful events. In addition, timely detection of immune-mediated rejection in the clinical setting would facilitate prompt intervention and treatment of acute immunologic events. Regardless of the nature of the immunologic events after transplantation, microvascular endothelium and endothelial cells have been shown to be primary targets of activated leukocytes. By using intravital microscopy, Menger et al (7) showed a reduction in size of the

microvascular network and an increase in microvascular permeability, capillary widening, intravascular accumulation of white blood cells, and finally, breakdown of the integrity of the microcirculatory system after xenogenic islet transplantation. Immunosuppressive drugs that prevent leukocyte accumulation in the graft also prevent endothelial damage and capillary perfusion failure (8).

Our group showed that noninvasive imaging techniques could have potential for the detection of various pathologic events associated with diabetes mellitus development, such as lymphocyte infiltration (9,10) and β -cell death (11). Furthermore, we developed an MR imaging approach that allows for monitoring of vascular changes in endogenous islet cells in patients with type 1 diabetes mellitus by using a protected graft copolymer (PGC) that is covalently linked to gadolinium-diethylenetriaminepentaacetic acid labeled with fluorescein isothiocyanate (Gd-DTPA-F) (12). PGC mechanism of action includes passive targeting to sites of enhanced vascular permeability because of openings in the blood vessels that make them porous to large molecules followed by extravasation to the interstitial area (13). Our study results showed changes in vascular permeability associated with early stages of type 1 diabetes mellitus in several animal models at PGC-Gd-DTPA-enhanced MR imaging (12,14). The goal of this study was to detect an adoptively transferred immune attack in a mouse model

of islet cell transplantation by using the long-circulating paramagnetic T1 contrast agent PGC-Gd-DTPA-F which accumulates in the sites of inflammation characterized by vascular disruption.

Materials and Methods

Imaging Agent

PGC is based on a conjugate of a polylysine backbone to which methoxypoly (ethylene glycol) chains are covalently and randomly linked via *N*- ϵ -amino groups (15). PGC-bearing covalently linked gadolinium–diethylenetriaminepentaacetic acid residues conjugated to the fluorescent dye fluorescein isothiocyanate (Gd-DTPA-F) was obtained from PharmaLN (Seattle, Wash).

The contrast agent has an average calculated molecular weight of approximately 430 kDa as determined by means of gel filtration. The diameter of PGC-Gd-DTPA is $16 \text{ nm} \pm 4$ in phosphate-buffered saline solution with a pH of 7 at 25°C as determined by means of submicron particle analysis. The agent has an r_1 value in the range of $2000\text{--}2500 \text{ L} \cdot \text{mmol}^{-1} \cdot \text{sec}^{-1}$ in water (13,15). It has been previously used in imaging studies in animal models of cancer (16),

Advance in Knowledge

- Vascular leakage in transplanted islet cells can be identified by using a long-circulating paramagnetic T1 contrast agent, which passively accumulates in the sites of inflammation characterized by vascular disruption. MR imaging demonstrated a significantly greater accumulation of contrast agent in the graft area after immune attack initiated by adoptive transfer of splenocytes compared with that of the same area before the transfer (T1 values were $137.2 \text{ msec} \pm 39.3$ and $239.5 \text{ msec} \pm 17.6$, respectively; $P < .001$).

Implications for Patient Care

- Noninvasive assessment of vascular damage could aid in monitoring posttransplant events.
- A real-time monitoring approach can be used for the detection of the early stages of the rejection cascade and can provide an important opportunity for the prompt rescue of the graft, potentially benefiting the clinical outcome of islet cell transplantation in patients with type 1 diabetes mellitus.

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Abbreviations:

DM = diabetes mellitus

Gd-DTPA-F = gadolinium–diethylenetriaminepentaacetic acid labeled with fluorescein isothiocyanate

PGC = protected graft copolymer

Author contributions:

Guarantors of integrity of entire study, J.F.M., A.M.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; approval of final version of submitted manuscript, all authors; literature research, P.W., C.S., J.F.M., A.M.; clinical studies, J.F.M.; experimental studies, P.W., C.S., A.R., G.D., A.M.; statistical analysis, P.W., C.S.; and manuscript editing, P.W., C.S., A.R., A.M.

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Conflicts of interest are listed at the end of this article.

See also Science to Practice in this issue.

inflammation (17), arthritis (18), and diabetes mellitus (12,14).

Animals and Treatment

All animal experiments were performed in compliance with institutional guidelines and were approved by the Subcommittee on Research Animal Care at Massachusetts General Hospital. Human islet cells were obtained from the Integrated Islet Distribution Program centers (including the Clinical Islet Transplantation centers, and centers funded by the Juvenile Diabetes Research Foundation). The viability and purity of the islet cells were both higher than 85%. On arrival at our facility, islet cells were placed in a cell culture medium (CMRL-1066; GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum and 100 mg/mL penicillin-streptomycin. Human pancreatic islet cells were implanted under the left kidney capsule (1500 islet equivalents per kidney) of 6-week-old female nonobese diabetic severe combined immunodeficiency mice ($n = 6$).

To induce immune rejection in transplanted islet cells, the grafts were challenged with adoptively transferred splenocytes. This animal model mimics immune rejection of the xenografts, in which human islet cell grafts are severely challenged by exposure to a sudden onslaught of primed T cells that were isolated from the spleens of immunocompetent mice (19,20). For this procedure, splenocytes from 6-week-old nonobese diabetic mice were isolated ($n = 8$) by mashing spleens through the cell strainer followed by purification on a magnetically activated cell-sorting column (Miltenyi Biotec, Auburn, Calif). After separation, the lymphocyte compartment of splenocytes was evaluated by means of fluorescence-activated cell sorting with phycoerythrin-labeled rat antimouse CD8 monoclonal antibody (BD Biosciences, Bedford, Mass) and fluorescein isothiocyanate-labeled antimouse CD3 monoclonal antibody (Cedarlane laboratories, Hornby, Ontario, Canada). Data on purity and content of the transferred cell fraction are presented in Figure E1 (online).

Three to four weeks after islet cell transplantation, 5×10^6 of the isolated

splenocytes (naïve cells) were adoptively transferred to the recipients. These six animals also served as control subjects for comparison between imaging of pre- and postadoptive transfer grafts. Two mice that received only phosphate-buffered saline solution were included as nonadoptive transfer control subjects. All the islet cell transplantation and splenocyte adoptive transfer experiments were performed by P.W. (radiologist), and C.S. (surgeon), who had 8 and 10 years of experience, respectively.

In Vivo MR Imaging

To monitor rejection-induced microvascular changes in the islet cell graft, we used the long-circulating paramagnetic T1 contrast agent PGC-Gd-DTPA-F, which accumulates in islet cell interstitium after leakage through porous vasculature. In vivo MR imaging was performed 1 day before and 7 days after adoptive transfer. During each session, the six animals were imaged before and 17 hours after intravenous injection of fluorescein-labeled PGC-Gd-DTPA-F (0.2 mmol Gd/kg) on a 9.4-T horizontal bore imaging unit (Bruker, Billerica, Mass) equipped with a home-built radiofrequency transmit-receive 3×4 -cm elliptical surface coil and ParaVision 5.1 Software (Bruker). T1 maps were acquired by using a rapid acquisition with relaxation enhancement inversion recovery sequence with the following parameters: echo time, 7.253 msec; repetition time, 10000 msec; inversion time, 0.001, 200, 400, 800, 1600, 3200, and 6400 msec; field of view, 25.6×25.6 mm²; spatial resolution, 0.2×0.2 mm · pixel⁻¹; matrix size, 128×128 ; section thickness, 1 mm; rapid acquisition with relaxation enhancement factor (number of echoes), 16; reconstruction matrix size, 128×128 ; and total imaging time, 16 minutes and 5 seconds. At the same time points, T1-weighted images of the same mice were obtained by using a multisection multiecho sequence with the following parameters: echo time, 7.841 msec; repetition time, 100 msec; field of view, 25.6×25.6 mm²; spatial resolution, 0.2×0.2 mm · pixel⁻¹; matrix size, 128×128 ; section thickness, 1 mm;

number of echoes, 1; reconstruction matrix size, 128×128 ; and total imaging time, 3 minutes and 24 seconds. For quantitative analysis of T1 relaxation, T1 color-coded maps were constructed by using software (Marevisi 3.5; Institute for Biodiagnostics, National Research Council, Winnipeg, Manitoba, Canada). T1-weighted images were analyzed on a voxel-by-voxel basis by fitting the T1 measurements in a standard exponential decay curve, defined by the following equation: $y = A[1 - 2 \cdot \exp(1 - t/T1)]$. The islet cell graft-associated T1 relaxation times were analyzed by manually drawing a region of interest around the grafts and averaging the T1 relaxivity of the voxels. For the time-course analysis of T1 relaxation times in transplanted islet cells, animals that received adoptively transferred splenocytes ($n = 3$) were imaged before PGC-Gd-DTPA-F injection and 1 hour, 17 hours, and 40 hours after injection. Precontrast T1 maps and PGC-Gd-DTPA-F-enhanced T1 maps were obtained and analyzed as described above. MR imaging experiments were primarily performed by P.W. and A.R. (animal technologist who has 5 years of experience). One author (P.W.) identified the regions of interest in a blinded manner.

Dual Contrast Agent-enhanced MR Imaging of the Islet Cell Graft Rejection

To demonstrate colocalization of the graft site with the PGC-Gd-DTPA-F-enhanced signal intensity on MR images, human pancreatic islet cells were labeled with superparamagnetic dextran-coated iron oxide nanoparticles as described previously (21), and transplanted to the area under the left kidney capsule (1500 islet cell equivalents per kidney) of 6-week-old female nonobese diabetic severe combined immunodeficiency mice ($n = 2$). Three to four weeks after transplantation, animals received 5×10^6 splenocytes that were adoptively transferred from 6-week-old nonobese diabetic mice. One day before and 7 days after adoptive transfer, animals were imaged with a 9.4 T MR imaging unit. Gradient echo T2*-weighted sequence fast low angle shot images were obtained with the following parameters: repetition time,

150 msec; echo time, 15 msec; field of view, $3.2 \times 3.2 \text{ cm}^2$; matrix size, 128×128 ; averages, eight; section thickness, 1 mm; number of echoes, one; spatial resolution, $0.25 \times 0.25 \text{ mm} \cdot \text{pixel}^{-1}$; reconstruction matrix size, 128×128 ; and total imaging time, 2 minutes and 33 seconds. Next, precontrast T1-weighted images and PGC-Gd-DTPA-F-enhanced T1-weighted images were obtained. Overlay of T1-weighted and T2*-weighted images was performed with software (SPOT 4.0 advanced version; Diagnostic Instruments, Sterling Heights, Mich). The colocalizations of the images were evaluated by P.W., and A.M. (20 years experience in molecular imaging).

Histologic Examination

Left kidneys from experimental and control animals were removed immediately after the second imaging session, embedded in paraffin, and cut into 5- μm sections. After antigen retrieval, tissue sections were blocked in a solution of 5% normal goat serum in phosphate-buffered saline and were stained for insulin by incubation with a rabbit antihuman insulin primary antibody (1:100 dilution; Santa Cruz Biotechnology, Santa Cruz, Calif), followed by a Texas red-labeled goat antirabbit secondary immunoglobulin G (IgG) antibody (1:100 dilution, Santa Cruz Biotechnology, Santa Cruz, Calif), and mounted with a 4,6-diamidino-2-phenylindole-containing mounting medium (Vectashield, Vector Laboratories, Burlingame, Calif) for nuclear staining. Fluorescence microscopy was performed in three channels (fluorescein isothiocyanate for PGC-Gd-DTPA-F, Texas red for insulin, and 4,6-diamidino-2-phenylindole for nucleus) by using a fluorescence microscope (Eclipse 50i; Nikon Metrology, Brighton, Mich). Images were acquired by using a charge-coupled device camera (SPOT 7.4 Slider RTKE; Diagnostic Instruments) and analyzed by using software (SPOT 4.0 advanced version; Diagnostic Instruments). All the tissue sections were evaluated by observers blinded to the treatments. Evaluation was performed primarily by P.W. and A.M.

Figure 1

Figure 1: PGC-Gd-DTPA-F-enhanced T1-weighted images of islet grafts under the kidney capsule obtained 17 hours after injection of contrast agent in mice that received islet cell transplants and adoptively transferred splenocytes. (a) No enhancement was observed before adoptive transfer. (b) After transfer, islet cell graft area under left kidney capsule is clearly enhanced in experimental mice (arrow). (c) No enhancement was seen in control mice.

To verify the presence of the adoptively transferred splenocytes, consecutive kidney sections were blocked in a solution of 5% normal goat serum in phosphate-buffered saline. One section was stained for insulin with rabbit antihuman insulin polyclonal antibody (1:200 dilution) followed by a Texas Red-labeled goat antirabbit secondary IgG antibody (1:100 dilution). For T cell staining, separate sections were incubated with rat antimouse anti-CD3 monoclonal antibody (1:50 dilution; Abcam, Cambridge, Mass) followed by an Alexa Fluor 350 conjugated secondary goat antirat IgG antibody (1:50 dilution; Invitrogen, Carlsbad, Calif). For endothelial-cell staining, separate sections were treated with rabbit antihuman CD34 monoclonal antibody or rat antimouse CD34 monoclonal antibody (1:100 dilution; Abcam). After incubation with a Texas red conjugated goat antirabbit secondary IgG antibody

(1:100 dilution) or Alexa Fluor 350 conjugated goat antirat secondary IgG antibody (1:50 dilution), the sections were analyzed by using fluorescence microscopy.

Statistical Analysis

Data were presented as means $\pm$ standard deviation. Statistical differences were analyzed by means of the paired Student *t* test (GraphPad Prism 5; GraphPad Software, La Jolla, Calif). For the time-course analysis, the repeated two-way analysis of variance was applied, followed by Bonferroni multiple comparison adjustment (GraphPad Prism 5; GraphPad Software). A value of $P < .05$ was considered to indicate a statistically significant difference.

Results

In Vivo Imaging of Islet Cell Graft Rejection

As shown in Figure 1a no changes in signal intensity on T1-weighted images in the graft region under the kidney

Figure 2: Analysis of T1 relaxation times of graft. **(a)** T1 color-coded map obtained before and 7 days after adoptive transfer of splenocytes shows accumulation of contrast agent in graft area under kidney capsule (arrow) after transfer. Colored bars show scale of T1 relaxation time. **(b)** Bar graph shows significant difference in T1 relaxation times in graft area between pre- and posttransfer of splenocytes ($239.5 \text{ msec} \pm 17.6$ and $137.2 \text{ msec} \pm 39.3$, respectively; $P < .001$) but no difference in T1 values in control animals. **(c)** Graph shows time-course analysis of T1 relaxation times of graft region and significant difference in T1 between pre- and post-transfer values, which was especially pronounced at 17 hours after injection of PGC-Gd-DTPA-F, reflecting higher accumulation of contrast agent in damaged grafts ($n = 3$). $* = P < .05$ at 1 hour or 40 hours after contrast agent injection, $** = P < .01$ at 17 hours after contrast agent injection. Complete view of axial MR images is shown in Figure E2 (online).

capsule that would be indicative of vascular leakage were noted before T-cell transfer. However, high signal intensity due to the T1-shortening effect of Gd-DTPA was detected after adoptive transfer (Fig 1b). As expected, control animals showed no changes in signal intensity after PGC-Gd-DTPA-F injection before or after administration of phosphate-buffered saline solution (Fig 1c; Fig E2d [online]).

T1 Relaxation Time Analysis in Islet Cell Grafts

To quantify signal intensity changes in animals into which splenocytes were adoptively transferred, we constructed

color-coded T1 maps (Fig 2a). Region-of-interest analysis showed that the T1 relaxation times of the graft area after adoptive transfer were significantly lower than those before the transfer ($137.2 \text{ msec} \pm 39.3$ and $239.5 \text{ msec} \pm 17.6$, respectively; $P < .001$; Fig 2b). In control animals, the T1 values remained unchanged from pre- to post-adoptive transfer ($230.0 \text{ msec} \pm 28.85$ and $232.0 \text{ msec} \pm 12.65$, respectively). Time-course analysis of T1 relaxation times in transplanted islet cells showed vascular enhancement as early as 1 hour after injection both before and after the transfer that reflected the presence of the contrast agent in the blood

pool. However, in animals that received transferred splenocytes, there was substantial leakage of the agent, which was shown by a prominent drop in T1 (Fig 2c). The peak of contrast-agent bioavailability (and hence the signal enhancement) in the grafts was reached 17 hours after injection, followed by a gradual washout by 40 hours.

In Vivo Confirmation of the Signal Localization

T1-weighted images of the animals injected with PGC-Gd-DTPA-F before adoptive transfer showed no enhancement (Fig 3a). However, after adoptive splenocyte transfer, PGC-Gd-DTPA-F

Figure 3

injection caused notable high intensity of the graft area on T1-weighted images (Fig 3b). This signal colocalized with a signal from a distinct hypointense region on T2*-weighted images produced by iron oxide-labeled grafts, which confirms that area of vascular dysfunction defined by PGC-Gd-DTPA-F originated from the islet cell graft underneath the kidney capsule (Fig 3c). T1-weighted images of the labeled grafts before PGC-Gd-DTPA-F injections produced no notable T1 effect (Fig E4 [online]).

Histologic Correlation of the Imaging Data

Infiltration of T cells into islet cell grafts (Fig E5 [online]) that ultimately led to vascular damage identified by means of PGC-Gd-DTPA-F (Fig E5 [online]) was confirmed histologically. Figure 4 demonstrated substantial leakage of PGC-Gd-DTPA-F into the islet cell interstitium after adoptive transfer. Kidney

graft sections of control animals showed that the agent was restricted to the kidney vasculature outside of the graft or at the graft periphery. Staining for endothelial marker CD34 on donor and host endothelial cells demonstrated that cells from both sources were involved in the forming of new blood vessels in the islet cell graft area (Figs E6, E7 [online]). Staining of the consecutive sections revealed that, in the animals challenged with splenocytes, most of the agent was located in islet cell interstitium and not colocalized with blood vessels. Insulin staining confirmed CD34 staining localization to the grafts.

Discussion

Islet cell damage due to immunologic and nonimmunologic factors after transplantation is the main reason for graft failure in diabetic patients. The issue of immune rejection is a major

contributor to early posttransplantation islet cell loss (22). Previous studies have shown that 50% of islet cell xenografts are rejected 9 days after transplantation without immunosuppression in animal models (23). Current strategies for assessment of immune function that correlates with graft survival include detection of de novo alloimmune and autoimmune antibodies in graft recipients (24,25), and ex vivo monitoring of T cell reactivity against grafts after transplantation (26–28), and tissue biopsy. However, these assessments have several limitations. It remains unclear whether the detected antibodies are the cause or the consequence of graft damage (2). Long incubation times and low correlation with the clinical outcome are some of the disadvantages related to the T cell-based assays (2). Tissue biopsy is the standard method for accessing immune cell infiltration in the graft, but the method is both invasive and inadequate when temporal information is needed to characterize the immune reaction that progresses dynamically over time (6). Noninvasive assessment of this damage would greatly aid in monitoring recurrent posttransplant events and in devising therapeutic interventions. The goal of this study was to detect vasculature leakage as a biomarker of graft damage due to immune rejection by using the long-circulating blood pool contrast agent PGC-Gd-DTPA-F, which can penetrate through the damaged parts of the endothelium and accumulate in the interstitium (29).

To model recurrent immune damage in islet cell grafts, we adoptively transferred splenocytes from nonobese diabetic mice to nonobese diabetic severe combined immunodeficiency mice that received transplanted human islet cells underneath the kidney capsule. This was done 3–4 weeks after transplantation, which ensured the establishment of the graft at the time of the transfer. Noninvasive assessment of the graft vasculature by using PGC-Gd-DTPA-F before the T-cell challenge showed that the vasculature was intact judging by the absence of leakage in the graft area. However, 7 days after the challenge, there

Figure 4: (a, b) Fluorescence microscopic images of islet cell grafts after adoptive transfer shows considerable leakage of contrast agent (green) into the islet cell interstitium. (c, d) However, in control animals, PGC-Gd-DTPA-F was mainly restricted to kidney vasculature at islet cell graft periphery. Red = insulin; green = PGC-Gd-DTPA-F; blue = cell nucleus. Scale bar in a, c = 100 μm; scale bar in b, d = 40 μm.

was a noticeable change in T1 relaxation times in the grafts, which indicated the presence of vascular leakage. Concurrently, histologic studies confirmed that accumulation of the contrast agent was restricted to the graft area in challenged animals, whereas in control animals, vascular enhancement of the kidney parenchyma was not accompanied by interstitial accumulation of the agent in the islet cells. We found that the time course of PGC-Gd-DTPA-F accumulation and washout in transplanted grafts was very similar to the pattern of the agent kinetics observed in endogenous islet cells (12). In both cases, accumulation of the agent was especially pronounced at 17 hours after injection followed by a gradual washout by 40 hours, which

confirmed the potential versatility of our approach.

Although we expected that after PGC-Gd-DTPA-F administration the signal would originate in the islet cell graft area of the animals that received transferred splenocytes, T1-weighted images alone do not provide information about the precise localization of that signal. To prove that the enhancement on T1-weighted images was caused by PGC-Gd-DTPA-F accumulation that originated from the transplanted grafts, we used dual contrast-enhanced MR imaging of the animals that received adoptively transferred T cells. Before transplantation, pancreatic islet cells were labeled with T2 contrast agent (superparamagnetic iron oxide

nanoparticles), which produces signal loss on T2-weighted images (21). Multiple previous studies have proved the utility of superparamagnetic iron oxide nanoparticles for detection of transplanted islet cells by means of MR imaging in small and large animal models (21,30–32). In our study, a hypointense area on T2*-weighted images, which was produced by the T2-shortening effect of the iron particles, colocalized with hyperintense signal intensity from PGC-Gd-DTPA-F, which confirmed that the islet cell graft served as a target for the T-cell challenge and resulted in vascular damage. These results attest to the feasibility of dual-contrast agent MR imaging for monitoring islet graft survival and possible immune events.

Revascularization of the islet cell graft included endothelial cells of the donor (33,34) and intraislet endothelial cells from the transplant recipient, which may account for up to 40% of the endothelial cells lining capillaries in a revascularized graft (34,35). These functionally integrated vessels are involved in the revascularization of pancreatic islet cells after transplantation and are believed to be an additional source of nutrients (33). In our study, we confirmed that the cells from both origins contributed to PGC-Gd-DTPA-F accumulation in the islet cell graft interstitium. This finding is important because it attests to the utility of this contrast agent in the definition of areas of vascular damage in islet grafts regardless of the origin of the vasculature.

In our previous studies, we used superparamagnetic iron oxide nanoparticles for labeling pancreatic islet cells before transplantation (21). Immune rejection of islet cell grafts in immunocompetent animals was evident at MR imaging because the gradual disappearance of the islet cell-associated hypointense signal intensity (21). However, this method only allowed direct visualization of graft destruction at a late stage of immunologic rejection. In a clinical setting, a method for detecting immunorejection at an early stage, which we tried to establish in this study, would allow for the prompt intervention in acute immunologic events.

Our study had limitations. Our sample size was small ($n = 6$). In addition, vascular leakage could be observed in other inflammatory conditions. In addition, the histologic and qualitative localization process was subjective in nature. Finally, in clinical scenarios, islet cell grafts are susceptible to allo-rejection, recurrence of autoimmunity, or a combination of both. Here, we demonstrated the feasibility of dual-contrast in vivo MR imaging for the detection of the damage caused by adoptively transferred host-versus-graft xenorejection. We plan to further validate this noninvasive imaging tool in allo- or autoimmune rejection animal models.

Overall, we believe that this real-time MR imaging monitoring approach

can be used to detect the early stages of the rejection cascade, and thus, can provide an opportunity for the prompt rescue of the graft, which could result in higher rates and greater durability of insulin independence in patients with type 1 diabetes mellitus. Importantly, this noninvasive technique may also be applied for detection of the early rejection events in other models of solid organ transplantation.

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