PROLONGED SURVIVAL OF RAT ISLET XENOGRAFTS IN MICE AFTER CD45RB MONOTHERAPY

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Background. **Pancreatic islet transplantation can correct the disordered glucose metabolism of type 1 diabetes, but the number of successful transplants has been low because of the need for long-term immunosuppression and the limited availability of human islets. New approaches, such as the use of tolerance-inducing treatment modalities and the use of islets of nonhuman sources, can possibly improve the success of islet transplantation. In the present study, the authors investigated the effect of anti-CD45RB treatment on the survival of islet xenografts.**

Methods. **Chemically induced diabetic mice underwent xenografting with rat islets and were treated with CD45RB antibodies on days −1, 0, and 5. Immunohistology and real-time polymerase chain reaction were used to study the effect of the treatment in the xenografts. The effect of anti-CD45RB treatment in peripheral blood of normal mice was measured with flow cytometry.**

Results. **In the treated mice, survival of the grafts was prolonged substantially. In the treated mice with functioning grafts, no lymphocytes were found infiltrating the transplanted islets on day 6; whereas in the untreated animals with functioning grafts, signs of rejection were evident. In the grafts of the treated animals, significantly less mRNA for interleukin (IL)-2, interferon-γ, and IL-4 was found compared with the untreated mice. After CD45RB treatment, there was depletion or decrease of CD45RB^bright cells from the peripheral blood.**

Conclusions. **Our results show that a short course of anti-CD45RB monotherapy prolongs the survival of rat islet xenografts in C57BL/6 mice.**

The major challenge in the treatment of type 1 diabetes mellitus is to prevent or delay the onset of diabetic complications and thereby improve the quality of life of the patient. The onset of diabetic complications can be delayed by tight control of blood glucose levels (*1, 2*). However, tight control of glucose levels by insulin therapy is frequently associated with episodes of hypoglycemia, and daily glucose profiles approach that of healthy humans but are still not normal.

It has been shown by several groups that adequate metabolic control can be achieved by successful transplantation of a pancreatic islet graft (*3, 4*). Unfortunately, however, the large-scale application of islet transplantation in type 1 diabetes has been hampered by the low success rates in humans, which are usually not more than 11%. Major factors have been responsible for these low success rates, including quantitative insufficiencies in the number of islets available for transplantation and the destruction of the islet graft as a consequence of ineffective immunosuppression (*5*). Only recently were successful islet transplants reported by the Edmonton group (*6*). However, the requirement for long-term immunosuppression limits the application of this protocol to a small subgroup of patients.

In another recent study, three of seven patients retained their grafts longer than 52 weeks. All three patients had a history of antithymocyte globulin (ATG) therapy at the time of prior kidney transplantation. In these patients, an absence of auto- and alloreactivity to islets was found. ATG is hypothesized to deplete autoreactive memory T cells (*7*). It has been shown that antibodies against CD45 are a main ingredient of polyclonal antisera such as ATG (*8–10*). Early studies showed that rabbit antisera to pure CD45 could completely suppress renal allograft rejection in rats, whereas large doses of a mouse monoclonal antibody to a common determinant of rat CD45 was not immunosuppressive (*11*).

The effective use of antibodies against CD45RB has been previously reported in transplantation and autoimmune disease. CD45RB antibody MB23G2 protects against rejection and reverses rejection in a mouse model for kidney transplantation. Two injections of the monoclonal antibody (mAb) on days 0 and 1 induced indefinite allograft acceptance and subsequent tolerance to skin transplants (*12*). Similar results were found in islet allografts in mice (*13, 14*) and in an experimental allergic encephalomyelitis model (*15*).

The mechanism by which CD45RB antibodies induce tolerance is still unclear, but the depletion of a subset of CD45RB^bright T cells from the peripheral blood appears to play an important role. As a consequence, the composition of the infiltrates and the cytokine profile in the grafts is also changed. In the islet allografts, IL-4 and IL-10 mRNA were found to be increased (*13*), indicating that the T-helper (Th)1-Th2 balance had shifted toward a Th2 immune response by the anti-CD45RB treatment.

In the present study, we tested the efficacy of CD45RB antibody MB23G2 in a xenograft model for islet transplantation. To this end, we compared the success rates of CD45RB-treated mice and untreated controls. In functioning and nonfunctioning grafts, we studied and compared the type and quantity of infiltrating cells and the expression of mRNA for cytokines. In a separate experiment, we investigated the kinetics of the depletion of CD45RB^bright cells from peripheral blood after treatment with CD45RB.

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MATERIALS AND METHODS

Study Design

Islet grafts composed of 1,000 Albino Oxford (AO) rat islets were transplanted under the right kidney capsule of streptozotocin-induced diabetic C57BL/6 mice. One group of mice received three intravenous injections with rat anti-mouse monoclonal antibody MB23G2 (HB220) against CD45RB on days −1, 0, and 5. The other group of mice received no treatment and served as the control group. Recipients of islet grafts were followed and monitored for blood glucose levels every second day until the recurrence of hyperglycemia.

For studies of the infiltrating cells and for cytokine expression in functioning and nonfunctioning grafts, we killed anti-CD45RB–treated mice and control mice on day 6 after transplantation and after the recurrence of hyperglycemia. The kidneys containing the islet grafts were explanted and processed for histologic examination and for RNA isolation.

In a separate experiment, we studied the effect of CD45RB treatment on the composition of peripheral blood lymphocytes. To this end, the expression of CD45RB on the peripheral blood lymphocytes of mice treated with CD45RB was measured during several days by flow cytometry.

Animals and Induction of Diabetes

Male AO rats (Harlan CPB, Zeist, The Netherlands) weighing 300 to 320 g served as donors. Male inbred C57BL/6 mice (Harlan) weighing 18 to 20 g were used as recipients of islet grafts. Diabetes was induced by intraperitoneal injection of 180 to 200 mg/kg (*16*) of streptozotocin (Sigma, St. Louis, MO). Glucose concentration in blood was determined with glucose test tapes (Reflolux; Boehringer Mannheim, Germany). A second injection of 180 mg/kg of streptozotocin was administered if the blood glucose level was lower than 20 M at 5 days after the first injection. Only animals with severe weight loss, polyuria, polydipsia, and blood glucose levels exceeding 20 M were used as recipients.

Islet Isolation

Islets were isolated as previously described (*17*). Briefly, under halothane anesthesia, the abdomen was opened and the common bile duct was cannulated under nonsterile conditions. The donor pancreas was distended with 10 mL of sterile Krebs-Ringer-HEPES supplemented with 25 M HEPES buffer and containing 10% bovine serum albumin. Subsequently, the pancreas was excised and brought into a laminar flow cabinet. All further procedures were performed under sterile conditions.

The pancreas was chopped and digested using a two-stage incubation of 20 min at 37°C with 1.0 and 0.7 mg/mL, successively, of collagenase (Sigma type XI; Sigma). Islets were separated from exocrine tissue by centrifugation over a discontinuous dextran gradient (*18*) and further purified by handpicking. The islets were divided into portions of 1,000 islets.

Transplantation and Explantation of the Grafts

Transplantation was performed immediately after the islet isolation. Transplantation under the kidney capsules was performed under the upper pole by carefully expelling the islets from a polyethylene tube introduced at the lower pole of the kidney. In a previous study, we found that in xenografts with AO islets, 1,000 islets resulted in an optimum outcome; as a consequence, 1,000 islets were transplanted per mouse. Glycemia of less than 10 M and greater than 20 M on 3 successive days defined primary graft function and graft loss, respectively.

Explantation was performed by laparotomy and careful but fast excision of the right kidney containing the islet graft. The islet graft was split by bisecting the kidney perpendicularly through the graft. One portion was immediately fixed for histologic examination, and the other portion was snap-frozen to be used for isolation of RNA and immunohistology. Pancreas biopsy specimens were checked for the presence of islets.

Treatment Protocols

Anti-CD45RB antibody MB23G2 (HB220) (*19*) was obtained from American Type Culture Collection (ATCC, Rockville, MD), and antibody was purified over protein G. C57BL/6 recipients received 100 μg of antibody MB23G2 (CD45RB) administered intravenously on days −1, 0, and 5 under general isoflurane anesthesia. Untreated recipients served as controls. Two anti-CD45RB–treated mice and two controls were killed on day 6 for immunohistology and real-time polymerase chain reaction (PCR) of cytokines. Twelve treated and four untreated animals were killed after the recurrence of hyperglycemia. In a second identical experiment, four anti-CD45RB–treated mice, three untreated controls, and two immunoglobulin-treated controls (rat immunoglobulin [Ig] G2a isotype control; BD Biosciences, San Diego, CA) were killed on day 6 for immunohistology and real-time PCR. Statistical analysis of survival was performed with Kaplan-Meier analysis, and differences were assessed with log-rank testing.

Histology

Half of the right kidney containing the islet graft was snap-frozen and used for immunophenotyping. Sections were prepared at 5 μm and processed for immunohistochemical staining according to standard procedures. The monoclonal antibodies used were CD3 (KT3; Serotec, Oxford, United Kingdom), CD4 (L3T4), CD8 (Lyt2), CD25 (PC61-5.3), B220 (RA3-3A1), and CD45RB (MB4G4) (ATCC). To visualize the bound mAb, we used peroxidase-conjugated goat anti-rat IgG (Jackson Immunoresearch, West Grove, PA) and 3-amino 9-ethylcarbazole and hydrogen peroxide (Sigma) for immunoperoxidase staining. The presence and type of infiltrating cells was scored semiquantitatively by microscopy.

Real-Time PCR Analysis for Cytokines

Total RNA was isolated with the Absolutely RT-PCR Miniprep Kit (Stratagene, La Jolla, CA); cDNA synthesis was primed with random hexamers using the buffer provided by the manufacturer for 1 hr at 37°C (Gibco BRL, Paisley, United Kingdom). Primers and probes used for the amplification were described previously (*20*). Reactions were performed in 20 μL in 384-well plates (Applied Biosystems, Foster City, CA) using real-time PCR MasterMix (Eurogentec, Liege, Belgium) supplemented with 900 nM of each primer and 200 nM of probe. Standard cycling conditions (40 cycles: 25 sec at 95°C and 60 sec at 60°C) including a preamplification step (2 min at 50°C and 10 min at 95°C) were performed on the ABI PRISM 7900 Sequence Detection System instrument (PE Applied Biosystems). All samples were analyzed in triplicate. Mean cycle threshold values (Ct) and standard deviations (SD) were calculated for cytokine and housekeeping genes. The amount of cytokine target was normalized relative to the amount of housekeeping gene ($\Delta Ct = Ct_{(gene)} - Ct_{(GAPDH)}$) and the SD of the ΔCt [$SD(\Delta Ct)$] was calculated [$SD(\Delta Ct) = \sqrt{((SD_{gene})^2 + (SD_{GAPDH})^2)}$]. The relative amount of cytokine was measured by determining the $\Delta\Delta Ct$ ($\Delta\Delta Ct = \Delta Ct_{calibrator} - \Delta Ct_{testsample}$) and the factor difference is calculated ($2^{-\Delta\Delta Ct}$). The range is given as $2^{-\Delta\Delta Ct + SD\Delta Ct}$ and $2^{-\Delta\Delta Ct - SD\Delta Ct}$.

Depletion of CD45RB^{bright} Peripheral Blood Lymphocytes

C57BL/6 mice (Harlan) were used to test the effect of MB23G2 on the expression of CD45RB in peripheral blood. Mice received 100 μg MB23G2 intravenously on days 0, 1, and 6. Blood samples were taken by orbital punction on days −3, 3, 7, 11, 14, and 17. White blood cell counts were performed on a Sysmex counter (TOA Medical Electronics, Hamburg Germany). Statistical analysis was performed. Blood was separated by Ficoll-Paque (Amersham Pharmacia Biotech, Roosendaal, The Netherlands) density centrifugation and the mononuclear cells were collected. Cells were stained with MB23G2

and phycoerythrin-labeled anti-rat Ig (Southern Biotechnologies Associates, Birmingham, AL). Cells were analyzed by flow cytometry (Elite; Coulter, Hialeah, FL).

RESULTS

No animals were lost because of primary nonfunctioning transplants. All 12 anti-CD45RB–treated streptozotocin-induced diabetic mouse recipients of a rat xenograft became normoglycemic within 3 days after transplantation. The survival was 22 to 75 days (22, 22, 26, 29, 31, 32, 38, 40, 42, 50, 61, and 75 days) (Fig. 1), with a median of 32 days. The untreated control animals also became normoglycemic within 3 days but started to reject their graft within the next week, as evidenced by an increase in the blood glucose levels starting at day 8. Statistical analysis showed significant differences in the survival between the treated and untreated groups ($P<0.005$).

Cellular Infiltrates in the Grafts

In the still functioning nontreated control grafts at day 6, many lymphocytes infiltrating (i.e., insulitis) and surrounding the islets were found. The lymphocytes were mainly T cells (CD3$^+$) (Fig. 2A), but also some B cells were present. Both CD4$^+$ and CD8$^+$ cells were present, some activated as shown by expression of CD25. In the 6-day-old grafts of anti-CD45RB–treated animals, fewer lymphocytes were found, there were no cells infiltrating the islets, and most of the cells were CD3$^+$ (Fig. 2B).

After graft failure, in all grafts, remains of β cells and fibrotic tissue were present. In the tissue surrounding the graft, CD4$^+$ and CD8$^+$ T cells were observed, of which some expressed CD25. There were some B cells present.

Cytokine Profiles in the Graft

Two untreated and two treated animals, all normoglycemic, were killed on day 6 to compare cytokine mRNA levels by real-time PCR. In a second experiment, three untreated, two immunoglobulin-treated, and four CD45RB-treated animals, all normoglycemic, were killed at the same time point. The PCR results are given for both experiments separately (Table 1). The Ig-treated animals show results comparable to
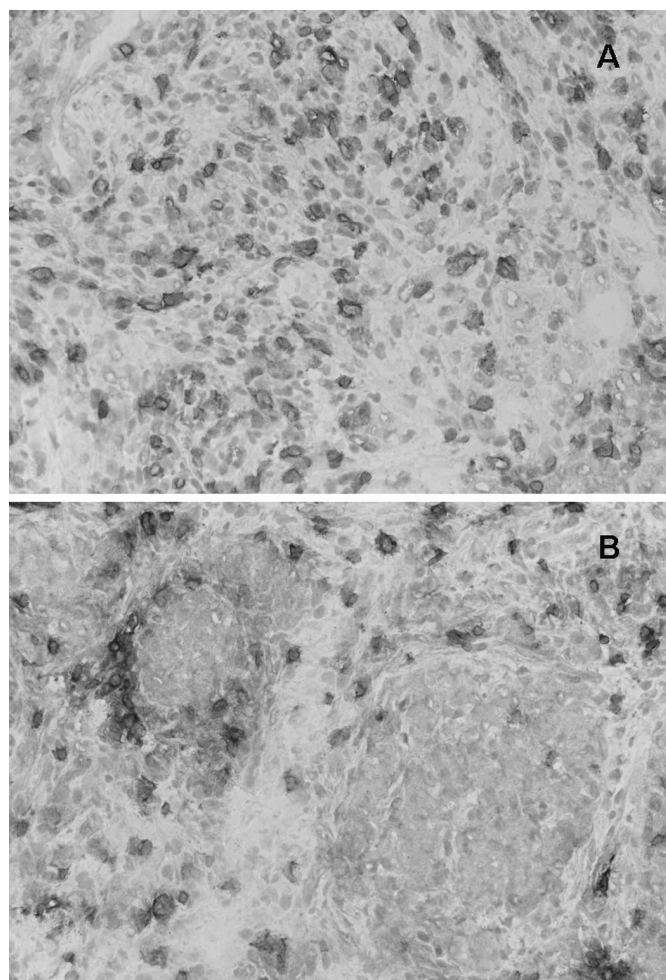
FIGURE 2. Immunoperoxidase staining for CD3 on explants of (A) an untreated animal, with a diffuse infiltrate of CD3$^+$ cells, and (B) a CD45RB-treated animal, with CD3$^+$ cells surrounding intact islets.
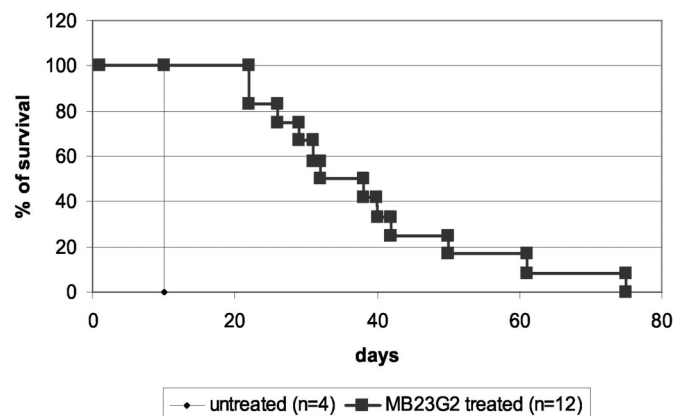
FIGURE 1. Survival of diabetic mice that underwent transplantation with rat islets. Untreated controls (n=4) survived 10 days after transplantation. Survival was prolonged after treatment with a CB45RB antibody (MB23G2) on days −1, 0, and 5 (n=12).

the untreated animals in interferon (IFN)-γ, IL-4, and IL-10. The IL-2 data show lower levels in the Ig-treated animals than in the untreated group. For IL-4, we found no signal after 40 cycles of amplification in five of six CD45RB-treated animals, whereas in the untreated and Ig-treated animals, IL-4 mRNA was definitely present. We performed statistical analyses of the untreated and CD45RB-treated animals (Fig. 3). The number of Ig-treated animals is too low to be included. There is a significant difference in the IL-2, IFN-γ, and IL-4 mRNA amounts; the CD45RB-treated mice show a decrease compared with the untreated mice. For IL-10, the change in mRNA expression is not significant, but a trend toward a lower level is seen.

### Depletion of CD45RB^{bright} Peripheral Blood Lymphocytes

Treatment with the anti-CD45RB antibody MB23G2 causes depletion of peripheral blood lymphocytes. On days 3 and 7, a significant decrease in white blood cell counts was seen after treatment. On day 3, the counts dropped to 50% and on day 7 to 30% of the counts on day 0 (Fig. 4). Although the white blood cell counts stayed low up to day 14, there was no significant change on days 11 and 14 compared to the

TABLE 1. Real-time PCR data for the quantitative expression of cytokines expressed in the factor difference normalized to an untreated control

Mouse	Treatment	Factor (range)			
		IL-2[a]	IFN-γ[a]	IL-4[a]	IL-10[a]
Experiment 1					
1	None	1.00 (0.90–1.11)	1.00 (0.93–1.08)	1.00 (0.73–1.38)	1.00 (0.82–1.22)
2	None	0.53 (0.41–0.70)	1.79 (1.71–1.87)	2.78 (2.11–3.67)	4.64 (4.37–4.92)
3	CD45RB	0.40 (0.23–0.68)	0.29 (0.27–0.32)	[b]	0.87 (0.66–1.15)
4	CD45RB	0.22 (0.15–0.30)	0.09 (0.07–0.11)	0.38 (0.15–0.97)	0.33 (0.14–0.78)
Experiment 2					
1	None	1.00 (0.89–1.12)	1.00 (0.92–1.09)	1.00 (0.60–1.67)	1.00 (0.44–2.26)
2	None	0.99 (0.86–1.15)	0.86 (0.82–0.89)	4.09 (2.17–7.68)	2.87 (2.49–3.30)
3	None	0.34 (0.14–0.81)	1.14 (1.05–1.22)	1.53 (0.57–4.08)	3.39 (2.43–4.71)
4	Ig	0.59 (0.30–1.13)	1.40 (1.38–1.43)	2.06 (1.40–3.02)	4.67 (4.32–5.04)
5	Ig	0.26 (0.08–0.83)	1.49 (1.47–1.51)	3.44 (2.73–4.32)	6.51 (6.02–7.01)
6	CD45RB	0.12 (0.09–0.16)	0.02 (0.02–0.02)	[b]	0.28 (0.19–0.42)
7	CD45RB	0.53 (0.38–0.74)	0.20 (0.19–0.22)	[b]	1.79 (1.34–2.40)
8	CD45RB	0.21 (0.15–0.29)	0.05 (0.04–0.06)	[b]	0.61 (0.43–0.85)
9	CD45RB	0.43 (0.33–0.56)	0.04 (0.04–0.05)	[b]	1.67 (1.10–2.55)

[a] Expressed as the factor difference normalized to a housekeeping gene and relative to the calibrator (one of the untreated animals).
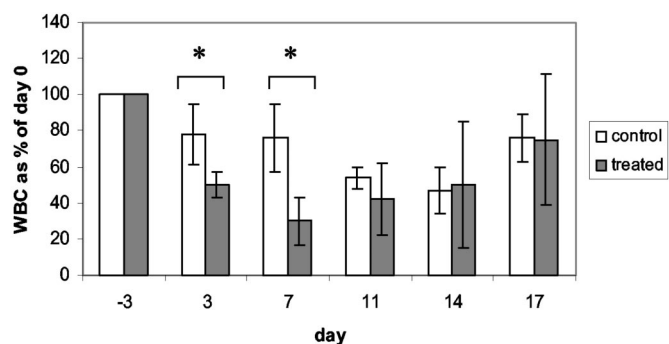[b] No signal was obtained after 40 cycles of amplification.
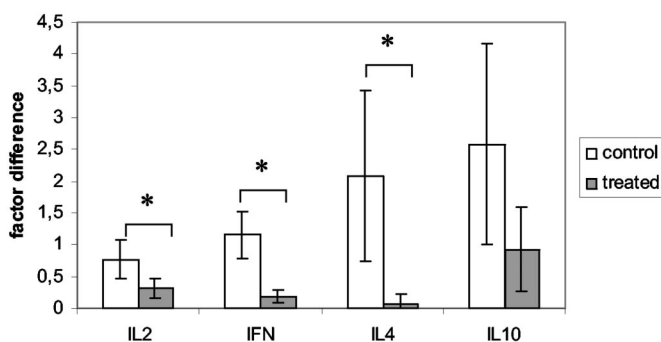
FIGURE 3. Comparison of the means of the factor differences as found with real-time PCR for quantitative expression of cytokines. There is a significant decline of IL-2 ($P=0.03$), IFN-γ ($P=0.002$), and IL-4 expression ($P=0.03$). IL-10 expression shows a lower trend but is not significant ($P=0.08$)

FIGURE 4. Cell counts in peripheral blood as a percentage of initial counts on day −3. The CD45RB-treated animals show a decrease on days 3 and 7 in cell counts (medians for both groups, n=7). When comparing the counts of the individual days in a Student's *t* test, the decrease on days 3 and 7 are significant ($P=0.04$ and $P=0.01$, respectively). WBC, White blood cell count.

untreated controls. At the start of the experiment, 93% of peripheral blood lymphocytes were positive for CD45RB and 55% of the lymphocytes showed bright staining. On day 3, after treatment on days 0 and 1, 60% were positive for CD45RB, but only 5% stained bright for CD45RB. On day 7, after a third treatment on day 6, we found similar percentages (6% and 68%). On day 11, we found 87% of cells positive for CD45RB, and 35% were CD45RB^bright cells. At the measurement on day 14, we found 78% CD45RB+ cells and 59% with bright expression. Thus, treatment of C57BL/6 mice with the rat anti-mouse CD45RB antibody MB23G2 reduced the CD45RB^bright population for a week, but the population had already partially returned 5 days after the last injection (Fig. 5). In a separate set of experiments in BALB/C mice, we found similar results.

DISCUSSION

This study shows that treatment with antibodies against CD45RB on days −1, 0, and 5 prolongs the survival of xenografted rat islets in chemically induced diabetic mice. Survival in untreated mice was approximately 10 days, with 7 days of normoglycemia, whereas the CD45RB-treated animals remained normoglycemic from 17 to 70 days (survival, 22–75 days). Prolonged xenograft survival in treated mice was associated with a lack of lymphocyte infiltration of the graft and differences in intragraft cytokine mRNA levels.

Although the untreated animals showed extensive lymphocytic infiltration and insulitis as a sign of rejection on day 6, the grafts of the treated mice contained less lymphocytes and no insulitis. Rejection was thus postponed several days. It has been hypothesized that the mechanisms involved in anti-CD45RB therapy include depletion and redistribution or decrease of cell surface molecules on a subset of peripheral blood lymphocytes (*12, 21*). The anti-CD45RB antibody MB23G2 reduces the CD45RB^bright T-cell population for at least a week. This population contains mainly the naive and Th1 subsets and most CD8 cells (*22*). These subsets contain the allo- and xenoreactive precursor cells and the cells helping to maintain a Th1 response. The CD45RB^dim cells, which include Th2 and immunoregulatory cells, remain (*23–25*).
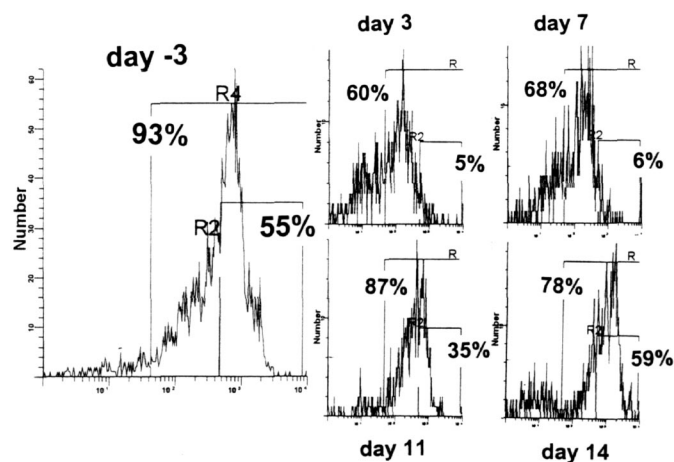
FIGURE 5. Flow cytometry results of CD45RB expression in peripheral blood lymphocytes. Percentages of positive cells in histograms (at left for total CD45RB$^+$ cells; at right for CD45R$^{bright+}$ cells) are given. The CD45R^{bright} population is absent at days 3 and 7, after treatment with MB23G2 on days 0, 1, and 6. Graphs of representative mice are shown.

The presence of mRNA for IL-2, IFN-γ, IL-4, and IL-10 in the control mice on day 6 is an indication for the occurrence of rejection. In the treated mice, a decrease in IFN-γ mRNA and IL-2 is found on day 6. Of the Th2 cytokines, the IL-4 mRNA is also significantly decreased, with no signal detected after 40 cycles in five of six mice. IL-10 also shows a less definite decline. It can be concluded that Th1 and Th2 cytokines are decreased after CD45RB treatment in this model.

The findings in the islet xenograft model are different from those in the islet allograft model, because mRNA for IL-4 and IL-10 was increased on day 6 in the treated allografts, whereas the expression of IL-2 and IFN-γ was unchanged (*13*). This suggests that in the allograft model, a shift toward a Th2 immune response occurred, whereas in the xenograft model, apparently the Th1 and Th2 responses were suppressed. This lack of a Th2 response may explain the absence of long-term tolerance in the xenograft model.

The xenograft survival in this model is modestly prolonged in the majority of the animals, whereas in the islet allograft model, long-term survival (>120 days) was achieved in 50% of the animals (*13*). The same treatment protocol and the same recipient strain of mice were used, so the difference in survival is probably attributable to differences in the response to the xenografts. The CD45RB antibody MB23G2 has been tested in other models for xenotransplantation. In a heart and kidney model, both of which were also rat-to-mouse models, the antibody by itself had no effect on graft survival, whereas in our study, the survival of islets improved significantly. Prolonged survival in both the heart and kidney xenograft model was achieved with a treatment protocol of 9 days of monoclonal antibody in combination with cyclophosphamide for 7 days. In the kidney model, 33% of graft survival was indefinite after treatment with a combination of CD45RB and cyclophosphamide (*26, 27*). Cyclophosphamide used in combination with anti–T-cell monoclonal antibodies also prolongs survival of fetal pig islet grafts in nonobese diabetic mice (*28*). The addition of cyclophosphamide to the anti-CD45RB treatment protocol can possibly further improve the survival of islet xenografts. In two recently published models in which a pig kidney cell line (*29*) or pig metanephroi were transplanted into the peritoneum or omentum of mice (*30*), a combination of CD45RB and costimulation blockade improved graft survival and reduced T-cell infiltration.

CONCLUSION

A short course of anti-CD45RB therapy significantly prolonged the survival of islet xenografts. The absence of a Th2 infiltrate in the early phase constitutes a difference with islet allografts and may explain the finding that no long-term tolerance develops in this model. Data in the literature suggest that a combination of anti-CD45RB with cyclophosphamide or costimulation blockade may significantly improve the results.

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EFFECT OF FK506 ON DONOR T-CELL FUNCTIONS THAT ARE RESPONSIBLE FOR GRAFT-VERSUS-HOST DISEASE AND GRAFT-VERSUS-LEUKEMIA EFFECT

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Background. FK506 is a potent immunosuppressive agent that is used in human graft-versus-host disease (GvHD) prevention. However, the precise mechanisms for GvHD prevention and the effect on graft-versus-leukemia (GvL) activity are unknown. This study was undertaken to determine the effect of FK506, given at clinically relevant doses, on donor T-cell functions responsible for GvHD and GvL activity.

Methods. The effect of FK506 on GvHD prevention and GvL activity was investigated using a murine model of allogeneic bone-marrow transplantation in which mice were injected with a P815 leukemic cell line. The regulatory role of FK506 on donor T cells was tested by analysis of donor T-cell expansions in the spleen and donor anti-host T-cell proliferative and cytotoxic responses. mRNA expression of type 1 T helper (Th1), Fas ligand (L), and granzyme B were also evaluated in target organs of GvHD.

Results. FK506 significantly prolonged the survival of GvHD mice when given at the trough level of 17.6 ng/mL, whereas it also blocked GvL effect in P815-injected GvHD mice. FK506 reduced the expansion of donor CD8$^+$ and, to a lesser extent, CD4$^+$ T cells in the spleen and inhibited donor anti-host T-cell proliferative and cytotoxic responses. It also inhibited the induction of Th1, FasL, and granzyme B mRNA expression in target organs of GvHD.

Conclusions. FK506 inhibits both GvHD and GvL activity when given at clinical doses by inhibiting donor T-cell expansion, donor anti-host T-cell reactivity, and Th1 immune responses.

FK506 is known as a potent immunosuppressive agent, and its mechanism of action is thought to be caused by the inhibition of calcineurin phosphatase (*1, 2*). The inhibition of calcineurin phosphatase in T cells leads to impairment of interleukin (IL)-2 gene transcription, and thus this inhibition results in immunosuppression (*3, 4*). FK506 is currently used clinically to prevent rejection of organ allografts and to block graft-versus-host disease (GvHD) after allogeneic hematopoietic stem-cell transplantation (HSCT) (*5–7*). The immunosuppressive potential of FK506 has been tested using animal models of GvHD, and it has been found effective in preventing the mortality of GvHD mice (*8, 9*). However, limited information is available concerning details of the protective effect of FK506 on host target organ damage caused by GvHD or on donor T-cell functions that are responsible for GvHD in animal models of GvHD. Furthermore, in view of the facts that graft anti-host reactions caused by donor T cells induce graft-versus-leukemia (GvL) effect and that FK506 suppresses T-cell functions, we questioned whether FK506 administered at doses effective in preventing GvHD would have any adverse effects on GvL activity. The present study was undertaken to determine (1) whether the GvL effect was reduced by FK506 administration at doses used in the clinic,

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