Ultraviolet Light Immunomodulation of Canine Islets for Prolongation of Allograft Survival

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Ultraviolet (UV) light treatment of donor islets has been shown to be effective for the prolongation of islet allograft survival in rodent models. This study evaluated UV as an immunomodulator of canine islets. The effects of UV irradiation on islet secretory function in vitro revealed a trend of increasing basal insulin release with increasing doses of UV and a corresponding significant decrease in glucose-mediated insulin release (expressed as percentage of basal fractional insulin release) beginning at UV light exposures of 200–300 J/m^2 (n = 3, P < 0.05). Proliferative responses to UV-irradiated allogeneic peripheral blood leukocytes and islets were significantly decreased by 53–112% (P < 0.05) in 27 of 29 mixed-lymphocyte cultures and by 35–74% (P < 0.05) in 4 of 5 mixed-lymphocyte islet culture experiments, respectively, beginning at 200–600 J/m^2. Autotransplantation of nonirradiated (n = 8) and irradiated islets (600 J/m^2, n = 6) resulted in a 1-mo graft survival rate of 75% for the control group and 50% for the irradiated group. Allotransplantation of irradiated islets (600 J/m^2) into either nonimmunosuppressed recipients (1 donor to 1 recipient, n = 8) or recipients of subimmunosuppressive doses of cyclosporin (2 donors to 1 recipient, n = 4) resulted in 100% rejection by day 10. In contrast, when islets were cultured for 24 h postirradiation and transplanted into cyclosporin-treated pancreatectomized recipients (2 donors to 1 recipient), 3 of 7 grafts were prolonged beyond day 10 to days 16, 26, and >100. We conclude that direct UV irradiation of islets decreases canine islet immunogenicity and in combination with subtherapeutic dosages of cyclosporin can result in prolongation of islet allograft survival in this large-animal model. *Diabetes* 39:305–11, 1990

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Received for publication 23 August 1989 and accepted in revised form 30 October 1989.

Observations of ultraviolet (UV) light–induced alterations in the skin and the subsequent interest in the effects of UV light on the immune system have led to active investigation in the areas of photomedicine and photoimmunology (1–4). UV-irradiated lymphocytes do not stimulate mixed-lymphocyte culture (MLC) reactions (5), and UV irradiation of canine dendritic cells abrogates their ability to cluster with lymphocytes and induce mitogenesis (6).

Such observations and the idea that passenger leukocytes or antigen-presenting cells are responsible for allograft rejection (7–9) led Lau et al. (10,11) and Hardy et al. (12,13) to analyze the effectiveness of direct UV irradiation as an immunomodulator of rat islets before transplantation. A dose of UV light that consistently abrogated the MLC stimulatory capacity of donor-strain dendritic cells but that did not destroy the ability of UV-irradiated islets to reverse hyperglycemia in syngeneic diabetic rats was used (10–13). Although islet UV irradiation alone was sufficient to prolong graft survival for weakly allogeneic donor-recipient strains (10), stronger combinations required the brief peritransplant administration of cyclosporin A (CsA) to recipients (11–13).

In this study, we investigated the effectiveness of UV light as an immunomodulator of canine islets in an effort to define conditions that might consistently lead to long-term prolongation of allograft survival in unrelated beagles. Our results provide evidence that UV irradiation of islets, in combination with 24 h of postirradiation culture and recipient treatment with subimmunosuppressive doses of CsA, can prolong canine islet allograft survival. The prolongation observed is not as consistent as that seen with the use of UV irradiation in rodent models, and the possible reasons for this are discussed.

RESEARCH DESIGN AND METHODS

Male and female adult beagles, ranging in age from 6.5 to 14 mo and weighing 5.75–12.25 kg, were obtained from Marshall (North Rose, NY) to serve as donors and recipients.

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Heparinized peripheral blood for in vitro immunological studies was obtained by venipuncture.

Islet isolation and assessment. In a modification of the procedure previously described by our laboratory (14,15), enriched canine islets were obtained by ductal distension and digestion with collagenase (type V, Sigma, St. Louis, MO), followed by density-gradient centrifugation on bovine serum albumin (Sigma) gradients (16) in a Cobe 2991 blood processor (Denver, CO) (R.A., P. Zucker, D.H. Mintz, unpublished observations). The preparations consisted of 20–90% endocrine tissue, as assessed by dithizone staining (17), and nonendocrine tissue consisted of endothelial, ductal, and acinar tissues.

Immediately before transplantation, the enriched islet preparations were suspended in a total volume of 40 ml of tissue culture medium 199 (Gibco, Grand Island, NY) supplemented with 1× antibiotic-antimycotic (Gibco), and 0.5 ml (1/80 of preparation) was removed for assessment as previously described (16). Preparations were assessed for endocrine volume by counting the number of islet particles in the 30- to 50-, 51- to 100-, and 101- to 150-μm size range in a 20-μl aliquot (5 individual slides and minimum of 300 dithizone-positive isletlike particles counted to determine mean number of islets per group). For individual islet preparations, volume was determined with the formula $V = I \times \frac{4}{3}\pi(r^3)$, where radius (r) is equal to 20 μm for the 30- to 50-μm size islets, 37.5 μm for the 51- to 100-μm size islets, and 62.5 μm for the 101- to 150-μm size islets, and I is mean number of islets counted per group. These preparations were contained in a packed cell volume of <3 ml.

Islet cell transplantation. Recipients were pancreatectomized (18) and splenectomized, and intrahepatic islet auto- and allotransplantations were performed as previously described (15,19,20). The animals received cephalothin (Lilly, Indianapolis, IN) for 5 days postoperatively, and the transplanted dogs received i/d dog food (Hill Pet Products, Topeka, KS) supplemented with 3–5 tablets of Viokase-V (Robins, Richmond, VA). Fasting blood glucose (FBG) levels were monitored daily for the first 30 days postoperatively and weekly thereafter with a Beckman glucose analyzer (Fullerton, CA). Grafts that failed to reverse hyperglycemia for the first 2 days posttransplantation were regarded as technical failures and excluded from calculations of survival times. Thereafter, a graft was considered to have failed or undergone rejection when FBG rose >250 mg/dl for 2 consecutive days. CsA (Sandoz, Basel) was started (10 mg · kg^{-1} · day^{-1} i.m.) 5 days before transplantation and was maintained at a dose of 5–10 mg · kg^{-1} · day^{-1} for 30 days posttransplantation. This treatment protocol resulted in CsA trough levels of 100–300 ng/ml, a range that is not sufficient to prolong canine islet allograft survival (21). CsA levels were measured twice weekly with a radioimmunoassay kit with a polyvalent sheep anti-CsA serum (Sandoz). In conducting this research, we adhered to the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences, National Research Council.

Metabolic studies. At 1 mo posttransplantation, oral glucose tolerance tests (OGTTs), intravenous glucose tolerance tests, and glucagon tolerance tests (GTTs) were performed as previously described (22) on dogs that received nonirradiated and UV-irradiated (600 J/m^2) islet autografts. Blood samples were collected in tubes containing 500 KIU/ml Trasylol and 1.2 mg/ml EDTA and kept on ice until the plasma was separated. Plasma samples for immunoreactive insulin determination were stored at −70°C until assayed (22).

UV irradiation of peripheral blood leukocytes (PBLs) and islets. Leukocytes were suspended at a concentration of 2×10^6 cells/10 ml in Waymouth's medium (Gibco) supplemented with 15% normal dog serum (NDS; Colorado Serum, Denver, CO) and 1× antibiotic-antimycotic. For islets destined for transplantation, a packed-cell volume of 0.2 ml of islet preparation was suspended in 10 ml RPMI-1640 (Gibco) supplemented with 10% NDS and 1× antibiotic-antimycotic; islets for in vitro experiments were evenly divided into the needed number of aliquots and suspended in 10 ml medium. Ten-milliliter volumes of cells or islets were plated into 100 × 15-mm plastic petri dishes (Falcon, Becton Dickinson, Linden Park, NJ), and a stir bar was added to the dish to facilitate even distribution of cells during irradiation. The dishes were placed on a magnetic stir plate at a distance of ~10 cm from the UV source, which consisted of two Westinghouse FS-20 sun lamps that emit 60% of their energy in the 280- to 320-nm UVB range (13). The distance from the source was adjusted to deliver 10 J · m^{-2} · s^{-1} as determined with a UVX-Radiometer (Ultra-Violet Products, San Gabriel, CA). For in vitro experiments, islets and PBLs were irradiated at doses varying from 0 to 1500 J/m^2; the control group consisted of cells stirred in the absence of UV irradiation for the maximum amount of time of irradiation for that particular experiment. Islets destined for transplantation were stirred for 60 s without exposure to UV irradiation (control group) or with an exposure equivalent to 600 J/m^2 (UV group). Cells were subsequently washed and prepared for transplantation or in vitro experiments as described.

Canine islet monolayer cultures. Culture medium consisted of RPMI-1640 (11.2 mM glucose) supplemented with 10% NDS and 1× antibiotic-antimycotic. Islets used for in vitro experiments were *1*) irradiated on the day of isolation, cultured overnight, and plated the next day (experiment 3; Table 1) or *2*) isolated and cultured overnight, irradiated the following day, cultured an additional 24 h, and subsequently plated (experiments 1 and 2; Table 1). No consistent differences attributable to time of irradiation were detected with these two protocols. On the day of plating, UV-irradiated and control islets were dispersed into single-cell suspensions, resuspended at a concentration of 5×10^5 cells/ml in culture medium, and 20 replicates/UV dose (0.1 ml/well) were plated into 96-well half-area tissue-culture clusters (Costar, Cambridge, MA) as previously described (23). Dispersed islets consisted of >95% single cells, and viability, as determined by trypan blue exclusion, was >90% for islets UV irradiated with ≤800 J/m^2. Above 800 J/m^2, increasing cytotoxicity was evident. After 5–7 days, the newly established monolayer cultures were washed twice with RPMI-1640 containing 2.8 mM glucose, 1× antibiotic-antimycotic, and 10% NDS (basal medium) and subsequently incubated with basal medium for 2 h. After two further washes in basal medium, 10 wells were incubated in basal medium and 10 wells in medium containing 16.7 mM glucose to assess glucose-mediated insulin release at each UV dose that was tested. At the end of a 4-h incubation, medium was collected and

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TABLE 1
Effect of ultraviolet (UV) light on canine islet insulin release in vitro

UV dose (J/m²)	Fractional insulin release (mM) 2.8	16.7	Percent of basal release
Experiment 1			
0	3.1 ± 0.4	15.1 ± 1.0	488.1 ± 33.5
200	6.7 ± 0.8	22.4 ± 3.2	334.8 ± 47.8*
400	14.0 ± 1.3	21.8 ± 1.2	155.8 ± 8.6*
600	28.8 ± 8.1*	22.8 ± 1.8	79.2 ± 6.2*
800	33.2 ± 4.2*	25.0 ± 2.4	75.2 ± 7.3*
Experiment 2			
0	7.7 ± 1.1	29.3 ± 1.6	380.8 ± 20.9
200	13.6 ± 7.6	30.5 ± 1.7	224.1 ± 12.5*
400	7.8 ± 0.9	23.0 ± 1.2	294.9 ± 14.8*
600	11.5 ± 1.1	23.4 ± 1.4	203.8 ± 12.3*
800	20.4 ± 2.7	31.1 ± 1.8	152.6 ± 9.0*
Experiment 3			
0	6.5 ± 1.0	23.2 ± 1.2	356.9 ± 18.1
300	7.8 ± 1.1	14.1 ± 1.5	181.4 ± 18.5*
500	9.4 ± 1.3	14.8 ± 2.7	157.6 ± 29.2*
700	11.1 ± 1.3*	14.4 ± 2.7	129.6 ± 23.9*

Values are means ± SE.
*$P < 0.05$ vs. control (0 J/m²).

frozen at −20°C until assayed for immunoreactive insulin. The cultures were washed twice in basal medium, and acid alcohol was added for 18 h at 4°C to extract insulin for the subsequent determination of islet insulin content. Antiporcine insulin (Novo, Wilton, CT) and ^{125}I-labeled porcine insulin (Lilly) were used in a standard radioimmunoassay procedure to determine insulin concentration (24). Data (mean ± SE of data obtained for 10 replicate wells) were expressed as *1*) μU/ml insulin, *2*) fractional insulin release [(release/release + content) × 100], or 3) percentage of basal fractional insulin release [(stimulated fractional insulin release/basal fractional insulin release) × 100].

MLC and mixed-lymphocyte islet cultures (MLIC). Culture medium (CM) consisted of Waymouth's medium supplemented with 15% NDS, 1× antibiotic-antimycotic, 10 mM HEPES buffer (Gibco), 1× nonessential amino acids (Sigma), and 1× sodium pyruvate (Whittaker Bioproducts, Walkerville, MD). PBLs were isolated by diluting heparinized whole blood 1:2 with medium 199, layering 35 ml diluted blood over 15 ml Ficoll-Paque (Pharmacia, Piscataway, NJ) and centrifuging at 800 × *g* for 25 min. The cells at the interface were collected, washed 3 times with medium 199, resuspended in CM, and kept overnight at 4°C. PBLs were subsequently washed, UV irradiated as needed, and resuspended at a concentration of 10^6 viable cells/ml in CM. In experiment 1, PBLs were UV treated the day of isolation, kept overnight, and plated in MLC the subsequent day; alteration of PBL immunogenicity did not appear to be dependent on time of irradiation before plating. For the MLC, 10^5 responder PBLs were plated with 10^5 γ-irradiated (3000 rads) stimulator cells in quadruplicate or triplicate cultures in a total volume of 0.2 ml in a 96-well round-bottom tissue-culture cluster (Costar). Controls consisted of responder cells alone, responder cells versus γ-irradiated responder cells, responder cells versus unstirred/non-UV–treated stimulator cells, and responder cells versus non-UV–treated stimulators that had been stirred for an amount of time equal to that needed to deliver the maximum UV dose used in that particular experiment.

Islets used in the MLIC experiments were either *1*) UV irradiated on the day of isolation and plated the subsequent day for experiments 1 and 5 or *2*) isolated and cultured overnight, followed by UV irradiation and plating the subsequent day for experiments 2–4 (Table 2). No differences in the effectiveness of UV irradiation were detected with these methods of study. For the MLIC, islet stimulators were γ-irradiated (3000 rads), and 10^5 dispersed islet cells/well (experiments 2–4; Table 2) or 50 handpicked whole islets/

TABLE 2
Effect of ultraviolet (UV) irradiation on canine leukocyte and islet immunogenicity assessed by ability to stimulate proliferative responses

	Without UV irradiation		Initial effective dose of UV*				Effect of 600 J/m²		
	Δcpm†	SI	Δcpm	SI	UV dose (J/m²)	Percent decrease	Δcpm	SI	Percent decrease‡
Experiment 1									
MLC	5807	51.5	−70	0.4	300	101	31	1.3	99
MLIC	14,274	123.0	3743	33.0	300	74	677	6.8	95
Experiment 2									
MLC	2453	10.9	26	1.1	200	99	−27	0.9	101
MLIC	26,271	106.9	17,055	69.8	200	35	281	2.1	99
Experiment 3									
MLC	31,393	12.2	2840	2.0	200	91	1010	1.4	97
MLIC	18,680	12.9	8057	6.1	200	57	5555	4.5	71
Experiment 4									
MLC	26,380	126.0	13,587	65.4	400	48	4327	20.1	84
MLIC	7440	36.3	3810	19.1	600	49	3810	19.1	49
Experiment 5									
MLIC	32,402	17.2					18,979	10.5	41

Percent decrease determined with Δcpm. cpm, Counts per minute; SI, stimulation index (mean cpm experimental culture/mean cpm background culture); MLC, mixed-lymphocyte culture; MLIC, mixed-lymphocyte islet culture.
*Initial significant decrease in proliferative response observed ($P < 0.05$).
†Mean cpm experimental culture − mean cpm background culture.
‡$P < 0.05$ in experiments 1–4.

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well (experiments 1 and 5; Table 2) were incubated with 10^5 responder PBLs in a total volume of 0.2 ml in a 96-well round-bottom tissue-culture cluster. Controls were as for the MLC. Plates were incubated for 6 days at 37°C under 5% CO_2 followed by an 18-h pulse with 1 μCi [^{3}H]thymidine. Cells were harvested and counted in 3a20 scintillation cocktail (Research Products International, Mt. Prospect, IL) with an LKB β-counter. The mean counts per minute (cpm) were calculated for quadruplicate or triplicate cultures, and the mean cpm obtained from cultures of responder cells versus self were used as the background cpm; mean cpm obtained from cultures of responder cells versus allogeneic cells (PBLs or islets) were used as experimental cpm. Results are represented as *1*) mean cpm ± SE, *2*) Δcpm (mean cpm experimental culture − mean cpm background culture), or *3*) stimulation index (mean cpm experimental culture/mean cpm background culture).

Statistical analysis. Repeated-measures analysis of variance and the Student-Newman-Keuls *t* test were used to analyze MLC, MLIC, and in vitro islet functional data for significant differences between control and UV-irradiated cultures (25). Comparisons of pooled data at 0 and 600 J/m^2 were done with the paired *t* test (25). Secretagogue data, microliters of islet transplanted per kilogram of body weight, and islet purity for the different transplantation groups were assessed with one-way analysis of variance and the Student-Newman-Keuls *t* test (25). The BMDPIL Life Tables and Survivor Functions Program was used to analyze survival times between transplantation groups (27).

RESULTS

UV irradiation of canine islets alters glucose-mediated insulin release in vitro. The effect of UV irradiation on islet functional capacity in vitro is summarized in Table 1. To minimize possible variability due to differences in islet cell number and/or content per well, the data were analyzed and expressed as a function of content (fractional insulin release). For all three experiments, basal fractional insulin release was increased with increasing doses of UV irradiation, although the increase was not statistically significant in experiment 2. However, stimulated insulin release expressed as percentage of basal fractional insulin release was significantly decreased in all three experiments beginning at doses of 200, 200, and 300 J/m^2 ($P < 0.05$, experiments 1–3, respectively). The mean of the data from the three experiments is shown in Fig. 1. A significant decrease in glucose-mediated fractional insulin release was seen at 200–300 J/m^2.

UV irradiation of canine lymphocytes and islets. With UV-irradiated PBLs as stimulators, significant decreases of 53–112% were observed in 27 of 29 MLC proliferative responses ($P < 0.05$, data not shown) beginning at doses of UV light that varied from 200–600 J/m^2. The mean decrease in MLC proliferative responses at 600 J/m^2 is shown in Fig. 2.

The ability of UV-irradiated islets to stimulate lymphoproliferative responses was also assessed in MLIC ($n = 5$, Table 2). The effect of UV irradiation on MLC and MLIC was analyzed concurrently in four of five of these experiments. The four MLC responses were significantly decreased by 48–101% beginning at UV doses of 200–400 J/m^2, and the corresponding MLIC responses were significantly de-

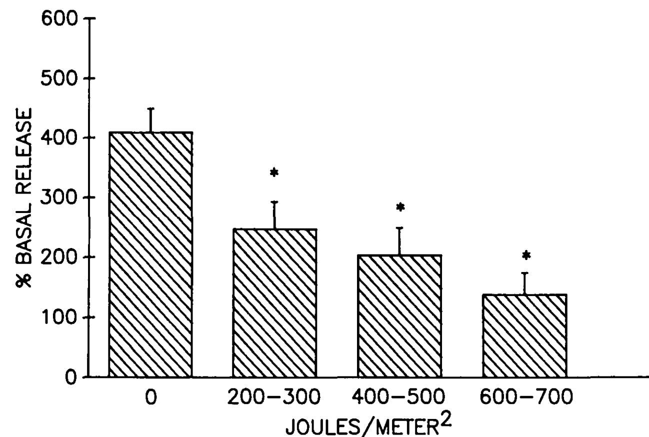

FIG. 1. Pooled data from 3 experiments depicting effect of ultraviolet irradiation on glucose-mediated fractional insulin release (expressed as percentage of basal fractional insulin release). Release was significantly decreased vs. control (0 J/m^2) beginning at 200–300 J/m^2. *$P < 0.05$.

creased by 35–74% beginning at UV doses of 200–600 J/m^2 ($P < 0.05$, experiments 1–4). Although a dose of 200–300 J/m^2 was sufficient to abrogate the MLC in three of four experiments, a higher UV dose of 600 J/m^2 was needed to similarly decrease the MLIC by 71–99%. In experiment 4, a dose of 600 J/m^2 significantly lowered the MLC by 84% but only decreased the MLIC by 49% (both at $P < 0.05$). Similarly, in one additional MLIC experiment (an MLC was done but not with UV), a decrease of only 41% was seen at a dose of 600 J/m^2 (not statistically significant, experiment 5). Results from the five MLIC experiments yielded a mean Δcpm of 19,813 at a dose of 0 J/m^2, which was significantly decreased by 70% to 5860 Δcpm at a UV dose of 600 J/m^2 ($P < 0.02$).

Autotransplantation of UV-irradiated canine islets. A series of autotransplantations with nonirradiated or 600 J/m^2 irradiated islets was undertaken (Table 3). Beagles autotransplanted with nonirradiated islets demonstrated a 75% graft survival rate at 1 mo ($n = 8$) with two islet grafts failing at days 12 and 22, whereas transplantation of UV-irradiated

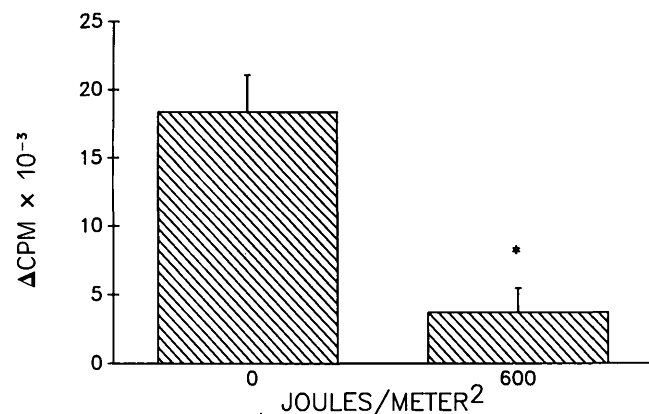

FIG. 2. Pooled data from 29 experiments depicting effect of 600 J/m^2 ultraviolet (UV) irradiation on mixed-lymphocyte culture stimulatory capacity of canine peripheral blood leukocytes. Mean Δcounts per minute (cpm) at UV dose of 0 J/m^2 of 18,354 was significantly decreased by 80% to mean Δcpm of 3699 at UV dose of 600 J/m^2. *$P < 0.001$.

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TABLE 3
Effect of ultraviolet (UV) irradiation on canine islet autograft survival

UV dose (J/m²)	n	Survival (days)	Mean ± SE survival (days)	Transplanted islet (µl/kg body wt)
0	8	12,22,100,100,100, 100,100,100	79.5 ± 13.6	5.28 ± 1.25
600	6	9,17,20,69,100,100	52.5 ± 17.3	3.87 ± 0.99

Differences between groups not significant.

islets (n = 8) resulted in two grafts that failed initially to reverse hyperglycemia and three additional grafts that failed at days 9, 17, and 20. The 1-mo graft survival was therefore 50% for technically successful transplants. Of the three grafts that functioned beyond 1 mo, one failed at day 69 and the other two remained normoglycemic for 100 days for an overall graft survival rate of 33% and a mean survival time (MST) of 52.5 ± 17.3 days. All six dogs in the control group retained graft function to 100 days with an overall MST of 79.3 ± 13.6 days.

Secretagogue studies done 1 mo posttransplantation revealed no significant difference in blood glucose levels, glucose disappearance rates, or the areas under the glucose response curves to oral and intravenous glucose or intravenous glucagon between recipients of control (n = 6) and UV (n = 3) islet autografts (Fig. 3). Similarly, the areas under the insulin response curves in the OGTT and GTT were not significantly different for the two groups. However, the UV autografts demonstrated a significantly decreased area under the insulin response curve for intravenous glucose (Fig. 3).

Allotransplantation of UV-irradiated canine islets. Islet allografts were done between pairs of MLC-reactive animals (n = 8). Allotransplantation of islets irradiated with 600 J/m² resulted in initial reversal of hyperglycemia in all cases (n = 8), with subsequent failure or rejection of 100% of the grafts between days 3 and 10 and an MST of 5.25 ± 0.88 days (Table 4).

A series of islet allografts (4 of 5 prescreened and known to be MLC reactive to donor PBLs) was also conducted in which recipients were administered subimmunosuppressive doses of CsA beginning 5 days before and continuing 30 days after transplantation (n = 5). In these experiments, we used two donors for each recipient. We have previously shown that two or more donors can be used to increase the transplantable islet mass without immunologically jeopardizing graft functions in recipients that receive continuous immunosuppressive doses of CsA (19,20; Table 4). One graft failed to induce normoglycemia, and the remaining four grafts were rejected between days 6 and 10 with an MST of 7.5 ± 0.9 days (Table 4).

In another similar series of islet allografts (5 of 8 prescreened and known to be MLC reactive to donor PBLs), the islets were cultured before transplantation for 24 h after UV irradiation (n = 8; Table 4). One graft failed to induce normoglycemia, and four others were rejected between 3 and 10 days. However, an additional 24 h of culture after UV irradiation resulted in prolongation of graft survival in three dogs to 16 (euthanized at day 16), 26, and >100 days to give an MST of 24.6 ± 12.9 days for the seven technically successful experiments. The dog surviving to day 26 was highly MLC reactive against each donor individually and against the two donors combined.

DISCUSSION

Although administration of CsA to recipients of UV-irradiated islets has been a consistently successful protocol for prolongation of islet allo- and xenografts in rodents (10–13), our results in a canine model indicate that this strategy requires considerably more investigation if a potential for its use in large mammals, including humans, is to be realized. UV irradiation of canine islets at doses that result in downmodulation of islet immunogenicity may damage islets and lead to a decrease in the functional islet mass transplanted. A UV dose of 600 J/m², which uniformly decreased PBL and islet

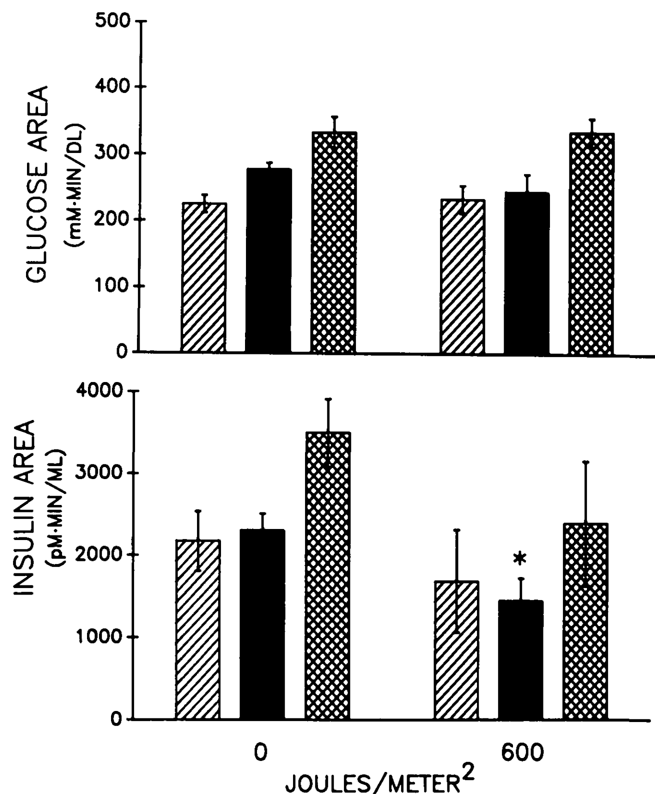

FIG. 3. Areas under glucose (*top*) and insulin (*bottom*) response curves from 0 to 30 min in response to oral glucose tolerance tests (*hatched bars*), intravenous glucose tolerance tests (IVGTT; *solid bars*), and glucagon tolerance tests (*cross-hatched bars*) for recipients of control (0 J/m²) and ultraviolet-irradiated (600 J/m²) islet autografts. Area under insulin response curve in response to IVGTT was significantly decreased in recipients of irradiated islets vs. control dogs that received nonirradiated islets. *P < 0.05.

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TABLE 4
Effect of ultraviolet (UV) irradiation on survival of canine islet allografts

Cyclosporin	*n*	Survival (days)	Mean ± SE survival (days)	Donors (*n*)	Transplanted islet (μl/kg body wt)	24 h of culture postirradiation
None	8	3,3,3,4,6,6,7,10	5.25 ± 0.9	1	3.44 ± 0.77	No
10 mg · kg⁻¹ · day⁻¹	4	6,7,7,10	7.5 ± 0.9	2	14.34 ± 2.36	No
10 mg · kg⁻¹ · day⁻¹	7	7,8,8,8,16,26,>100	24.6 ± 12.9*	2	9.04 ± 0.67	Yes

*$P < 0.005$ vs. no cyclosporin.

immunogenicity assessed in MLC and MLIC, respectively, was shown to alter in vitro glucose-responsive islet secretory capacity. Expressed as percentage of basal fractional insulin release, glucose-mediated fractional insulin release was significantly decreased between 200 and 300 J/m^2 in three of three experiments. The nature of the defects in the insulin-secretory mechanism in 5- to 7-day-old monolayer cultures of UV-irradiated canine islets was not delineated in this study, and we did not obtain direct evidence that the defect was reversible after transplantation. In fact, a detrimental effect of UV on islet function may be supported by the autograft data; although autografts of nonirradiated islets resulted in a 75% survival rate at 1 mo and >100 days, autografts of UV-irradiated islets resulted in a 50% survival rate at 1 mo that fell to 33% by 100 days. In addition, the UV-induced islet defect in glucose-mediated insulin release detected with intravenous glucose but not oral glucose or intravenous glucagon may also reflect a decreased and/or diminishing functional islet mass (22). These data are in agreement with preliminary results from other large-animal studies in which cynomolgus monkey, baboon, and human islets were adversely affected by a UV dose of 600 J/m^2, the dose needed to abrogate stimulatory responses in the appropriate MLC (12).

Although a UV dose of 600 J/m^2 consistently resulted in attenuation of PBL and islet immunogenicity as assessed in MLC and MLIC, this dose did not routinely result in abrogation of proliferative responses (Table 2). In addition, it appeared that a greater dose of UV was necessary to abrogate and/or attenuate islet immunogenicity compared with PBLs. Because islets are irradiated as whole structures, it is likely that the penetration of UV light to dendritic and other antigen-presenting cells within the islets is incomplete, whereas the antigen-presenting cells in single-cell suspensions of PBLs are all accessible to UV. Alternatively, islets may be more immunogenic than PBLs, but this notion was not directly assessed in this study.

Transplantation of UV-irradiated islets from a single donor into nonimmunosuppressed MLC-reactive recipients resulted in 100% graft rejection between days 3 and 10. These results are consistent with studies in rodent models in which strongly allogeneic recipient-donor combinations required the addition of CsA to prolong graft survival (11–13). Therefore, we performed experiments with islets that were UV irradiated and transplanted into recipients who received immunosuppressive doses of CsA that alone are inadequate to prevent islet allograft rejection (19,20). In addition, we elected to use two donors/recipient to increase the transplanted functional islet mass. Allotransplantation under these conditions also led to 100% graft rejection by day 10.

Although our experiments demonstrated that UV decreased PBL and islet immunogenicity, independent of whether the irradiated cells were cultured before setting them in an MLC or MLIC, in vivo studies in rodents have shown that islets must be cultured for 24 h after UV irradiation to achieve prolongation of graft survival (13). Therefore, we performed a series of allografts in which irradiated islets from two donors were cultured for 24 h postirradiation before transplantation into recipients who were administered low doses of CsA. In contrast to the previous results, this protocol resulted in prolongation of allograft survival in three of seven animals to 16, 26, and >100 days. Prolongation of islet allograft survival with the addition of 24 h of culture of islets after UV irradiation indicates that a certain period of time is necessary for the complete downregulatory effects of UV to be exerted on allogenic passenger antigen-presenting cells. For example, human lymphocytes are able to mobilize Ca^{2+} after stimulation with the mitogen phytohemagglutinin if tested immediately after UV irradiation but are incapable of doing so by 3 h postirradiation (26). Therefore, it appears that some of the effects of UV irradiation on antigen-presenting cells are immediate, whereas some are dependent on an as-yet undefined time period.

In summary, UV irradiation effectively downmodulates canine PBL and islet immunogenicity as assessed in vitro and, when used in combination with a 24-h culture postirradiation, can result in prolongation of canine islet allograft survival in CsA-treated unrelated pancreatectomized recipients. These are unique observations in a large-animal model for islet transplantation. Our results contrast with the consistent success of this strategy when handpicked rodent islets are used in similar protocols. The lack of consistent prolongation of islet allograft survival may be a consequence of the use of relatively impure islet cell preparations characteristic of islet-enriched fractions that are usually isolated from large-animal pancreases. Therefore, the potential utility of UV light as an immunodulator of large-animal islets, including humans, must await the availability of highly purified islet preparations in these species.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health Grants DK-25802 and DK-07346, the Diabetes Research Institute Foundation, and Sandoz Pharmaceuticals.

Special thanks are extended to Dr. David Winter for support and encouragement during these studies and to the Diabetes Research Institute staff.

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