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Donor Bone Marrow Infusion in Deceased and Living Donor Renal Transplantation

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The infusion and persistence in a transplant recipient of donor-derived bone marrow cells (DBMC) of multi-lineage can lead to a state of permanent chimerism. In solid vascular organ transplantation, the donor bone marrow lineage cells can even be derived from the transplant organ, and these cells can be detected in very small numbers in the recipient. This has been called microchimerism. Much controversy has developed with respect to the function of chimeric cells in organ transplantation. One idea is that the occurrence of these donor cells found in microchimerism in the recipient are coincidental and have no long-term beneficial effect on engraftment. A second and opposing view, is that these donor cells have immunoregulatory function that affect both the acute and chronic phases of the recipient anti-donor responses. It follows that detecting quantitative changes in chimerism might serve as an indication of the donor-specific alloimmune or regulatory response that could occur in concert with or independent of other adaptive immune responses. The latter, including autoimmune native disease, need to be controlled in the transplant organ.

The safety and immune tolerance potential of DBMC infusion with deceased and living donor renal transplants was evaluated in a non-randomized trial at this center and compared with non-infused controls given identical immunosuppression.

Overall DBMC infusions were well tolerated by the recipients. There were no complications from the infusion(s), no episodes of graft-vs-host disease (GVHD) and no increase infections or other complications. In the deceased DBMC-kidney trial, actuarial graft survival at 5 years was superior especially when graft survival was censored for recipient death. Acute rejections were significant reduced in patients given two DBMC infusions, and chronic rejection was dramatically reduced in all DBMC treated patients. The most interesting finding was that the degree of microchimerism slowly increased over the years the DBMC group that had exhibited no rejection episodes. In the DBMC-living related trial, the incidence of acute rejection did not differ between groups. However, DBMC chimerism in recipient iliac crest marrow had increased more rapidly than might be predicted from results previously seen in the cadaver group, despite four times fewer DBMC infused, with the generation of T-regulartory cells *in-vitro* assays.

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INTRODUCTION

An elusive goal in human solid organ transplantation has been the induction of a state of permanent tolerance to the allograft, free of long-term immunosuppressive therapy. Several centers are attempting to induce donor-specific hypo- or unresponsiveness using donor bone marrow cell (DBMC) infusions to augment or eventually replace chronic immunosuppression in solid organ transplantation,[1-5] based on early work in rodents[6] and the hints of some recent results in human recipients.[7] Until recently, attempts to use DBMC to induce tolerance have not been frequent in clinical studies. Monaco was the first to administer donor-specific bone marrow simultaneously with kidney transplantation using antilymphocyte globulin (ALG) "induction" therapy[8] similar to a protocol he had described in inbred mice.[9] In discussing his results, the experience using blood transfusion in organ transplantation was pointed to emphasizing the number of transfusions and time interval as critical variables in allograft outcome.

Barber et al.[9] in a more recent series (in the cyclosporine era) from the University of Alabama, reported somewhat encouraging short-term results using kidney plus bone marrow from human cadaver organ donors. They presented the results of 57 cadaveric renal allograft recipients who received Minnesota ALG (MALG) followed by the transfusion of cryopreserved donor-specific bone marrow. There were initial encouraging results. However, the long term follow-up from this study showed that the course and frequency of chronic rejection was not significantly affected in either the infused or non-infused controls by the presence of circulating cells of donor origin detected using molecular methods.[10]

Subsequently, the Pittsburgh group has been attempting to induce a tolerant (chimeric) state in recipients of organ allografts using a tacrolimus-based immunosuppression regimen and bone marrow infusions from the donors.[3,4] Previous observations[11,12] documented long-term microchimerism in kidney and liver transplant recipients, some of whom had stopped immunosuppression for several years. With a combination of molecular and cellular methods, donor chimerism was detected 10 to 19 years post-transplantation in the blood, skin, and lymph nodes. It was hypothesized as a result of these observations that cell migration, repopulation, and chimerism are seminal events that define graft acceptance and ultimately can lead to acquired donor-specific nonresponsiveness (tolerance) and that the DBMC might augment this process.

In order to explore the regulatory mechanism involved and to follow the clinical outcome in the hope of eventually reducing the burden of generalized immunosuppression, a series of kidney transplant recipients, perioperatively infused with DBMC in the absence of a bone marrow ablation protocol, have been followed at our center since September 1994.[5,13] The rationale was to produce a chimeric state that would lead to down-regulation of donor-specific alloimmunity, thus eventually enabling a significant reduction in continuous immunosuppressive therapy. As mentioned, the protocol was initiated after seminal observation by Starzl et al.[11,12] of occasional persistence of life-sustaining organ transplants with normal function after decades of total absence of immunosuppressive therapy, coupled with the detection of "microchimerism" of donor-derived cells putatively of bone marrow origin in the recipients. It also evolved on the basis of much supportive evidence of the *in vitro* immunoregulatory effects of several cellular elements of the donor bone marrow demonstrated in our own laboratory.[14-16]

MACROCHIMERISM AND MICROCHIMERISM

There are quantitative distinctions in the definitions of the chimeric state that have very significant functional consequences. Macrochimerism usually is produced after bone marrow (BM) (or a related cell type) transplantation; to achieve macrochimerism invariably requires some type of ablative pre-conditioning. Hematologic stem-cell (HSC) engraftment occurs, giving rise usually to multilineage chimerism. Donor-specific cells are easily detectable by flow cytometry at levels 2% or 3% to 100%. Theoretically, macrochimerism states can be produced by syngeneic, allogeneic, or xenogeneic HSC transplants. In contrast, microchimerism refers to the state in which donor-specific cell levels are low (1% or less), frequently detectable only by molecular techniques[17] and usually consisting of class HLA II antigen bearing dendritic cells. The potential role or significance of microchimerism has been debated. Microchimerism could reflect merely effective immunosuppression; alternatively, such cells might mediate tolerance by functioning as immature antigen presenting cells (i.e. in the absence of co-stimulation)

CLINICAL STUDIES WITH MICROCHIMERISM

Clinical analyses have not uniformly substantiated an association between microchimerism and tolerance and donor-specific hyporesponsiveness. Indeed, in some reports allograft rejection occurred in the presence of microchimerism, and in other long-term graft survival occurred in the absence of microchimerism. Most importantly, thus far microchemerism could not be used as a marker for immunosuppression withdrawal. One

study,[18] only one third of long-term successful patients (20 - 30 years) and only 14% of short-term successful patients (> 2 years) were microchimeric. The detectable microchimerism (1 in 104 donor-recipient cell ratio) did not correlate with human leukocyte antigen matching, rejection, or various clinical and immunologic parameters. Using more sensitive methodology (1 in 10^5 donor-recipient cell ratio), Hisanga et al.[19] showed that 75% of heart and 72% of liver allograft recipients were microchimeras at some time in the posttransplant period; Devlin et al.[20] subjected 18 microchimeric liver allograft recipients with stable function (> 5 years) to a staged program of immunosuppressive drug withdrawal. When the frequencies of achieving complete or partial drug withdrawal were compared, there was no statistical difference between the number of patients who were chimeric versus the number who were nonchimeric. Schlitt et al.[21] reported the extraordinary case of the occurrence of extensive donor-type microchimerism associated with graft rejection 8 years after liver rejection.

RESULTS AT THE UNIVERSITY OF MIAMI

Donor bone marrow infusions in cadaveric renal transplant patients

Between September of 1994 and May of 1998, 63 cadaver renal transplant (CAD) recipients of either 1 or 2 post-operative donor bone marrow cell (DBMC) infusions have been followed, compared with 219 non-infused controls given equivalent immunosuppression. The immunosuppression regimen included a 10 - 14 day course of OKT3 induction, and Tacrolimus, Mycophenolate Mofetil, and methylprednisolone maintenance. A total $7.01 \times 10^8 \pm 1.9 \times 10^8$ DBMC/kg was infused into the CAD recipients on either days 4 and 11 (n=42) or one half of that dose on day 4 (n=21) post-operatively. Follow-up has ranged from 2.5 to 5.5 years thus far (mean follow-up of 3.7 years).

There were 8/63 biopsy-proven acute rejection episodes (AR) in the DBMC-infused group, and 33/219 AR in the control group (P=N.S.). However, only 2/63 DBMC recipients had biopsy-proven chronic rejection (CR), while 41/219 showed CR in the controls ($p=<0.01$). In both groups mortality was not associated with rejection. The actuarial graft survival at 5.5 years in the CAD DBMC group was 90% compared with 82% in the control group ($p=0.5$). However, if death with a functioning graft was excluded, graft survival was 97.4% in the DBMC group and 87.2% in the controls at 5.5 years ($p=0.032$). Forty patients in the control group (40/219) have continued to have deteriorating renal function (increasing serum creatinine concentrations to 2 mg/dl and higher), compared with 2 patients in the DBMC group ($p=0.04$). In the DBMC group chimerism as measured by the quantitative PCR-Flow method[17] in iliac crest marrow aspirates has increased in yearly sequential measurements between 1 and 4 years post-operatively averaging 1.3% at this time, with 5 patients now having between 1.5 and 3% chimeric DBMC in this bone marrow compartment. This has not occurred in the control group who did not receive DBMC. These results point towards a modulatory effect of DBMC infusions on chronic rejection and allograft survival.[22]

Donor bone marrow infusion in living related kidney transplant recipients

Prompted by the results of DBMC infusions in cadaver kidney transplant recipients,[22] we embarked on a study of DBMC infusion in living-related donor (LRD) kidney transplant recipients. Between November 1996 and May 2000, 47 LRD kidney transplant recipients received donor iliac crest marrow ($1.8 \times 10^8 \pm 1.9 \times 10^8$ cells/kg body weight ± SD) in a single infusion 4 days postoperatively. Either OKT3 (n=26) or Daclizumab (n=21) were used for induction therapy, with maintenance tacrolimus, mycophenolate mofetil, and methylprednisolone immunosuppression. These recipients were prospectively compared with 39 noninfused LRD kidney transplants (control group), which received equivalent immunosuppression in the same time period. Clinical follow-up ranged from 19.0 months to 61.6 months (mean 33.2 months). Polymerase chain reaction-flow chimerism analysis[17] and *ex vivo* assays of immunoregulatory activity of chimeric cells were performed.[23,24] The incidence of acute rejection

over this period of time was 10.6% and 10.3%, respectively (i.e., did not differ between groups). Immunosuppressive dosages were somewhat (but not statistically) lower over time in the DBMC group. Four-year actuarial patient and graft survival for the DBMC-infused group was 98% and 98%, and 98% and 95% for the control group, respectively (p=NS). DBMC infusion was well tolerated, with no increase in infectious episodes. DBMC chimerism in recipient iliac crest marrow increased more rapidly than might be predicted from results previously seen in the cadaver group, despite four fold fewer DBMC infused. DBMC and (donor) peripheral blood mononuclear cells purified by immunobeads from recipient bone marrow or PBL (recipients-derived donor cells) inhibited mixed leukocyte responses of the recipient to the donor more strongly compared with (non-chimeric) or freshly obtained peripheral blood cells bone marrow cells aspirated from the donors of experiments.[25] (Matthew 2000 Tx) Additionally, similarly immunobead purified recipient-derived recipient cells from the same chimeric recipient more strongly inhibited the same mixed leukocyte response reactions autologously than a large group of nonchimeric (autologous) bone marrow modulating cells in similar reactions. These observations supported the notion that an immunoregulatory process was generated by DBMC infusion, encouraging a further decrease in immunosuppressive dosing using such assays in the future.[26]

In vitro studies

Mathew et al. and Jin et al. also reported that a number of cell subpopulations of even non- chimeric bone marrow, including $CD34^+$ stem cells, down-regulated these *in vitro* responses. Moreover, when co-cultured with allogeneic stimulators, $CD34^+$ cells were found to give rise directly to both $CD3^+$ TCR $\alpha\beta^+$ as well as $CD3^-$ TCR $\alpha\beta^+$ cells *in vitro* in the absence of a thymus gland, responding weakly in MLR to allogeneic stimulation (but not generating cytotoxic effector cells).[27] Whether these cells become T cell receptor excision circle (TREC) positive or not (in the absence of thymic processing) has not yet been established. Additionally, non-chimeric bone marrow cells inhibited the proliferative and cytotoxic responses of autologous T cells to Epstein-Barr virus (EBV) antigens, inducing T regulatory cells, which in turn could inhibit autologous anti-EBV cytotoxic lymphocyte (CTL) generation[28] and even B-cell anti-cytomegalovirus antibody production (infectious tolerance *in vitro*) through a reduction of T-cell help.[29] Recently we found that specific *in vitro* MLR inhibitory activity could be augmented by autologous human bone marrow transduced with a foreign class II MHC gene sequence of the stimulating cell donor.[30] These studies all suggested a strong inhibitory property of a number of bone marrow cell subpopulations with the notion that they could promote clinical unresponsiveness.

CONCLUSIONS

The potentially important question of the role of DBMC chimerism might be answered if it be convincingly demonstrated that chimeric cells cause a regulatory effect in the organ transplant recipient *in vivo*. Thus, assessment of the quantitative and temporal relationships between the level of chimerism and the regulation of recipient anti-donor responses becomes important. Knowledge of these relationships would allow one to predict that at optimum regulation immunosuppressive therapy could be drastically reduced or withdrawn. The issue may be one of quantitative and functional threshold in the assays of DBMC lineages which need to be determined if grafts can be permanently accepted in the recipient without immunosuppression. Thus far, this was only been addressed on the basis of trial and error when (irreversible) graft loss is potentially at stake.

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