Experimental Short-term Immunosuppression After Bowel Transplantation and Donor-Specific Bone Marrow Infusion

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Hypothesis: We previously showed in a large animal pig model that unmodified donor-specific bone marrow infusion (DSBMI) did not facilitate total bowel engraftment; in contrast, it increased the risks of rejection, infection, and graft-vs-host disease (GVHD) posttransplant. We hypothesize that continuous immunosuppression, in combination with DSBMI, might contribute to—or even trigger—these unwarranted immune responses by both host and graft; therefore, discontinuing immunosuppression might decrease these risks and prolong survival.

Methods: Six groups of outbred, mixed lymphocyte culture–reactive pigs underwent a total (small and large) bowel transplant: group 1, nonimmunosuppressed control pigs (n=5); group 2, nonimmunosuppressed DSBMI pigs (n=6); group 3, tacrolimus (indefinite) pigs (n=7); group 4, tacrolimus (indefinite) plus DSBMI pigs (n=7); group 5, tacrolimus (10 days only) pigs (n=5); and group 6, tacrolimus (10 days only) plus DSBMI pigs (n=6).

Results: The combination of short-term immunosuppression and DSBMI (group 6) significantly prolonged survival, compared with short-term immunosuppression only (group 5) or DSBMI only (group 2). Short-term immunosuppression and DSBMI (group 6) did not prolong overall survival, compared with indefinite immunosuppression with (group 4) or without (group 3) DSBMI: survival rates at 7, 14, and 28 days posttransplant were 100%, 100%, and 67% in group 6; 100%, 100%, and 71% in group 3; and 100%, 67%, and 47% in group 4 (*P*=.14). Short-term immunosuppression and DSBMI (group 6) increased the incidence of rejection, infection, and GVHD, compared with indefinite immunosuppression without (but not with) DSBMI.

Conclusions: Short-term immunosuppression and DSBMI did not prolong survival and did not reduce the incidence of death from rejection, infection, or GVHD, compared with indefinite immunosuppression without DSBMI. But short-term immunosuppression and DSBMI resulted in a lower incidence of death from infection and GVHD, compared with indefinite immunosuppression and DSBMI. When immunosuppression was discontinued 10 days posttransplant, the effect of DSBMI was insufficient to avert death from rejection.

Clinical Relevance: The clinical results of bowel transplantation trail those of other solid organ transplants. It reduced the rates of infection and GVHD. Our study shows that systemically infused donor-specific bone marrow with short-term or indefinite immunosuppression does not improve outcome after bowel transplantation. It seems necessary to modify the time, dosing, routing, and/or composition of donor-specific bone marrow before it can be successfully used in clinical bowel transplantation.

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EXPERIMENTAL models have shown that unmodified donor-specific bone marrow infusion (DSBMI) with and without cytoablation can induce specific graft tolerance after kidney,[1] heart,[2] liver,[3] and islet[4,5] transplantation. The benefit, however, has been less obvious after clinical kidney,[6,7] liver,[8] and pancreas[9] transplantation. Thus far, clinical studies of DSBMI without cytoablation have shown no significant improvement in graft survival, although augmentation of chimerism has been demonstrated.[10,11]

In contrast to most other solid organs, the role of DSBMI has rarely been studied in bowel transplantation. We have previously shown in a pig model of total (ie, small and large) bowel transplantation using standard immunosuppression that DSBMI without cytoablation, rather than promoting engraftment, can sensitize recipients and cause rejection. In addition, DSBMI aggravated the risk of generalized graft-vs-host disease (GVHD) and infection, and reduced graft and pig survival.[12] Using standard immunosuppression, we added a cytoablative regimen (nonlethal whole body irradiation and antithymocyte globulin) to our DSBMI protocol, in an attempt to make space for cotransplanted bone marrow cells to fully expose their tolerogenic potential. But, cytoablation combined with DSBMI further

MATERIALS AND METHODS

For this randomized, prospective large animal study, we used 72 outbred nonrelated Yorkshire Landrace pigs. Mean±SD donor weight was 26.5±0.6 kg, and mean±SD recipient weight was 29.6±1.3 kg. The pigs were randomized as donors (n=36) or as recipients (n=36). We studied 6 groups of total bowel transplant recipients according to the use of (1) indefinite vs short-term immunosuppression and (2) DSBMI without cytoablation.

Group 1 was composed of nonimmunosuppressed control pigs (n=5); group 2, nonimmunosuppressed DSBMI pigs (n=6). In groups 3 and 4, tacrolimus was given indefinitely; DSBMI was not given in group 3 (n=7) but was in group 4 (n=7). In groups 5 and 6, tacrolimus was given for only 10 days posttransplant; DSBMI was not given in group 5 (n=5), but was in group 6 (n=6).

MIXED LYMPHOCYTE CULTURE

We used only reactive (per mixed lymphocyte culture [MLC]) donor-recipient pairs. The standard technique of MLC has been used in our previous studies and described in detail.[12,13]

ANIMAL PREPARATION, SURGICAL PROCEDURES, AND POSTTRANSPLANT CARE

For all surgical procedures, pigs were premedicated with atropine (0.2 mg/kg intramuscularly) and thiopental sodium (30 mg/kg intravenously); general anesthesia was maintained with 3% isoflurane. Donor and recipient pigs were fasted for 72 hours before surgery and maintained on intravenous fluids.

Donor and recipient operations are detailed elsewhere.[12-14] In the donor, the bowel graft (duodenum to sigmoid colon) was procured with the portal vein and an aortic tube containing the superior mesenteric artery.

In the recipient, a subtotal enterectomy (third portion of the duodenum to sigmoid colon) was done. The donor aortic conduit was anastomosed end-to-side to the recipient infrarenal aorta. The donor portal vein was anastomosed to the recipient portal vein in a piggyback fashion. Intestinal continuity was directly restored through construction of a duodenojejunostomy and a colocolostomy. A Bishop-Koop ileostomy was constructed to procure daily biopsy specimens of the intestinal graft.

Antibiotic prophylaxis posttransplant was with cephalothin (500 mg every day), ticarcillin (1 g twice daily), and metronidazole (250 mg every day) for 7 days. Pigs losing more than 30% of their initial body weight were killed according to the guidelines of the University of Minnesota's Research Animal Resource Committee.

BONE MARROW PREPARATION

Fresh donor bone marrow was obtained from the exsanguinated donor at the time of procurement. Bilateral long bones served as donor bones for marrow collection. After removing the bone fragments and debris by centrifugation, we prepared single-cell suspensions by multiple pipetting. Subsequently, mononuclear cells were isolated from the bone marrow cell suspensions by Ficoll-Hypaque density gradient separation work.[5] Mononuclear cells were washed in minimal essential medium, checked for viability by trypan blue exclusion, and counted. Bone marrow mononuclear cells (5×10^8 cells/kg) were infused intravenously into recipient pigs within 2 to 4 hours posttransplant.

POSTTRANSPLANT IMMUNUSUPPRESSION

Tacrolimus was started at 0.2 mg/kg per day, and then adjusted to maintain trough levels, determined by a microparticle enzyme immunoassay (ABBOTT IMX; Abbott Laboratories, Abbott Park, Ill) of 10 to 30 ng/mL posttransplant. Tacrolimus was given indefinitely in groups 3 and 4, but only for 10 days posttransplant in groups 5 and 6. In groups 3 and 4, prednisone, used for induction and maintenance, was started at 2 mg/kg per day, then reduced by 50% at 8 days and again at 15 days. In groups 5 and 6, it was also started at 2 mg/kg per day and reduced by 50% at 8 days, but then it was discontinued at 10 days. Immunosuppressants were given intravenously. Rejection episodes were not treated in any group.

POSTTRANSPLANT BIOPSIES AND AUTOPSIES

Biopsies from the ileostomy were done daily to assess interstitial and vascular rejection. Skin samples were obtained weekly to assess cutaneous graft-vs-host reactions. At autopsy, the following tissues were examined histologically: the transplanted intestine, the native duodenum and colon, the liver, the lungs, and the skin. We used our previously published scoring system[15] to grade the extent of both interstitial and vascular rejection of the bowel. Histologic studies were done by a single pathologist (R.E.N.) in a blinded fashion.

For each pig, the cause of death (rejection, infection, GVHD, and other) was defined by the clinical course, as well as microscopic and macroscopic findings. Histologic changes had to be graded as severe to account for animal death. Death from rejection was defined by the presence of grade 3 (severe) interstitial rejection in the bowel at autopsy. Death from GVHD was defined by the typical clinical course (lethargy, cachexia, skin rashes) and by the typical histologic features of severe GVHD: in the liver by a pronounced mononuclear infiltrate within the portal tracts with invasion and damage to the bile ducts; in the native intestine (duodenum and sigmoid colon) by inflammation of the lamina propria with individual necrosis of enterocytes in the crypts; and in the skin by dermal infiltration by mononuclear cells and keratinocyte necrosis. Death from infection was defined as death from pneumonia or peritonitis.

STATISTICAL ANALYSIS

Death from rejection, infection, or GVHD was analyzed according to the Kaplan-Meier method. Multiple causes of death were listed if clinical and histologic changes were considered severe for more than 1 condition. The log-rank test was used to determine late differences. The Wilcoxon test was used to determine early differences.

increased the incidence of death from rejection, GVHD, or infection.[13] We then hypothesized that continuous immunosuppression after bowel transplantation, in combination with DSBMI, might contribute to—or even trigger—unwarranted immune responses by both host and graft. The purpose of this study was to investigate the effect of short-term immunosuppression on the incidence of rejection, GVHD, and infection in our pig model of total bowel transplantation and DSBMI.

RESULTS

OVERALL SURVIVAL

In group 1 (control pigs), survival rates at 7, 14, and 28 days posttransplant were 60%, 0%, and 0%; group 2 (DSBMI pigs), 69%, 0%, and 0%; group 3 (pigs receiving tacrolimus indefinitely), 100%, 100%, and 71%; group 4 (pigs receiving tacrolimus indefinitely plus DSBMI), 67% and 47%; group 5 (pigs receiving tacrolimus for 10 days), 100%, 20%, and 0%; and group 6 (pigs receiving tacrolimus for 10 days plus DSBMI), 100%, 100%, and 67%, respectively. The difference in survival between the 6 groups was highly significant ($P \le .0001$) (**Figure 1**).

In group 6, overall survival was not different compared with group 3 ($P \le .88$) or group 4 ($P \le .17$). Overall survival was significantly higher in group 6 than in group 5 ($P < .002$). In group 5, overall survival was not different compared with group 1 ($P = .80$) or group 2 ($P = .90$).

Median survival times were as follows: group 1, 8 days (range, 4-13 days); group 2, 8 days (range, 7-14 days); group 3, 37 days (range, 21-49 days); group 4, 21 days (range, 9-61 days); group 5, 12 days (range, 10-16 days); and group 6, 39 days (range, 17-195 days).

DEATH FROM REJECTION

For this analysis, only deaths from rejection were counted as graft failures. In group 1, the death rate from rejection at 7, 14, and 28 days was 40%, 100%, and 100%; group 2, 31%, 100%, and 100%; group 3, 0%, 0%, and 0%; group 4, 0%, 27%, and 34%; group 5, 0%, 60%, and 100%; and group 6, 0%, 0%, and 33%, respectively ($P \le .0001$) (**Figure 2**).

In group 6, the death rate from rejection was not different compared with group 4 ($P \le .71$). The death rate from rejection was higher in group 6 than in group 3 ($P < .08$), but did not reach significance. The death rate from rejection was significantly lower in group 6 than in group 5 ($P \le .007$).

In group 5, the death rate from rejection was not different compared with group 1 ($P = .90$) or group 2 ($P = .80$).

DEATH FROM INFECTION

For this analysis, only deaths from infection were counted as graft failures. In group 1, the death rate from infection at 7, 14, and 28 days was 0%, 0%, and 0%; group 2, 8%, 26%, and 26%; group 3, 0%, 0%, and 17%; group 4, 0%, 23%, and 40%; group 5, 0%, 50%,
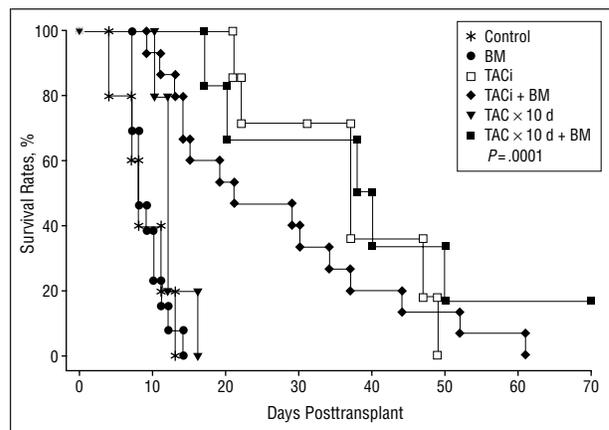

Figure 1. Overall pig survival rates. TACi indicates indefinite tacrolimus; BM, bone marrow.
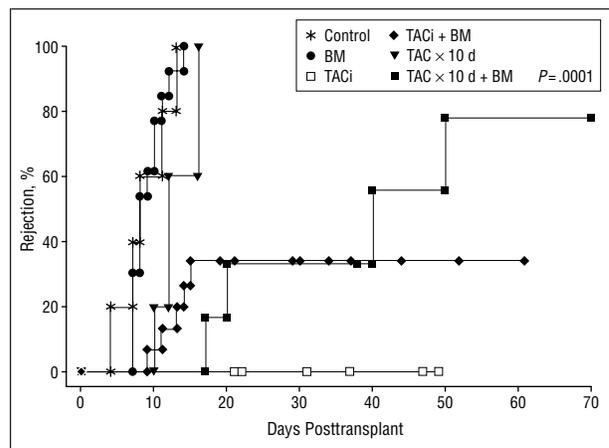

Figure 2. Death from rejection. TACi indicates indefinite tacrolimus; BM, bone marrow.
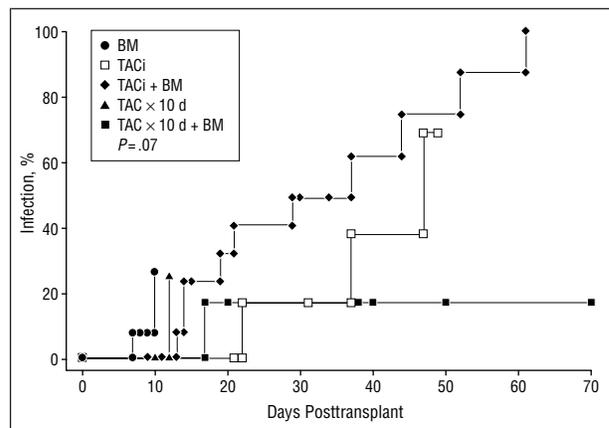

Figure 3. Death from infection. TACi indicates indefinite tacrolimus; BM, bone marrow.

and 50%; and group 6, 0%, 25%, and 25%, respectively ($P = .10$, Wilcoxon; $P = .07$, log-rank) (**Figure 3**).

In group 6, the death rate from infection was not different compared with group 3 ($P \le .19$), group 4 ($P \le .28$), or group 5 ($P \le .08$).

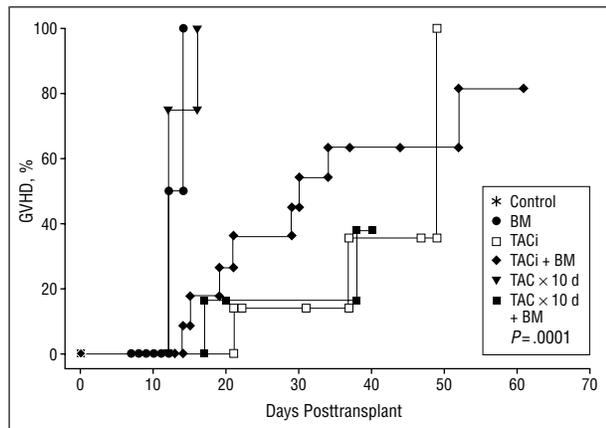In group 5, the death rate from infection was lower than in group 4 ($P = .13$, Wilcoxon; $P = .04$, log-rank).

Figure 4. Death from graft-vs-host disease (GVHD). TACi indicates indefinite tacrolimus; BM, bone marrow.

DEATH FROM GVHD

For this analysis, only deaths from GVHD were counted as graft failures. In group 1, the death rate from GVHD at 7, 14, and 28 days was 0%, 0%, and 0%; group 2, 0%, 100%, and 100%; group 3, 0%, 0%, and 14%; group 4, 0%, 8%, and 36%; group 5, 0%, 75%, and 100%; and group 6, 0%, 0%, and 17%, respectively ($P=.0001$).

In group 6, the death rate from GVHD was not different compared with group 3 ($P\leq.92$) or group 4 ($P\leq.24$). The death rate from GVHD was significantly lower in group 6 than in group 5 ($P\leq.001$) (**Figure 4**).

LONG-TERM SURVIVAL (>4 WEEKS)

There were no long-term survivors in group 1 (longest survival, 13 days), group 2 (longest survival, 14 days), or group 5 (longest survival, 16 days). All long-term survivors received either tacrolimus indefinitely with or without DSBMI, or tacrolimus for 10 days with DSBMI. In group 3, 71% survived 28 days (range, 31-49 days); they died from either GVHD or infection, but not from rejection. In group 4, 47% survived longer than 28 days (range, 29-61 days); they died from GVHD, infection, or both, but not from rejection. In group 6, 67% survived longer than 28 days (range, 38-195 days); they died from rejection or GVHD, but not from infection. The longest surviving pig (195 days) had to be killed because of its high weight in the absence of rejection, infection, or GVHD.

AUTOPSY RESULTS

At autopsy, groups without DSBMI showed evidence of the following immunologic conditions: (1) In group 1, cause of death was moderate or severe rejection, but with no concurrent infection or GVHD. (2) In group 3, cause of death was either infection or GVHD, but not rejection; GVHD and infection rarely occurred simultaneously. (3) In group 5, cause of death was most commonly rejection, frequently concurrent with GVHD; only 1 pig died from infection (in combination with GVHD, but without evidence of rejection).

At autopsy, groups with DSBMI showed evidence of the following immunologic conditions: (1) In group

2, all pigs died from rejection. The incidence of concurrent rejection and infection was 15%, as was the incidence of concurrent rejection and GVHD. (2) In group 4, only 33% of the pigs died from rejection. The incidence of concurrent GVHD and infection was 27%; concurrent rejection and infection, 7%; concurrent rejection and GVHD, 7%; and concurrent rejection, GVHD, and infection, 7%. (3) In group 6, 66% of the pigs died from rejection; only 7 pigs died after several immunologic events (rejection, infection, and GVHD).

COMMENT

The high lymphoid load associated with bowel grafts has long been seen as a major obstacle to the success of clinical bowel transplants. It renders the bowel not only immunogenic (capable of eliciting rejection), but also immunocompetent (capable of provoking GVHD). Furthermore, the bowel's large bacterial content is likely to aggravate the risk of infection. Despite the high immunologic risk, bowel transplantation has proven to be clinically feasible under current immunosuppressive strategies.[16] Nevertheless, even mild episodes of rejection or infection are frequently disastrous. Treatment of rejection episodes may cause life-threatening infections or posttransplant lymphoproliferative diseases. Treatment of infection episodes—requiring a decrease in immunosuppressive dosing—may cause subsequent rejection episodes.

To prevent rejection or infection and their associated complications, more specific immunomodulatory strategies are needed. One such strategy is to combine the bowel transplant with DSBMI.

But in our previous studies, DSBMI alone (without posttransplant immunosuppression) uniformly resulted in death from rejection 7 to 14 days posttransplant. Survival rates and death rates from rejection were not any different between nonimmunosuppressed DSBMI pigs and control pigs.[12,13] When tacrolimus-based immunosuppression was added, DSBMI significantly prolonged graft survival, compared with DSBMI without immunosuppression.[13] But the death rate from infection and GVHD was higher, possibly because of the indefinite use of standard immunosuppressive therapy.

Given our previous results, the purpose of this study was to investigate whether arbitrarily discontinuing immunosuppression (10 days after bowel transplantation and DSBMI) prevents the development of infection and GVHD and averts rejection. In our control group of pigs with short-term immunosuppression only (no DSBMI), survival times were no different than for nonimmunosuppressed control pigs or for nonimmunosuppressed DSBMI pigs; pigs in all 3 groups survived 4 to 16 days. In contrast, the combination of short-term immunosuppression and DSBMI significantly prolonged survival, compared with either short-term immunosuppression only or DSBMI only. Compared with DSBMI and indefinite immunosuppression, the rates of infection and GVHD were slightly lower for DSBMI and short-term immunosuppression; survival and rejection rates were similar in both groups. Thus, a 10-day course vs indefinite use of immunosuppression reduces the risks of infection and

GVHD, but is not sufficient to avert severe rejection, despite simultaneous use of DSBMI.

One possibility to decrease the risk of rejection is to recycle immunosuppression at certain intervals. This approach might decrease the death rate from rejection, but it might also increase death rates from infection and GVHD. Another approach is to modify the timing, dosing, or composition of the donor-specific bone marrow cells.[17] In our experience, unmodified bone marrow cells infused in the systemic circulation at the time of transplant have served more as immunogens, irrespective of the amount of immunosuppression posttransplant. But bone marrow that has been prepared to remove professional antigen-presenting cells better facilitates specific immune tolerance. And T cell–depleted bone marrow (resulting either from elutriation or from monoclonal antibody lineage depletion techniques) might reduce the risks of GVHD associated with bone marrow infusions. Such studies are currently under way.

One question we did not address in this study is whether DSBMI causes development of chimerism. All of our transplants were performed with male donors and female recipients, theoretically offering the possibility of detecting male chromosomes in some female tissue; however, at the time of our transplants, no probe specific to the pig Y chromosome was available. In addition, swine leukocyte antigen specificity was not known; we were not able to use specific swine leukocyte antigen probes for identifying donor and recipient antigens. Thus, the degree of chimerism was not assessed. However, we believe that development of GVHD is direct evidence of engraftment of donor-derived cells.[18] In fact, the chimeric concept (ie, mutual coexistence of 2 genetically distinct entities resulting in tolerance) still awaits convincing, reproducible confirmation in the clinical setting. Most evidence relating chimerism to tolerance is circumstantial. Chimerism may be the result of graft acceptance, rather than the cause. It is acknowledged that chimerism is not the sine qua non of tolerance induction. Nor does chimerism represent a stable immunologic state: it has been detected in human recipients of solid organ transplants who have rejection or GVHD.[19,20]

In contrast to other experimental studies of solid organ transplants, we have not been able to show that the addition of DSBMI after bowel transplantation is beneficial: in our current studies, DSBMI without (short-term or indefinite) immunosuppression did not improve outcome, compared with nonimmunosuppressed control groups. Compared with these 2 control groups, the addition of (short-term or indefinite) immunosuppression significantly improved survival but did not avert rejection, infection, or GVHD. Best results were obtained with indefinite immunosuppression and without DSBMI. In this model of bowel transplantation, unmodified DSBMI given intravenously at the time of transplant had a detrimental effect.

CONCLUSIONS

We have shown that the combination of short-term immunosuppression and DSBMI significantly prolonged survival after total bowel transplantation, compared with short-term immunosuppression only or DSBMI only. But short-term immunosuppression with DSBMI failed to prevent development of rejection once immunosuppression was discontinued. Unmodified DSBMI in combination with short-term immunosuppression reduced the risks of infection and GVHD (in contrast with indefinite immunosuppression with DSBMI), but was not sufficient to avert graft loss from rejection.

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REFERENCES

1. Thomas J, Carver M, Cunningham P, Park K, Gonder J, Thomas F. Promotion of incompatible allograft acceptance in rhesus monkeys given posttransplant antithymocyte globulin and donor bone marrow. *Transplantation.* 1987;43:332-338.
2. Colson YL, Zadach K, Nalesnik M, Ildstad ST. Mixed allogeneic chimerism in the rat. *Transplantation.* 1995;60:971-980.
3. Murase N, Starzl TE, Tanabe M, et al. Variable chimerism, graft-versus-host disease, and tolerance after different kinds of cell and whole organ transplantation from Lewis to brown Norway rats. *Transplantation.* 1995;60:158-171.
4. Li H, Colson YL, Ildstad ST. Mixed allogeneic chimerism achieved by lethal and nonlethal conditioning approaches induces donor-specific tolerance to simultaneous islet allografts. *Transplantation.* 1995;60:523-529.
5. Ricordi C, Murase N, Rastellini C, Behboo R, Demetris AJ, Starzl TE. Indefinite survival of rat islet allografts following infusion of donor bone marrow without cytoablation. *Cell Transplant.* 1996;5:53-55.
6. Barber WH, Mankin JA, Laskow DA, et al. Long-term results of a controlled prospective study with transfusion of donor-specific bone marrow in 57 cadaveric renal allograft recipients. *Transplantation.* 1991;51:70-75.
7. Brennan DC, Mohanakumar T, Flye MW. Donor-specific transfusion and donor bone marrow infusion in renal transplantation tolerance: a review of efficacy and mechanisms. *Am J Kidney Dis.* 1995;26:701-715.
8. Rolles K, Burroughs AK, Davidson BR, Karatapanis S, Prentice HG, Hamon MD. Donor-specific bone marrow infusion after orthotopic liver transplantation. *Lancet.* 1994;343:263-265.
9. Burke GW, Ricordi C, Karatzas T. Donor bone marrow infusion in simultaneous pancreas/kidney transplant recipients. *Transplant Proc.* 1995;27:3121-3122.
10. Zeevi A, Pavlick M, Banas R, et al. Three years of follow-up of bone marrow-augmented organ transplant recipients: the impact on donor-specific immune modulation. *Transplant Proc.* 1997;29:1205-1206.
11. Shapiro R, Rao AS, Fontes P, et al. Combined simultaneous kidney/bone marrow transplantation. *Transplantation.* 1995;60:1421-1425.
12. Pirenne J, Gruessner AC, Benedetti E, et al. Donor-specific unmodified bone marrow transfusion does not facilitate intestinal engraftment after bowel transplantation in a porcine model. *Surgery.* 1997;121:79-88.
13. Gruessner RW, Uckun FM, Pirenne J, et al. Recipient preconditioning and donor-specific bone marrow infusion in a pig model of total bowel transplantation. *Transplantation.* 1997;63:12-20.
14. Pirenne J, Benedetti E, Gruessner AC, et al. Combined transplantation of small and large bowel: FK 506 versus cyclosporin A in a porcine model. *Transplantation.* 1996;61:1685-1694.
15. Nakhleh RE, Gruessner AC, Pirenne J, Benedetti E, Troppmann C, Gruessner RW. Colon vs small bowel rejection after total bowel transplantation in a pig model. *Transpl Int.* 1996;9(suppl 1):S269-S274.
16. Grant D, for the Intestinal Transplant Registry. Intestinal transplantation: 1997 Report of the International Registry. *Transplantation.* 1999;67:1061-1064.
17. Kaufman CL, Colson YL, Wren SM, Watkins S, Simmons RL, Ildstad ST. Phenotypic characterization of a novel bone marrow-derived cell that facilitates engraftment of allogeneic bone marrow stem cell. *Blood.* 1994;84:2436-2446.
18. Pirenne J, Moon C, Gruessner A, et al. Bone marrow augmentation of kidney allografts can cause graft-versus-host disease in immunosuppressed recipients. *Transplant Proc.* 1996;28:941-942.
19. Schlitt HJ, Hundrieser J, Ringe B, Pichlmayr R. Donor-type microchimerism associated with graft rejection eight years after liver transplantation. *N Engl J Med.* 1994;330:646-647.
20. Sivasai KS, Alevy YG, Duffy BF, et al. Peripheral blood microchimerism in human liver and renal transplant recipients: rejection despite donor-specific chimerism. *Transplantation.* 1997;64:427-432.