2159

Infusion of donor leukocytes to induce tolerance in organ allograft recipients

S. K. Salgar,* R. Shapiro,* F. Dodson,* R. Corry,* K. McCurry,* A. Zeevi,† S. Pham,‡ K. Abu-Elmagd,* J. Reyes,* M. Jordan,* R. Keenan,* B. Griffith,* T. Sesky,* L. Ostrowski,* T. E. Starzl,* J. J. Fung,* and A. S. Rao,*†

*Section of Cellular Transplantation, Thomas E. Starzl Transplantation Institute and the Departments of *Surgery and †Pathology, University of Pittsburgh Medical Center, Pennsylvania; and the ‡Daughtry Family Department of Surgery, University of Miami, Florida*

Abstract: To further enhance chimerism, 229 primary allograft recipients have received perioperative intravenous infusion of a single dose of 3 to 6 × 10^8 unmodified donor bone marrow (BM) cells/kg body weight. In addition, 42 patients have been accrued in a concurrent protocol involving multiple (up to three) sequential perioperative infusions of 2 × 10^8 BM cells/kg/day from day 0–2 posttransplantation (PTx). Organ recipients (n = 133) for whom BM was not available were monitored as controls. The infusion of BM was safe and except for 50 (18%), all study patients have optimal graft function. Of the control patients, allografts in 30 (23%) have been lost during the course of follow-up. The cumulative risk of acute cellular rejection (ACR) was statistically lower in the study patients compared with that of controls. It is interesting that, 62% of BM-augmented heart recipients were free of ACR (Grade ≥ 3A) in the first 6 months PTx compared to controls. The incidence of obliterative bronchiolitis was also statistically lower in study lung recipients (3.8%) compared with the contemporaneously acquired controls (31%). The levels of donor cell chimerism were at least a log higher in the peripheral blood of majority of the study patients compared with that of controls. The incidence of donor-specific hyporeactivity, as determined by one-way mixed leukocyte reaction, was also higher in those BM-augmented liver, kidney, and lung recipients that could be evaluated compared to controls. *J. Leukoc. Biol.* 66: 310–314; 1999.

Key Words: *transplantation · chimerism · human*

INTRODUCTION

Billingham, Brent, and Medawar [1] first documented the induction of donor-specific tolerance (DST) to transplanted allografts in rodents. In human recipients of bone marrow (BM), this outcome was first documented by Mathe et al. [2] in 1963. However, despite these earlier observations, the acceptance of organ allografts still remained an enigma. The persistence of donor leukocytes (i.e. chimerism) in the tissues of long-term functioning organ allograft recipients prompted us to conclude that these cells may play a seminal role in graft acceptance [3, 4]. This hypothesis is supported by the observation that, similar to that of microorganisms, rejection or acceptance (tolerance) of transplanted allografts may be governed by the migration and localization of the antigen [5]. The idea of this trial came from the realization that the BM-derived leukocyte component of whole organs (commonly called passenger leukocytes) begins to migrate ubiquitously within a few minutes after transplantation and that these donor cells survive and constitute a small second cell population that is capable of defending itself against the immunological system of the recipient [3, 4, 6]. We have postulated, in what has been called the two-way paradigm, that the eventual induction of mutual nonreactivity of the coexisting cell populations, each to the other, is the essential basis of allograft acceptance [3, 4, 6]. Based on these earlier findings, it was therefore hypothesized that, if successful, adjuvant perioperative infusion of donor BM to organ allograft recipients would culminate (in a relatively higher number of patients) in the induction of DST resulting in reduced incidence of acute cellular rejection (ACR) and chronic rejection (CR), and would allow early weaning and/or withdrawal of *nonspecific* immunosuppression (IS).

MATERIALS AND METHODS

Patients and BM infusion

After obtaining informed consent, patients with end-stage organ failure who had no evidence of active infection, disseminated malignancy, or pregnancy were

Abbreviations: DST, donor-specific tolerance; BM, bone marrow; ACR, acute cellular rejection; CR, chronic rejection; IS, immunosuppression; Tx, transplantation; PTx, posttransplantation; VB, vertebral bodies; PBL, peripheral blood leukocytes; PCR, polymerase chain reaction; MLR, mixed lymphocyte reaction; LDA, limiting dilution assays; GvHD, graft-versus-host disease; MSOF, multiple system organ failure; POD, post-operative day; OB, obliterative bronchiolitis; PTLD, posttransplant lymphoproliferative disease; EBV, Epstein Barr virus; DSH, donor-specific hyporeactivity.

Correspondence: Abdul S. Rao, M.D., Ph.D., Section of Cellular Transplantation, Thomas E. Starzl Transplantation Institute, E1545 Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, PA 15213. E-mail:

Received February 10, 1999; revised April 15, 1999; accepted April 19, 1999.

accrued into this study. In the protocol mandating single perioperative inoculation, 3 to 6 × 10^8 unmodified donor BM cells/kg body weight were infused intravenously into 229 recipients of liver (n = 71), kidney (n = 85), heart (n = 27), lung (n = 19), small bowel (n = 23), and multi-organ (n = 4) transplantation (Tx). In addition, in a concurrent protocol, 42 recipients of liver (n = 6), kidney (n = 28), heart (n = 3), and lung (n = 5) allografts received multiple infusions (2 × 10^8 donor BM cells/kg body weight/day) of donor BM from day 0–2 posttransplantation (PTx). As contemporaneous controls, 133 recipients of liver (n = 33), kidney (n = 47), heart (n = 23), lung (n = 12), small bowel (n = 16), and multi-organ (n = 2) were also accrued. These were patients for whom BM cells were not available due to our inability to obtain consent to retrieve vertebral bodies (VB) from prospective donors. Recipient sex (male/female ratio), follow-up, age at transplant, human leukocyte antigen disparity, and other demographic parameters were comparable in patients in the study (BM-augmented) and the control groups. BM cells were harvested from the VB of the cadaveric donors by a method described previously [6]. Immunosuppression was with tacrolimus and steroid; CellCept was administered to 53 study and 17 control patients. For steroid-resistance ACR, an anti-thymocyte monoclonal antibody (OKT3) was used. No induction therapy was employed.

In vitro monitoring

Determination of chimerism

Genomic DNA was isolated from recipient's peripheral blood leukocytes (PBL) with a QIAamp Blood Kit (Qiagen Inc., Santa Clarita, CA) and quantitated spectrophotometrically. DNA samples with $OD_{260/280}$ 1.7–1.9 were used for polymerase chain reaction (PCR) amplifications. A Y-chromosome specific marker (*Sry* gene) was amplified using the following external primer pairs in the first round of PCR: SRY5.1, 5-GAATATTCCCGCTCTCCGGA-3; SRY 3.1, 5-GTACAACCTGTTGTCCAGTT-3 [7]. The amplified product [423 base pairs (bp)] was subjected to a second round of PCR containing the following nested primers: SRY5.3, 5-CAGTGTGAAACGGGAGAAAACAGT-3; SRY3.3, 5-CTTCCGACGAGGTCGATACTTAT A-3 [8]. To avoid false-negative outcomes, the quality of genomic DNA was controlled by effective amplification of an Alu sequence using A1, 5-GGCACTTTGGGAGGCCAAGG-3; A2, 5-TACAAGCTTGTGCCATGCCCAAC-3 primers [9]. For semi-quantitation, products of nested PCR (270 bp) were electrophoresed on 1.5% agarose gel and stained with SYBR Green 1 (FMC BioProducts, Rockland, ME). To determine band fluorescence, the gels were scanned on FlourImager (Molecular Dynamics, Sunnyvale, CA) and the band volume was quantitated by placing a rectangle around each band with the use of the ImageQuant program. Samples of female DNA (500 ng) artificially spiked with increasing quantities (0.0005–0.5 ng) of male DNA were used (**Fig. 1A**) to obtain a standard curve (Fig. 1B). The quantity of donor (male) DNA in test samples was determined by comparing its band volume with the standard curve and a ratio of donor/recipient DNA was calculated; the levels of donor cell (chimerism) in each patient were subsequently deduced.

Mixed leukocyte reaction (MLR)

Pre- and serially posttransplant (every other month) monitoring of recipient's immune status was carried out by evaluating the proliferative responses of their peripheral blood mononuclear cells (PBMCs) to mitogens (concanavalin A, phytohemagglutinin), recall antigens (tetanus toxoid), MLR, and limiting dilution assays (LDA). These assays as well as detailed studies of most of these same patients have been described elsewhere [6, 10].

Statistical analysis

Log rank and Fisher's exact test were used for statistical analysis.

RESULTS

Clinical outcome

Except for that in liver allograft recipients, both single and multiple infusion of unmodified donor BM cells was safe. However, unlike single infusions, multiple infusions of BM

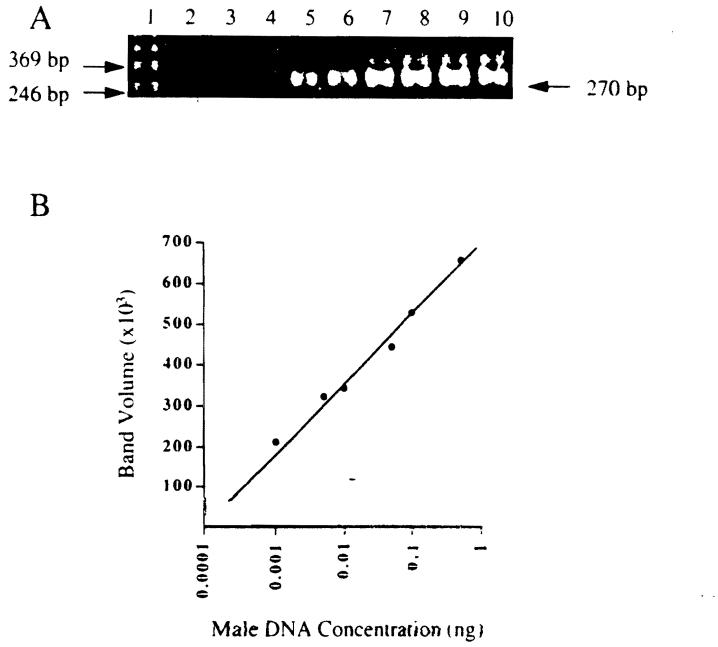

Fig. 1. Semi-quantitative PCR analysis of donor cell chimerism in the peripheral blood leukocytes of organ allograft recipients. (A) Agarose gel electrophoresis of nested PCR products obtained using female DNA (500 ng) artificially spiked with increasing quantities (0.0005–0.5 ng) of male DNA: lane 1, 123 bp molecular weight marker; lane 2, no template (negative control); lane 3, female DNA only (control); lanes 4–10, serial dilutions from 1 × 10^0 to 1 × 10^3 of spiked male:female DNA. (B) Standard curve derived by plotting band volume obtained by image analysis of the gel (A), using FlourImager and ImageQuant.

resulted in clinical manifestation of severe graft-versus-host disease (GvHD; grade III) in 1/6 (17%) liver transplant recipients; this patient ultimately succumbed to multiple system organ failure (MSOF) on postoperative day (POD) 147. The increased risk of morbidity and mortality of multiple BM infusions in liver transplant recipients has prompted the termination of this protocol in this particular group of patients. Patient and graft survival and graft function were comparable in study and control groups; allografts in 50/271 (18%) study and 30/133 (23%) control patients were lost during the course of their follow-up. For patients who were ≥12 months PTx, the dose of tacrolimus and steroids required to maintain a rejection-free state was lower in BM-augmented (6.4 ± 3.9 and 5.8 ± 3.3 mg/day, respectively) compared with that of controls (7.1 ± 5 and 6.2 ± 3.7 mg/day, respectively). In addition, a slightly higher number of study (64%) patients have been taken off-steroids compared with the controls (59%); none of these findings, however, approach statistical significance.

Rejection

The incidence of ACR was statistically lower in the study patients compared with controls [**Figure 2**; P = 0.0372 (log rank)]. It must be emphasized that during a comparable period of follow-up, the recipients of multiple infusion had a much lower cumulative risk of ACR (54%) compared with those receiving either single infusion or none at all (controls). This salutary effect of BM augmentation was more pronounced in heart allograft recipients. Sixty-two percent of study patients

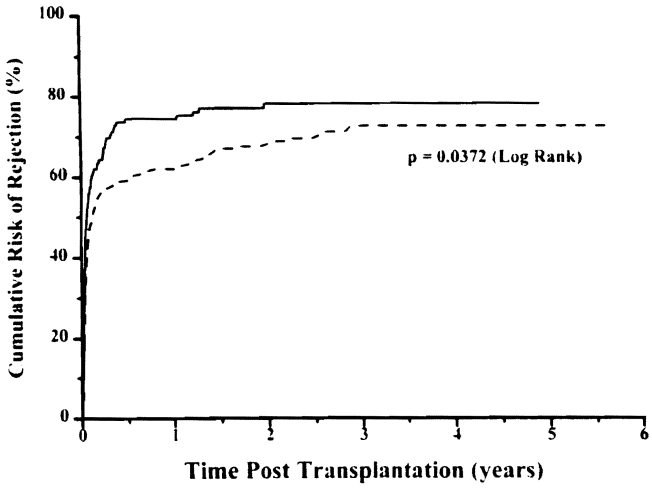

Fig. 2. Cumulative risk of acute cellular rejection in BM augmented (dashed lines) and contemporaneously acquired non-augmented (control) organ allograft recipients (solid lines). BM augmented, $n = 271$; controls, $n = 133$.

were free of ACR (grade ≥3A) in the first 6 months PTx compared with the controls (18%; $P = 0.006$; Fisher's exact test); this beneficial effect was sustained during the course of follow-up (>18 months PTx). The incidence of steroid-resistant ACR (which required OKT3 treatment for its resolution), was also lower in the study (6.7%) patients compared with the controls (8.6%). It is interesting that, despite having an analogous incidence of ACR, that of obliterative bronchiolitis (OB) was statistically lower in the BM-augmented lung allograft recipients (1/26; 3.8%) compared with contemporaneously acquired controls (4/13; 31%).

Incidence of posttransplant lymphoproliferative disease (PTLD) in Epstein-Barr virus-positive (EBV^+) → EBV^- BM recipients

There was some concern that adjuvant infusion of donor BM in $EBV^+ \rightarrow EBV^-$ organ transplant recipients may increase both the incidence and the severity of PTLD, thus obviating the beneficial effect of this therapy. The incidence of clinically proven PTLD in adult $EBV^+ \rightarrow EBV^-$ BM-augmented (22%) and control (20%) organ transplant recipients was found to be comparable (**Table 1**). It is interesting that none of the pediatric $EBV^- \rightarrow EBV^-$ BM-augmented organ allograft recipients developed PTLD. On the contrary, 2/2 (100%) concurrently acquired pediatric controls were diagnosed with this lesion: one died due to MSOF perhaps as a consequence of disseminated refractory PTLD (Table 1). Among the adult organ allograft recipients, refractory PTLD was not encountered in the $EBV^+ \rightarrow EBV^-$ controls, whereas 2/5 (40%) study patients developed a refractory lesion that was unresponsive to initial weaning and/or withdrawal of IS (Table 1). In one patient, this lesion resolved after immunotherapy with autologous lymphokine-activated killer cells; disseminated PTLD leading to MSOF was presumed to be the cause of death in the other patient (Table 1).

Donor cell chimerism

As reported previously [6], BM-augmented patients had much higher (~92%) incidence of chimerism in their PBL compared with controls (~50%). This finding was consistent over the course of serial follow-up. Similarly, using semi-quantitative PCR, the levels of donor cell chimerism were found to be at least a log higher in the majority of the evaluated (male → female Tx recipients) BM-augmented patients compared with the controls. It is interesting that, as compared to single, multiple perioperative infusions of donor BM were associated with further elevation of levels of serially determined chimerism in the PBL of organ allograft recipients.

In vitro immune reactivity

To undertake comparative analysis, one-way MLR was performed serially PTx only in organ allograft recipients from whom pre-transplant blood was procured and donor splenocytes were available. Using a previously outlined criterion [10], higher incidence of donor-specific hyporeactivity (DSH) was encountered in those liver, kidney, and lung transplant recipients that were evaluated in this study compared with the controls (**Table 2**). On the contrary, comparable levels of donor and third party-specific (data not shown) hyporeactivity were witnessed in study and control heart, small bowel (data not shown), kidney + pancreas, and multi-organ transplant (data not shown) recipients (Table 2).

DISCUSSION

The commonality between the body's immune responses against transplanted organ allografts and infections has long been recognized [11]. This hypothesis received major impetus when it was documented that, similar to that of Tx alloantigens,

TABLE 1. The Incidence of Clinically Diagnosed Post-transplant Lymphoproliferative Disease and Its Outcome in BM-augmented and Control Organ Transplant Recipients

Patients	n[a]	$EBV^- \rightarrow EBV^-$ transplants (n; %)	Developed PTLD	Refractory PTLD[b]	Clinical outcome
BM-Augmented					
Adult	209	23/209 (11%)	5/23 (22%)	2/5 (40%)	Resolved in one patient with LAK cell therapy; the other patient died of MSOF[d]
Pediatric	17	6/17 (35%)	0/6 (0%)	NA[c]	NA[c]
Control					
Adult	109	5/109 (5%)	1/5 (20%)	0/1 (0%)	NA[c]
Pediatric	11	2/11 (18%)	2/2 (100%)	1/2 (50%)	Died due to MSOF[d]

[a] Patients with known EBV status before transplant. [b] Unresponsive to reduction and/or withdrawl of immunosuppression. [c] Not applicable. [d] Multiple system organ failure possibly due to disseminated PTLD.

TABLE 2. The Incidence of Donor-Specific Immunomodulation in Bone Marrow-Augmented and Non-Augmented Organ Transplant Recipients in Whom *In Vitro* Immune Monitoring was Feasible

Organ type	*n*	Immune status [n (%)]	
		Hypo + intermediate	Reactive
Liver			
BM augmented (single infusion)	36	18 (50%)	18 (50%)
BM augmented (multiple infusion)	3	2 (66%)	1 (33%)
BM non-augmented	18	4 (22%)	14 (77%)
Kidney			
BM augmented (single infusion)	21	15 (71%)	6 (28%)
BM augmented (multiple infusion)	5	2 (40%)	3 (60%)
BM non-augmented	13	5 (38%)	8 (61%)
Kidney + Islets			
BM augmented (single infusion)	5	2 (40%)	3 (60%)
BM augmented (multiple infusion)	1		1 (100%)
BM non-augmented	1		1 (100%)
Kidney + Pancreas			
BM augmented (single infusion)	29	12 (41%)	17 (58%)
BM augmented (multiple infusion)	10	2 (20%)	8 (80%)
BM non-augmented	11	4 (36%)	7 (64%)
Heart			
BM augmented (single infusion)	16	3 (19%)	13 (81%)
BM augmented (multiple infusion)	2	0	2 (100%)
BM non-augmented	13	2 (15%)	11 (84%)
Lungs			
BM augmented	8	5 (62%)	3 (37%)
BM non-augmented	5	2 (40%)	3 (60%)

acquired immune responses against infectious agents were also MHC-restricted [12]. Because migration and localization of antigen was evidenced in both types of immune responses, it has been argued that in an otherwise naive host, this may be the seminal event resulting in the long-term acceptance of the allografts as well as the eradication of pathogenic infectious agents [5]. After Tx, migration of alloantigens from the graft into the host's lymphoid and non-lymphoid tissues has been unequivocally demonstrated in long-term kidney and liver transplant recipients [3, 4]. The role of these ubiquitously localized donor cells in the induction of DST has been corroborated in numerous pre-clinical models of Tx tolerance [13–15]. Although the precise phenotype of these migratory cells is as yet unknown, it was, however, determined that they were $CD45^+$ cells of donor BM origin [3, 4, 13–15]. Accordingly, it was proposed that willful augmentation of this phenomenon in organ allograft recipients by perioperative infusion of BM cells obtained from either cadaveric or living related donors, would greatly reduce the incidence of ACR and CR as well as allow early weaning and/or withdrawal of nonspecific IS [3, 4, 6].

In 1992, we initiated a prospective clinical trial to affirm or refute this hypothesis in recipients of various organ allografts. To replicate the natural events that predictably transpire perioperatively, the recipients were not conditioned and the infused BM was not modified. Due to ethical and fiscal concerns, this study was not randomized; availability (or otherwise) of VB from cadaveric donors determined the accrual of a prospective recipient as a study or a control patient. Except for recipients of liver allografts, the infusion of single or multiple doses of donor BM was safe. In the liver recipients, however, the perioperative infusion of multiple (but not single) doses of BM was associated with increased incidence of morbid GvHD, thus prompting precocious termination of this latter protocol. To prevent untoward complications (with the exception of the first six liver recipients), recipients of supposedly leukocyte-rich organs (i.e., small bowel, liver, or multivisceral), received only a single bolus infusion of donor BM immediately after organ Tx. On the contrary, since April 1996, recipients of all other organs have received multiple infusions of donor BM on 3 consecutive days (day 0–2) PTx.

During the course of follow-up, slightly higher patient and graft loss has been documented in the control group compared with that of the study. Although being monitored, these parameters are not primary end-points of this study; the large number of patients that need to be acquired in each organ group to conduct appropriate statistical analysis is considered prohibitive. As predicted, the cumulative risk of developing ACR was statistically lower in the BM-augmented patients compared with the controls. It is interesting that this risk was lowered further in recipients of multiple BM infusion in whom correspondingly higher levels of donor cell chimerism were documented. Although admittedly longer follow-up is required to confirm this finding, it nevertheless supports our earlier assertion that establishment of stable chimerism would result (in the majority of the patients) in the induction of DSH [3, 4, 6]. This benign effect of BM infusion is perhaps best exemplified in heart allograft recipients in whom, despite retention of donor-specific *in vitro* immune reactivity, the incidence and severity of ACR was markedly lower in study patients compared with controls. Because an increased incidence of ACR is considered a precursor to the eventual evolution of CR, it could be argued that BM augmentation would mitigate, if not abrogate, the development of the latter lesion in study heart allograft recipients.

In recipients of lung allografts, this predicted outcome has already been realized. Despite analogous incidence and severity of ACR, that of OB is remarkably lower in patients in the study group compared with that of the controls. It is interesting that, when evaluated serially, BM-augmented lung recipients exhibited a higher incidence of DSH compared with the controls. Correspondingly, in those who are ≥1 year PTx, a higher number (19%) of BM-augmented lung allograft recipients have been taken off steroids as compared to the concurrently accrued controls (11%).

In conclusion, infusion of donor BM was safe, with resultant increase in both the incidence and the levels of chimerism in the peripheral blood of organ allograft recipients. This latter finding translated into marked reduction in the incidence of ACR and CR with induction of DSH in BM-augmented liver, kidney, and lung recipients. Unlike that in rodents and/or non-human primates, it is our contention that much longer follow-up would be required to realize the entire spectrum of predicted efficacy of deliberate BM augmentation in human organ allograft recipients. In light of our previous experience of deliberately weaning IS [16], we have initiated a similar prospective trial in BM-augmented and non-augmented liver allograft recipients who exhibit stable graft function and are ≥5

years PTx. It is our assertion that experience acquired from this latter study would ultimately assist us to possibly wean and/or withdraw nonspecific IS in recipients of other organ allografts.

ACKNOWLEDGMENTS

This study was supported by National Institutes of Health Grant AI38899. We would like to thank Diana Zaldonis, R.N. for her help in monitoring cardiothoracic organ allograft recipients; Alison Logar for performing flow cytometry; Thomas Ploskina for BM and DNA isolation; Mary Pavlick and Richard Banas for their help in *in vitro* immune monitoring; Diana Reichenbach and Maria Valenti for data management; and Margie Lyle for preparation of the manuscript.

REFERENCES

1. Billingham, R. E., Brent, L., Medawar, P. B. (1953). "Actively acquired tolerance" of foreign cells. Nature 172, 603–606.
2. Mathe, G., Amiel, J. L., Schwarzenberg, L., Cattan, A., Schneider, M. (1963) Hematopoietic chimera in man after allogeneic (homologous) bone marrow transplantation. Br. Med. J. 2, 1633–1635.
3. Starzl, T. E., Demetris, A. J., Murase, N., Ildstad, S., Ricordi, C., Trucco, M. (1992) Cell migration, chimerism, and graft acceptance. Lancet 339, 1579–1582.
4. Starzl, T. E., Demetris, A. J., Murase, N., Trucco, M., Thomson, A. W., Rao, A. S. (1996) The lost chord: microchimerism. Immunol. Today 17, 577–584.
5. Starzl, T. E., Zinkernagel, R. M. (1998) Antigen localization and migration in immunity and tolerance. N. Engl. J. Med. 339, 1905–1913.
6. Fontes, P. S., Rao, A. S., Demetris, A. J., Zeevi, A., Trucco, M., Carroll, P., Rybka, W., Rudert, W. A., Ricordi, C., Dodson, F., Shapiro, R., Tzakis, A., Todo, S., Abu-Elmagd, K., Jordan, M., Fung, J. J., Starzl, T. E. (1994) Bone marrow augmentation of donor-cell chimerism in kidney, liver, heart, and pancreas islet transplantation. Lancet 344, 151–155.
7. Jäger, R. J., Anvert, M., Hall, K., Scherer, G. (1990) A human XY female with a frame shift mutation in the candidate testis-determining gene SRY. Nature 348, 452–454.
8. Nakagome, Y., Seki, S., Fukutani, K., Nagafuchi, S., Nakahori, Y., Tamura, T. (1991) PCR detection of distal Yp sequences in an XX true hermaphrodite. Am. J. Med. Genet. 41, 112–114.
9. Ivinson, A. J., Taylor, G. R. (1994) PCR in genetic diagnosis. In *PCR: A Practical Approach* (M. J. McPhearson., P Qirke, and G. R. Taylor, eds.). Oxford, UK: Oxford University Press, 18–19.
10. Zeevi, A., Pavlick, M., Lombardozzi, S., Banas, R., Pappo, O., Rao, A. S., Fontes, P., Demetris, A. J., Shapiro, R., Dodson, F., Carroll, P., Fung, J. J., Starzl, T. E. (1995) Immune status of recipients following bone marrow augmented solid organ transplantation. Transplant. 59, 3484–3486.
11. Lawrence, H. S. (1959) Homograft sensitivity. An expression of the immunologic origins and consequences of individuality. Physiol. Rev. 39, 811–859.
12. Doherty, P. C., Zinkernagel, R. M. (1975) A biological role for the major histocompatibility antigens. Lancet 1, 1406–1409.
13. Murase, N., Demetris, A. J., Matsuzaki, T., Yagihasi, A., Todo, S., Fung, J. J., Starzl, T. E. (1991) Long survival in rats after multivisceral versus isolated small bowel allotransplantation under FK506. Surgery 110, 87–98.
14. Demetris, A. J., Murase, N., Fujisaki, S., Fung, J. J., Rao, A. S., Starzl, T. E. (1993) Hematolymphoid cell trafficking, microchimerism, and GvHD reactions after liver, bone marrow, and heart transplantation. Transplant. Proc. 25, 3337–3344.
15. Qian, S., Demetris, A. J., Murase, N., Rao, A. S., Fung, J. J., Starzl, T. E. (1994) Murine liver allograft transplantation: tolerance and donor cell chimerism. Hepatol. 19, 916–924.
16. Mazariegos, G. V., Reyes, J., Marino, I. R., Demetris, A. J., Flynn, B., Irish, W., McMichael, J., Fung, J. J., Starzl, T. E. (1997) Weaning of immunosuppression in liver transplant recipients. Transplant. 63, 243–249.