
Rapid Publication

Calcineurin Inhibitor–Free CD28 Blockade-Based Protocol Protects Allogeneic Islets in Nonhuman Primates

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Recent success using a steroid-free immunosuppressive regimen has renewed enthusiasm for the use of islet transplantation to treat diabetes. Toxicities associated with the continued use of a calcineurin inhibitor may limit the wide-spread application of this therapy. Biological agents that block key T-cell costimulatory signals, in particular the CD28 pathway, have demonstrated extraordinary promise in animal models. LEA29Y (BMS-224818), a mutant CTLA4-Ig molecule with increased binding activity, was evaluated for its potential to replace tacrolimus and protect allogeneic islets in a preclinical primate model. Animals received either the base immunosuppression regimen (rapamycin and anti-IL-2R monoclonal antibody [mAb]) or the base immunosuppression and LEA29Y. Animals receiving the LEA29Y/rapamycin/anti-IL-2R regimen ($n = 5$) had significantly prolonged islet allograft survival (204, 190, 216, 56, and >220 days). In contrast, those animals receiving the base regimen alone ($n = 2$) quickly rejected the transplanted islets at 1 week (both at 7 days). The LEA29Y-based regimen prevented the priming of anti-donor T- and B-cell responses, as detected by interferon- γ enzyme-linked immunospot and allo-antibody production, respectively. The results of this study suggest that LEA29Y is a potent immunosuppressant that can effectively prevent rejection in a steroid-free immunosuppressive protocol and produce marked prolongation of islet allograft survival in a preclinical model. *Diabetes* 51:265–270, 2002

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ELISpot, enzyme-linked immunospot; IEQ, islet equivalents; IFN- γ , interferon- γ ; mAb, monoclonal antibody; MHC, major histocompatibility complex; MST, median survival time; POD, postoperative day.

Type 1 diabetes resulting from autoimmune destruction of the insulin-producing cells in the islets of Langerhans is a devastating disease with increasing incidence (1). Clinical islet cell transplantation has, in the past, produced less-than-promising results when compared with whole-organ pancreas transplantation (2,3). Recently, however, with the advent of steroid-free immunosuppressive regimens, outcomes in islet transplantation alone have significantly improved (2,4). This modification in immunosuppressive therapy coupled with improvements in isolation techniques and transplantation of adequate islet mass have resulted in dramatic improvements in islet allograft survival (5).

Currently, however, most (if not all) steroid-free regimens use a calcineurin inhibitor as the primary immunosuppressant. Calcineurin inhibitor therapy has significant concomitant side effects, including nephrotoxicity and diabetogenicity, even with low therapeutic target levels (6–9). The replacement of calcineurin inhibitors with an equally effective yet less toxic immunosuppressant is essential for the widespread application of islet transplantation.

Given the central role of T-cells in allograft rejection, therapies that block T-cell activation and function are common targets of immunosuppressive regimens. At least two signals are required for optimal T-cell activation, the first is transmitted as the T-cell receptor interacts with antigenic peptide bound to major histocompatibility complex (MHC), and the second is received through accessory costimulatory molecules (10). One of the best-characterized costimulatory pathways is the interaction of CD28 on T-cells and the B7 molecules expressed on antigen-presenting cells. This pathway has been intensely studied in both transplantation and autoimmunity.

Manipulation of CD28/B7 pathway has demonstrated extraordinary promise in experimental autoimmune disease models of diabetes, multiple sclerosis, and lupus (11–13). In addition to its beneficial effects in models of autoimmunity, blockade of the CD28 pathway has arisen as a promising strategy to prevent allograft rejection. In

many of these studies, a fusion protein consisting of the extracellular domain of the soluble CTLA4 molecule fused to the heavy chain of human IgG1 has been used to block CD28-B7 interactions (14). In rodents, this fusion protein construct has been shown to block T-cell proliferation in allogeneic mixed lymphocyte reaction (15) as well as inhibit allograft (16,17) and xenograft rejection (18). CTLA4-Ig therapy has also been shown to modestly prolong islet allograft survival in a nonhuman primate model (19). Although CTLA4-Ig monotherapy has only a minimal impact on renal allograft survival (median survival time [MST] 8 days) in rhesus macaques (20,21) when it is combined with cyclosporin and prednisone, we have shown a beneficial effect (MST 53 days) (20). More recently, CTLA4-Ig has been shown to have clinical efficacy in patients with psoriasis vulgaris, a T-cell-mediated autoimmune skin disorder (22). Nearly one-half of patients with documented disease demonstrated sustained improvements in clinical disease activity after treatment.

In an effort to improve the efficacy of CTLA4-Ig, a novel mutant form, LEA29Y, was constructed. LEA29Y is a second-generation CTLA4-Ig molecule that differs by two amino acid residues within the regions that bind to CD80 and CD86. These amino acid substitutions confer increased binding affinity to CD86 (slower rate of dissociation) without appreciably altering the interaction with CD80. The resultant improvement in binding affinity led to more potent immunosuppressive properties *in vitro* and *in vivo* (R. Peach, unpublished observations). Given the proven efficacy in experimental models of autoimmunity and transplantation, blockade of the CD28/B7 pathway represents an ideal target for use in islet transplantation, where presumably both barriers exist as diabetic patients receive allogeneic islets. In this study, we evaluated the capacity of LEA29Y to prevent islet allograft rejection in a steroid-free, calcineurin inhibitor-free regimen.

RESEARCH DESIGN AND METHODS

Animals. Captive bred adolescent rhesus monkeys (*Macaca mulatta*) (~3–18 kg) were used as recipients and donors. The absence of preformed donor-specific antibodies in the recipient was confirmed before transplant. All potential donors and recipients were tested for anti-cytomegalovirus antibodies, and sero-negative recipients were not paired with sero-positive donors.

Donor pancreatectomy and islet isolation. The donor pancreatectomy was performed under general anesthesia 1 day before transplantation. The splenorenal and splenicocolic ligaments were divided so as to mobilize the spleen and tail of the pancreas. The head of the pancreas and second portion of the duodenum were mobilized after Kocher maneuver. After administration of heparin (200 units/kg), the aorta was cannulated just above its bifurcation and the animal was exsanguinated. Cold slush was immediately placed in the lesser sac and behind the body of the pancreas. The body and neck of the pancreas were carefully excised, taking care not to violate the pancreatic capsule.

Rhesus monkey islet isolation was completed via minor modifications of the automated method for human islet isolation (23,24) by using Liberase (Boehringer Mannheim, Indianapolis, IN) at a concentration of 0.47–0.71 mg/ml. A three-layer discontinuous Eurofocoll gradient (densities 1.108, 1.097, and 1.037; Mediatech, Herndon, VA) and a Cobe 2991 blood cell processor (Gambro, Lakewood, CO) were used for purification of islets from the pancreatic digest. Samples of the final islet preparation were stained with dithizone (Sigma, St. Louis, MO), and the preparation was assessed by counting the number of islets in each of the following size ranges: 50–100, 100–150, 150–200, 200–250, 250–300, 300–350, and 350–400 μm . The data were mathematically converted to determine the number of islets with an average diameter of 150 μm and were expressed as islet equivalents (IEQ) (25).

Recipient pancreatectomy and intrahepatic islet-cell transplantation. Total pancreatectomy without duodenectomy or splenectomy was performed at least 1 week before transplant. The splenic, inferior and superior mesen-

teric, middle colic, and portal veins were identified and preserved during dissection of the body of the pancreas. The duodenum was mobilized, and branches of the pancreaticoduodenal vessels that entered the pancreas were ligated and divided, leaving the duodenal branches intact. The common bile duct was identified and preserved. The main and accessory pancreatic ducts were ligated and divided, and the pancreas was removed from the abdominal cavity. The procedure was well tolerated, and two forms of pancreatic enzyme replacement were administered postoperatively: Pancrease (pancrelipase enteric coated microspheres; Ortho-McNeil, Raritan, NJ) and Viokase V pancreatic enzyme supplement powder (Fort Dodge Animal Health, Fort Dodge, IA).

Overnight-cultured islets were washed in media and counted to determine the number of IEQ. Islets were then resuspended in 20 ml of media supplemented with 200 units of heparin. Intra-hepatic islet transplantation was performed via gravity drainage of islets into a sigmoid or branch of the left colic vein through a 22-gauge intravenous catheter.

Blood glucose monitoring and insulin administration. Fasting and post-prandial blood glucose levels were monitored (Glucometer Elite; Bayer, Elkhart, IN) twice daily (prebreakfast and postlunch) via ear-stick. Insulin (NPH, Ultralente; Eli Lilly, Indianapolis, IN) was administered three times daily in attempt to maintain fasting blood glucose <300 mg/dl in pretransplant pancreatectomized animals or in those who had rejected their allografts.

Experimental groups and immunosuppressive protocols. Two protocols were tested: LEA29Y-based protocol, rapamycin, and anti-IL-2R monoclonal antibody (mAb) (1) and control protocol treated with rapamycin and anti-IL-2R alone (2). LEA29Y was administered intravenously intra-operatively (10 mg/kg) and on postoperative day (POD) 4 (15 mg/kg). Additional doses of 20 mg/kg were given on POD 14 and every 2 weeks until POD 154. The drug was well tolerated, and there was never any evidence of gross toxicity. The chimeric anti-human IL-2R mAb (0.3 mg/kg *i.v.*) was administered intra-operatively and on POD 4. Rapamycin was administered orally in a Primaburger (Bio-Serv, Frenchtown, NJ) treat at 1.25 mg/kg bid POD 0–50 (average plasma drug levels 8–12 ng/ml), 1 mg/kg *q.d.* POD 50–100, and then tapered to terminate dosing by POD 121. The LEA29Y used in these experiments was provided by Bristol-Myers Squibb (Princeton, NJ). Rapamycin (Rapamune) and anti-human IL-2R mAb (Simulect) were purchased from the Emory University Hospital Pharmacy. Survival of the islet grafts among experimental groups was compared using the Mann-Whitney *U* test.

Detection of anti-donor antibodies. The presence of detectable donor-specific allo-antibody was determined using flow cytometry. Peripheral blood leukocytes served as the target cells for the pretransplant analysis. Leukocytes isolated from mesenteric lymph nodes obtained at the time of transplant were the target cells for the posttransplant assays.

Anti-donor enzyme-linked immunospot assay. Responses were measured by interferon- γ (IFN- γ) enzyme-linked immunospot (ELISpot) assay using peripheral blood leukocytes obtained from the recipient and donor animals. An equal number of irradiated stimulators (donor leukocytes) and responders (recipient leukocytes) were added to ester cellulose bottom plates (Millipore, Bedford, MA) coated with the capture antibody, mouse anti-human IFN- γ (clone GZ-4; Mabtech, Sweden). After 14–16 h incubation, biotinylated mouse anti-human IFN- γ (clone 7-B6-1; Mabtech, Sweden) was added, unbound antibody was removed, and horseradish peroxidase-Avidin D (Vector, Burlingame, CA) was added. Spots were developed with the substrate 3-amino-9-ethyl-carbazole (Sigma). Each spot represents an IFN- γ -secreting cell; the frequency of these cells can be determined by dividing the number of spots generated by the total number of responder cells plated.

RESULTS

CD28 pathway blockade-based therapy prolongs the survival of islet allografts in Rhesus macaques. Based on the current success of a protocol using the combination of tacrolimus, rapamycin, and anti-IL-2R mAb (5), we sought to develop a steroid-free, calcineurin inhibitor-free regimen. LEA29Y was used in combination with rapamycin and a chimeric antibody against IL-2 receptor α -chain. The dose and schedule of administration were selected based on previous pharmacokinetic studies of LEA29Y in primates and humans. Using these data, we selected a regimen designed to maintain serum trough LEA29Y concentrations ≥ 20 $\mu\text{g/ml}$. Diabetes was induced by surgical pancreatectomy of recipient animals and confirmed by pretransplant intravenous glucose tolerance test. Donor-recipient pairings were defined based on molecular typing

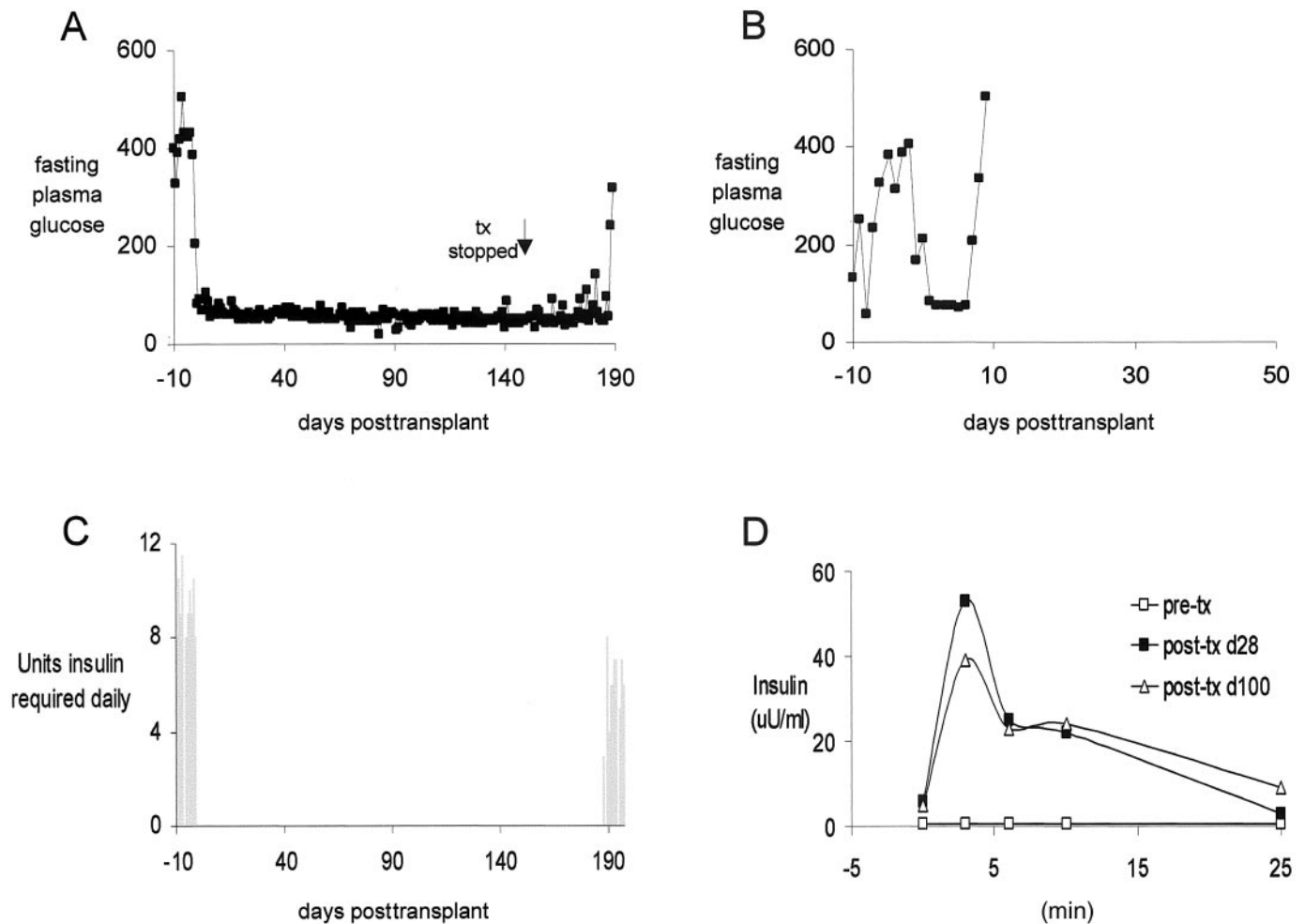


FIG. 1. Fasting blood glucose for LEA29Y treated (A) and control (B) recipients of allogeneic islets (representative animals) before and after transplantation. All animals underwent surgical pancreatectomy at least 2 weeks before transplantation (mean pretransplant insulin requirement of 8.76 ± 0.18 units/day) C: After intraportal infusion of allogeneic islets, recipients quickly became euglycemic requiring no exogenous insulin posttransplant. D: Diabetes induction and posttransplant islet function was confirmed by intravenous glucose tolerance test before transplantation and at 1 month and 3 months posttransplant.

using a panel of previously defined major histocompatibility alleles (8 class I and 12 class II) (26–28). Pairings maximized disparity at both class I and II loci. Rejection was defined as two consecutive fasting blood glucose values >125 mg/dl on subsequent days. Intra-portal infusion of allogeneic islets ($>10,000$ IEQ/kg) resulted in initial restoration of euglycemia and insulin independence in diabetic monkeys in both groups (Fig. 1A and B).

Treatment of pancreatectomized macaques with the LEA29Y-based regimen significantly prolonged islet allograft survival (204, 190, 216, >220 , and 56 days, respectively). In contrast, the control regimen (rapamycin and anti-IL-2R) did little to alter the tempo of rejection (both at 7 days) (Fig. 1C). Four of five animals receiving the LEA29Y regimen enjoyed rejection-free survival for the duration of the treatment period (Table 1). Intravenous glucose tolerance test with measurement of insulin and glucose levels confirmed islet function posttransplant (representative animal, Fig. 1D).

At 100 days posttransplant, the dosing of rapamycin was decreased and tapered to zero by day 121. Animals continued to remain insulin-independent while receiving LEA29Y monotherapy. At ~ 150 days posttransplant, the

remaining islet recipients received their final dose of LEA29Y, ceasing any additional immunosuppressive therapy. As expected, ~ 1 – 2 months after discontinuation of therapy, recipients became hyperglycemic and required exogenous insulin therapy. Histological analysis revealed a mononuclear infiltrate, strongly suggesting rejection as the etiology of the loss of glucose control (Fig. 2B).

LEA29Y therapy inhibits priming of anti-donor T- and B-cell responses. The frequency of primed alloreactive T-cells can effectively be detected by using the ELISpot assay, which can discriminate production of IFN- γ at the single-cell level. Peripheral blood samples from islet recipients were analyzed at various time points both pre- and posttransplant for their ability to generate IFN- γ in response to donor antigen. Animals treated with the base regimen alone quickly developed a measurable anti-donor response that coincided with rejection ~ 1 week after transplant. In contrast, the frequency of anti-donor IFN- γ -producing cells in animals receiving the LEA29Y-containing regimen was undetectable until therapy was withdrawn (representative animals, Fig. 3A and B). Thus, the LEA29Y regimen effectively blocked the generation of anti-donor T-cell responses as measured by the ability to produce IFN- γ .

TABLE 1
Islet allograft survival and treatment

	IEQ/kg	Survival*	Treatment	MHC mismatches (<i>n</i>)	
				Class I	Class II
RKf-7	22,250	204	LEA29Y/Rapa/ α IL-2R	2	ND
RUf-7	17,087	190	LEA29Y/Rapa/ α IL-2R	ND	3
RRe-7	20,266	216	LEA29Y/Rapa/ α IL-2R	2	6
RWt-6	16,033	56	LEA29Y/Rapa/ α IL-2R	2	3
RMv-6	8,201	>220	LEA29Y/Rapa/ α IL-2R	1	3
RQz-6	12,980	7	Rapa/ α IL-2R	2	5
RIb-7	10,903	7	Rapa/ α IL-2R	1	4

*Insulin independence. ND, none detected in alleles that were typed.

Flow cytometry was used to examine the development of anti-donor antibody responses. One animal within the control group generated a strong anti-donor Ab response, whereas the other failed to develop a detectable response, presumably because it was euthanized before the antibody response could be measured (Fig. 3C). In contrast, four of five animals failed to generate an antibody response while receiving LEA29Y therapy. This is consistent with previously reported results using CTLA4-Ig in an islet transplant model (19) as well as our experience in a renal allograft model where recipients failed to generate anti-donor antibodies (20). One animal of five recipients underwent a rejection episode while receiving the LEA29Y therapy and subsequently developed an anti-donor antibody response. As expected, the remaining four animals receiving the LEA29Y regimen consistently developed anti-donor antibody responses around the time of rejection (~200 days posttransplant, 50 days after the final dose of LEA29Y).

DISCUSSION

Islet transplantation is quickly becoming a viable treatment option for patients with brittle type 1 diabetes. Recent reports describing steroid-free immunosuppressive

regimens, which result in successful insulin independence after islet transplantation, have ushered in renewed optimism for the practical application of islet transplantation. Whereas the elimination of glucocorticoids from immunosuppressive regimens represents a major step forward in the effort to treat type 1 diabetes, the reliance on calcineurin inhibitor therapy for primary immunosuppression may limit the application of this approach. Calcineurin inhibitors have numerous unwanted side effects, including nephrotoxicity, diabetes, hypertension, impaired lipid metabolism, and hirsutism (6–8). Even when drug levels are kept low, significant side effects may develop. This is particularly true in the diabetic patient population where renal function may already be impaired. Indeed, in the most recent reports from Edmonton, two patients with mildly elevated pretransplant creatinine levels had significant decreases in renal function while on calcineurin inhibitor therapy and ultimately required withdrawal of this drug (9). In the same report, two-thirds of recipients developed some degree of glucose intolerance, with one-quarter developing frank posttransplant diabetes thought to be related to the use of tacrolimus. This underscores the appealing and essential nature of a calcineurin inhibitor-

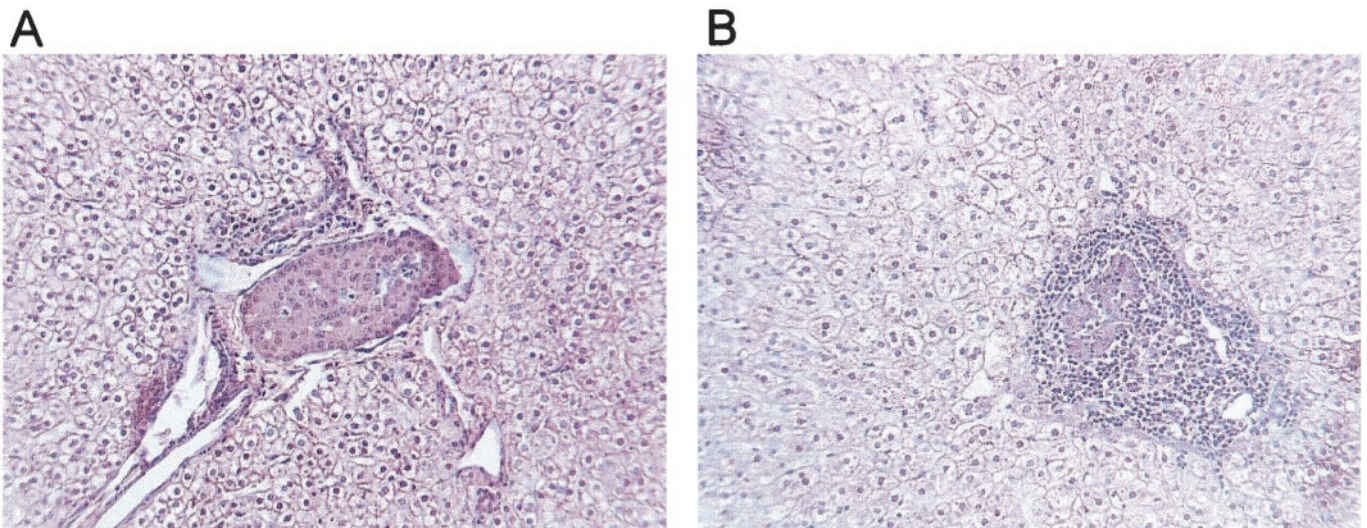


FIG. 2. **A.** Immunohistology of functional transplanted islet confirmed by positive staining for insulin. **B.** Islet from animal receiving control regimen surrounded by mononuclear infiltrate, indicating rejection.

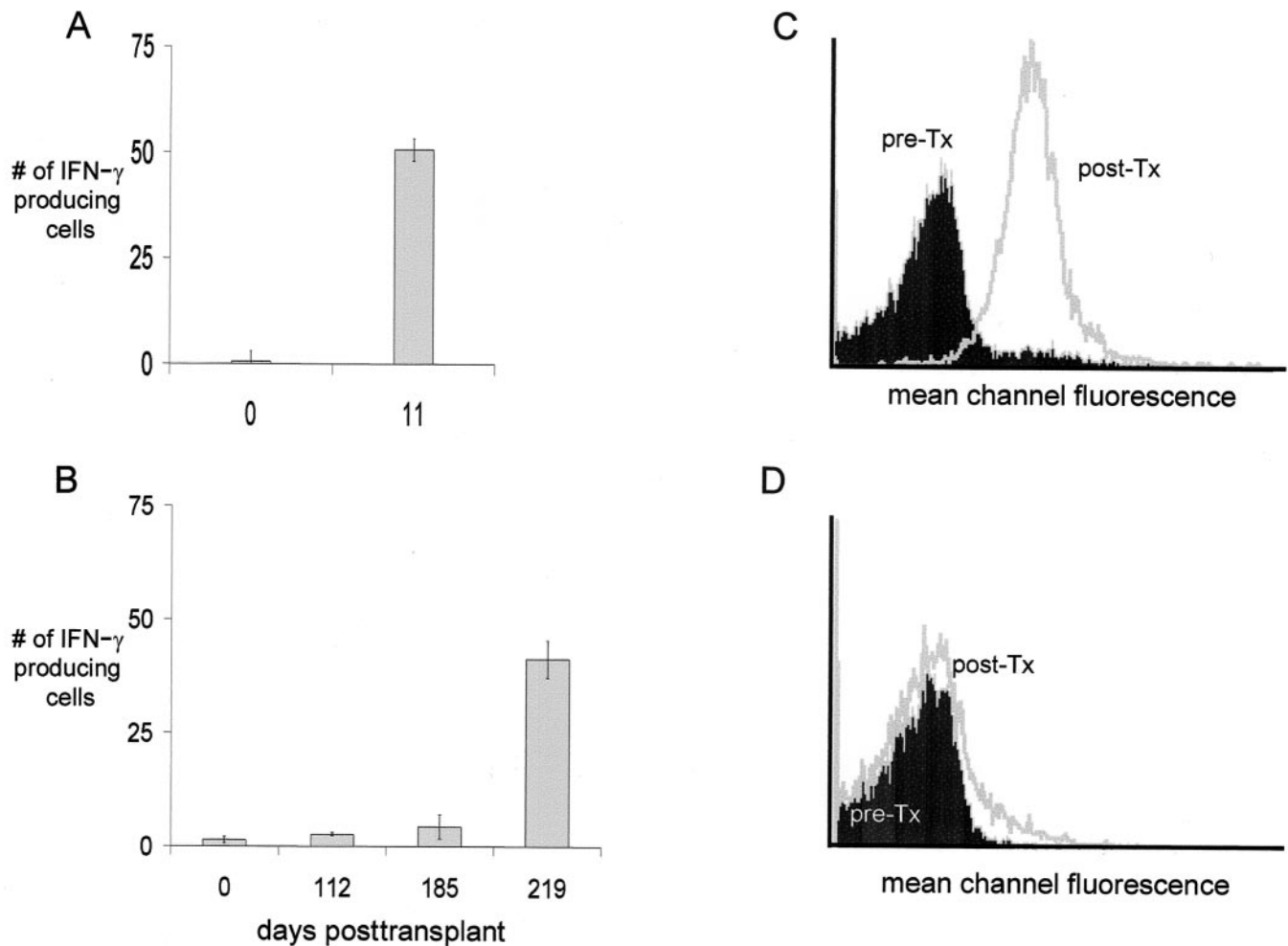


FIG. 3. Suppression of anti-donor T- and B-cell responses by LEA29Y regimen. **A:** Anti-donor IFN- γ ELISpot response corresponds to timing of rejection in the controls (\sim 1 week posttransplant). **B:** LEA29Y regimen effectively suppresses the generation of anti-donor T-cell response. **C:** Animals receiving rapamycin and anti-IL-2R mAb quickly produce detectable anti-donor antibody, as measured by flow cytometric methods at the time of rejection. **D:** Islet recipients receiving the LEA29Y-containing regimen fail to generate a detectable anti-donor antibody response while treated.

free immunosuppressive regimen, particularly for islet transplantation.

Blockade of T-cell costimulatory pathways is a promising strategy for the development of nontoxic immunosuppressive and potentially tolerogenic regimens. This approach targets those T-cells that receive "signal 1" during the period of drug administration. For example, treatment during the peritransplant period is thought to render allo-specific T-cells impotent upon encounter with the new organ or tissue, whereas other T-cells are left unimpaired (29). Blockade of the CD28/B7 pathway has demonstrated remarkable promise in experimental models of autoimmunity and transplantation, making it a particularly appealing immunosuppressive target in islet transplantation, where presumably both auto- and allo-immune obstacles exist. Bluestone and colleagues were the first to describe the potential of CD28 blockade in a large animal transplant model (19). Treatment with CTLA4-Ig was found to significantly, although modestly, prolong islet allograft survival in nonhuman primates (19). Similarly, we have shown that CTLA4-Ig monotherapy does little to prolong renal allograft survival (20). Recently, there have been several reports of long-term survival of islet allo-

grafts in nonhuman primate models. Anti-CD40L mAb therapy has shown the most impressive results thus far; however, similar to experiments using a renal transplant model, tolerance was not achieved, as withdrawal of therapy eventually resulted in rejection (30,31). In another encouraging report, Thomas et al. (32) recently described the use of an anti-CD3 immunotoxin and the immune modulatory agent DSG (15 deoxyspergualin) to dramatically prolong islet survival in streptozotocin-induced diabetic primates. Although promising, these reports used therapeutics whose clinical potential at the present time is still uncertain.

Our data using LEA29Y, a novel mutant form of CTLA4-Ig, in the Rhesus islet allograft model is consistent with *in vitro* evidence indicating that this second generation molecule is a more potent inhibitor of T-cell responses than the parent molecule. Given that CTLA4-Ig has already shown efficacy in a clinical trial of psoriasis patients (22), there is significant enthusiasm for the trials using LEA29Y as the primary immunosuppressant. It is clearly compatible, if not synergistic, with clinically approved immunosuppressive agents (anti-IL-2R mAb and rapamycin) facilitating the design of clinical trials (current study and T.C.P.,

unpublished observations). Initial human trials with LEA29Y are already underway in patients afflicted with rheumatoid arthritis and those undergoing renal transplant. Although a direct comparison of a tacrolimus-based protocol and the LEA29Y regimen was not attempted because of reported intolerable toxicities in nonhuman primates (33), our results suggest that LEA29Y has the potential to be at least as effective as tacrolimus as a primary immunosuppressant.

In summary, we have identified a novel calcineurin inhibitor/steroid-free immunosuppressive regimen that provides significant protection from rejection and prolongs the survival of islet allografts in nonhuman primates. Together, the encouraging results of clinical trials using the lower affinity parent molecule, CTLA4-Ig, in psoriasis and the results described here provide a strong rationale for clinical trials to test these strategies in human islet transplantation.

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REFERENCES

- Harris MI, Flegal KM, Cowie CC, Eberhardt MS, Goldstein DE, Little RR, Wiedmeyer HM, Byrd-Holt DD: Prevalence of diabetes, impaired fasting glucose, and impaired glucose tolerance in U.S. adults: the Third National Health and Nutrition Examination Survey 1988–1994. *Diabetes Care* 21:518–524, 1998
- Brendel M, Hering B, Schulz A, Bretzel R: *International Islet Transplant Registry Report*. Giessen, Germany, Giessen University Hospital, 2001, p. 1–20
- Gruessner AC, Sutherland DE: Report for the International Pancreas Transplant Registry 2000. *Transplant Proc* 33:1643–1646, 2001
- Inverardi L, Ricordi C: Tolerance and pancreatic islet transplantation. *Philos Trans R Soc Lond B Biol Sci* 356:759–765, 2001
- Shapiro AM, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV: Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 343:230–238, 2000
- Kahan BD: Cyclosporine. *N Engl J Med* 321:1725–1738, 1989
- Group TUSMFLS: A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation: the U.S. Multicenter FK506 Liver Study Group. *N Engl J Med* 331:1110–1115, 1994
- de Mattos AM, Olyaei AJ, Bennett WM: Nephrotoxicity of immunosuppressive drugs: long-term consequences and challenges for the future. *Am J Kidney Disease* 35:333–346, 2000
- Ryan EA, Lakey JR, Rajotte RV, Korbutt GS, Kin T, Imes S, Rabinovitch A, Elliott JF, Bigam D, Kneteman NM, Warnock GL, Larsen I, Shapiro AM: Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol. *Diabetes* 50:710–719, 2001
- Sayegh MH, Turka LA: The role of T-cell costimulatory activation pathways in transplant rejection. *N Engl J Med* 338:1813–1821, 1998
- Lenschow DJ, Ho SC, Sattar H, Rhee L, Gray G, Nabavi N, Herold KC, Bluestone JA: Differential effects of anti-B7-1 and anti-B7-2 monoclonal antibody treatment on the development of diabetes in the nonobese diabetic mouse. *J Exp Med* 181:1145–1155, 1995
- Miller SD, Vanderlugt CL, Lenschow DJ, Pope JG, Karandikar NJ, Dal Canto MC, Bluestone JA: Blockade of CD28/B7-1 interaction prevents epitope spreading and clinical relapses of murine EAE. *Immunity* 3:739–745, 1995
- Finck BK, Linsley PS, Wofsy D: Treatment of murine lupus with CTLA4-Ig. *Science* 265:1225–1227, 1994
- Linsley PS, Wallace PM, Johnson J, Gibson M, Greene JL, Ledbetter JA, Singh C, Tepper MA: Immunosuppression in vivo by a soluble form of the CTLA-4 T-cell activation molecule. *Science* 257:792–795, 1992
- Larsen CP, Ritchie SC, Pearson TC, Linsley PS, Lowry RP: Functional expression of the costimulatory molecule, B7/BB1, on murine dendritic cell populations. *J Exp Med* 176:1215–1220, 1992
- Turka LA, Linsley PS, Lin H, Brady W, Leiden JM, Wei R, Gibson ML, Zheng X, Myrdal S, Gordon D, Bailey T, Bolling SF, Thompson CB: T-cell activation by the CD28 ligand B7 is required for cardiac allograft rejection *in vivo*. *Proc Natl Acad Sci U S A* 89:11102–11105, 1992
- Lin H, Bolling SF, Linsley PS, Wei RQ, Gordon G, Thompson CB, Turka LA: Long-term acceptance of major histocompatibility complex mismatched cardiac allografts induced by CTLA4-Ig plus donor-specific transfusion. *J Exp Med* 178:1801–1806, 1993
- Lenschow D, Zeng Y, Thistlethwaite J, Montag A, Brady W, Gibson M, Linsley P, Bluestone J: Long-term survival of xenogeneic pancreatic islet grafts induced by CTLA4-Ig. *Science* 257:789–792, 1992
- Levisetti MG, Padrid PA, Szot GL, Mittal N, Meehan SM, Wardrip CL, Gray GS, Bruce DS, Thistlethwaite JR Jr, Bluestone JA: Immunosuppressive effects of human CTLA4-Ig in a non-human primate model of allogeneic pancreatic islet transplantation. *J Immunol* 159:5187–5191, 1997
- Pearson T, Routenberg K, Abrams J, Linsley P, Larsen C: Prolongation of renal allograft survival with CTLA4-Ig in rhesus macaque (Abstract). In *Programs and Abstracts of the 17th American Society of Transplant Physicians Annual Meeting, Chicago, 10–14 May 1997*. Chicago, American Society of Transplant Physicians
- Kirk AD, Harlan DM, Armstrong NN, Davis TA, Dong Y, Gray GS, Hong X, Thomas D, Fechner JH, Knechtle SJ: CTLA4-Ig and anti-CD40L prevent renal allograft rejection in primates. *Proc Natl Acad Sci U S A* 94:8789–8794, 1997
- Abrams JR, Leibold MG, Guzzo CA, Jegasothy BV, Goldfarb MT, Goffe BS, Menter A, Lowe NJ, Krueger G, Brown MJ, Weiner RS, Birkhofer MJ, Warner GL, Berry KK, Linsley PS, Krueger JG, Ochs HD, Kelley SL, Kang S: CTLA4-Ig-mediated blockade of T-cell costimulation in patients with psoriasis vulgaris. *J Clin Invest* 103:1243–1252, 1999
- Ricordi C, Lacy PE, Finke EH, Olack BJ, Scharp DW: Automated method for isolation of human pancreatic islets. *Diabetes* 37:413–420, 1988
- Ranuncoli A, Cautero N, Ricordi C, Masetti M, Molano RD, Inverardi L, Alejandro R, Kenyon NS: Islet cell transplantation: in vivo and in vitro functional assessment of nonhuman primate pancreatic islets. *Cell Transplant* 9:409–414, 2000
- Ricordi C, Gray DW, Hering BJ, Kaufman DB, Warnock GL, Kneteman NM, Lake SP, London NJ, Socci C, Alejandro R: Islet isolation assessment in man and large animals. *Acta Diabetol Lat* 27:185–195, 1990
- Lobashevsky A, Smith JP, Kasten-Jolly J, Horton H, Knapp L, Bontrop RE, Watkins D, Thomas J: Identification of DRB alleles in rhesus monkeys using polymerase chain reaction-sequence-specific primers (PCR-SSP) amplification. *Tissue Antigens* 54:254–263, 1999
- Knapp LA, Lehmann E, Piekarczyk MS, Urvater JA, Watkins DI: A high frequency of Mamu-A*01 in the rhesus macaque detected by polymerase chain reaction with sequence-specific primers and direct sequencing. *Tissue Antigens* 50:657–661, 1997
- Watkins DI: The evolution of major histocompatibility class I genes in primates. *Crit Rev Immunol* 15:1–29, 1995
- Li Y, Li XC, Zheng XX, Wells AD, Turka LA, Strom TB: Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T-cells and induction of peripheral allograft tolerance. *Nat Med* 5:1298–1302, 1999
- Kenyon NS, Chatzipetrou M, Masetti M, Ranuncoli A, Oliveira M, Wagner JL, Kirk AD, Harlan DM, Burkly LC, Ricordi C: Long-term survival and function of intrahepatic islet allografts in rhesus monkeys treated with humanized anti-CD154. *Proc Natl Acad Sci U S A* 96:8132–8137, 1999
- Kirk AD, Burkly LC, Batty DS, Baumgartner RE, Berning JD, Buchanan K, Fechner JH Jr, Germond RL, Kampen RL, Patterson NB, Swanson SJ, Tadaki DK, TenHoor CN, White L, Knechtle SJ, Harlan DM: Treatment with humanized monoclonal antibody against CD154 prevents acute renal allograft rejection in nonhuman primates [see comments]. *Nat Med* 5:686–693, 1999
- Thomas JM, Contreras JL, Smyth CA, Lobashevsky A, Jenkins S, Hubbard WJ, Eckhoff DE, Stavrou S, Neville DM Jr, Thomas FT: Successful reversal of streptozotocin-induced diabetes with stable allogeneic islet function in a preclinical model of type 1 diabetes. *Diabetes* 50:1227–1236, 2001
- Montgomery SP, Hale D, Xu H, Tadaki DK, Berning JP, Leconte J, Kirk AD: Toxicity of rapamycin, tacrolimus, and daclixumab in the non-human primate (Abstract). *Am J Transplant* 1 (Suppl. 1):438, 2001