Bone Marrow Augmentation in Renal Transplant Recipients

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THE goal of tolerance induction, although achievable in pre-clinical models, is not yet a clinical reality. One of the most important areas of experimental research in the field of tolerance induction has been the administration of donor-specific bone marrow in conjunction with whole organ transplantation. The same strategy has been tested in several clinical trials; all have included chronic immunosuppression as well.

This paper will review briefly the major published experience to date with bone marrow augmentation in renal transplantation, with passing reference to observations in pre-clinical experimental models. Although the results do not portend the end of the need for chronic non-specific immunosuppression, they suggest that bone marrow augmentation is reasonably safe, has an impact on chimerism and perhaps the outcome after transplantation, and may have a role in future immunosuppressive regimens.

SIGNIFICANCE OF CHIMERISM

The various efforts to augment the characteristic spontaneous microchimerism of whole organ transplantation can best be understood in the context provided by Starzl and Zinkernagel described earlier at this meeting.[1] In this concept, graft acceptance is analogous to an infectious carrier state and is accounted for by two straightforward mechanisms, both of which are governed by the migration and localization of the antigen, as opposed to the antigen per se. The first mechanism is T-cell (and probably also B-cell) clonal activation and then exhaustion (so called activation-associated tolerance) which occur exclusively in lymphoid organs or organized lymphoid collections.[13]

The second mechanism is immune indifference which requires movement of the live antigen to "hide outs" that are inaccessible to cytotoxic T lymphocytes and neutralizing antibodies. [3-6] Because the antigenic passenger leukocytes of organ grafts are widely disseminated and replaced by recipient leukocytes, "indifference" could occur peripherally, at the level of the graft, or at both sites. Negative regulators (ie, suppressor cells, veto cells, cytokine profile changes) may play an accessory role, but they are not essential.[1,3]

EXPERIENCE WITH ADJUNCT BONE MARROW IN ORGAN TRANSPLANTATION

It was not recognized until 1992 that whole-organ transplantation involved engraftment of migratory antigen (ie, passenger leukocytes producing microchimerism). Nevertheless, the induction of chimerism has been a historical theme of transplant research ever since the chimeric state was associated with acquired tolerance by Billingham et al.[7] in their neonatal mouse model.

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The Monaco Model

Some of the earliest and perhaps the most influential work in this area has been that of Monaco and his associates. His pre-clinical model involved the administration of induction antilymphocyte preparations and delayed infusion of bone marrow 2 to 3½ weeks after transplantation.[8-12] Monaco applied this strategy clinically in a 38-year-old sensitized patient who underwent cadaveric kidney transplantation in 1973, followed 25 days later by an intravenous infusion of cryopreserved donor bone marrow.[13] Immunosuppression was with azathioprine, ALG, and prednisone.

Donor cells in the peripheral blood were undetectable 2 months after transplantation. The patient had a relatively benign postoperative course for 8 months, with normal renal function and only one episode of mild histologic rejection (prior to bone marrow infusion); there was no evidence of graft versus host disease (GVHD). She then developed a perforated sigmoid diverticulitis, from which she died. At autopsy, there was no evidence of rejection.

Approximately a decade later, encouraged by Judy Thomas's work with the primate model, [14,15] Monaco performed three living-related kidney/bone marrow transplantations, using the same triple drug induction therapy (including ALG) and administration of 3 to 5×10^8 cryopreserved bone marrow cells/kg on postoperative day 21.[16] Two of the recipients did well, with no evidence of rejection at 11 and 13 months after transplantation, and had evidence of decreasing donor-specific responsiveness. The third allograft was lost to patient non-compliance.

The first large-scale trial of kidney/bone marrow transplantation with the Monaco model was carried out in Birmingham, Alabama by Barber and his colleagues under cyclosporine-based immunosuppression. Fifty-seven patients received cadaver kidneys with induction antilymphocyte therapy, and delayed (postoperative day 17–21) bone marrow infusion.[17] Fifty-four recipients of the contralateral kidneys were studied as controls, although assignment to the bone marrow and control groups was not formally randomized.

The bone marrow group had significantly better graft survival at 12 and 18 months (90% and 85% respectively) than the control group (71% and 67%). There also was a trend toward decreasing donor-specific reactivity in the bone marrow group. In a later study, less rejection was described in the recipients of adjunct bone marrow in whom blood chimerism was detectable,[18] and 1 year after transplantation, peripheral blood chimerism was seen in 56% of the bone marrow-treated patients, and 21% of the control patients. Barber and his group concluded that bone marrow augmentation was a useful modality.

The Miami kidney transplant experience of Miller and his associates under tacrolimus-based immunosuppression is discussed here, out of chronological order, because a modified Monaco strategy of induction antilymphocyte therapy with delayed bone marrow infusion was used. In their first 40 patients, two doses of cryopreserved marrow were given, the first between postoperative days 1 and 4 and the second between postoperative days 10 and 14.[19] In addition to tacrolimus and steroids, some of the patients were administered mycophenolate mofetil (MMF).

As in the Boston and Alabama experiences, this was a non-randomized trial. A comparison was made with 100 concurrent kidney recipients not receiving bone marrow. The observations included: (1) a ten-fold increase in donor cell chimerism in the recipient bone marrow; (2) less peripheral blood chimerism in bone marrow recipients experiencing rejection; (3) higher levels of chimerism in HLA DR identical recipients; and (4) the presence of pluripotent and/or precursor donor cells in recipient peripheral blood and bone marrow. This last finding has also been described by Rao et al. in liver/bone marrow recipients.[20,21]

There was, however, an unexpectedly higher mortality in the bone marrow group, and a higher incidence of viral complications. Although this was of concern to the Miami group, heightened morbidity or mortality has not been observed in subsequent cases, nor any graft losses to rejection (J. Miller, personal communication).

The Slavin/Strober and Sachs/Ildstad Models

The pre-clinical studies of these foregoing investigators represented cumulatively important contributions to the field. Slavin et al.[22] used recipient total lymphoid irradiation and bone marrow infusion to induce incomplete chimerism and stable tolerance in rodents. Because the cytoreduction left a substantial portion of the recipient immune function intact, the tolerance was achieved without GVHD. A small number of immunologically high risk cadaveric kidney transplantations were done with this approach in the pre-cyclosporine era,[23,24] but the series was terminated because of excessive morbidity and because the recipients, who were selected on the basis of high immunobiologic risk factors, could be better and more efficiently treated with cyclosporine-based immune suppression.

The work of Sachs and Ildstad is mentioned here for the sake of completeness, although there have been no published clinical applications. Both of these investigators used supra- or sublethal total body irradiation with hematolymphopoietic reconstitution with an infusion of mixed donor and recipient bone marrow to achieve partial chimerism and stable tolerance in mice without GVHD.[25]

The Pittsburgh Model

The origins of the Pittsburgh program in bone marrow augmentation began with the discovery that all long-surviving kidney (n = 5; 10 to 29 years) and liver (n = 25; 3 to 22 years) recipients had evidence of peripheral microchimerism in at least one of the following sites: skin, lymph nodes, and/or peripheral blood.[1,2,26] These findings led to the development of the two-way paradigm to explain the immunologic relationship between the donor and the recipient[1,2,27] and ultimately to the Starzl/Zinkernagel explanation of acquired immunologic tolerance.[3]

At the beginning, the simplistic therapeutic assumption was that if microchimerism was essential for long-term graft survival, the long-term prognosis, including achievement of drug-free tolerance, might be improved by augmenting the spontaneous chimerism with perioperative donor bone marrow. Thus, bone marrow augmentation without recipient preconditioning was begun in December 1992, and has been done with all of the transplantable organs (kidney, pancreas, islets, liver, heart, lung, and intestine).[28-32]

As with all of the other reported experiences, the logistic difficulties of bone marrow procurement have militated against a randomized trial, and in fact the only randomized trial of bone marrow augmentation is being performed in Miami, in liver recipients.[33] Between December 1992 and October 1996, 86 cases of bone marrow augmentation were accrued among the nearly 800 kidney transplantations at our center.

The Pittsburgh strategy, in which conventional tacrolimus-based immune suppression was used, has differed from previous models in that it attempted to mimic the conditions leading to spontaneous microchimerism. Thus, the recipient is not preconditioned before arrival of the bone marrow (prior immune suppression, cytoreduction, or cytoablation). Instead, a dose of 3 to 5×10^8 fresh unmodified bone marrow is given at the time of organ transplantation.

Although we believe that microchimerism is a requirement for long-term organ allograft acceptance,[1,2,26,27] there are limitations to the chimerism-inducing strategies. Recipient hematolymphopoietic cytoreduction or cytoablation unquestionably enhances the ease and extent of donor leukocyte engraftment, but the potential penalty with each further increment

in cytoreduction is proportionate weakening of the biologic safety device (against GVHD and rejection) that is provided by the nullification effect of the dual cell populations,[34] as has been learned with conventional bone marrow allotransplantation.

The use of multiple bone marrow doses increases the level of chimerism,[35] but to the extent donor specific nonreactivity is induced with the early infusions, subsequent ones place the recipient at jeopardy from GVHD as in a parent to defenseless offspring F_1 hybrid experiment. [36,37] In the first 200 bone marrow augmented liver recipients, there were no examples of clinically significant GVHD. The 18th patient given divided bone marrow doses in a modification of the main protocol died of uncontrollable GVHD.

Our results with the conservative single perioperative bone marrow dose in cadaver kidney recipients have been encouraging: 100% 1-year and 98% 3-year actuarial patient survival, and a 97% and 87% 1- and 3-year actuarial graft survival. Control patients have had 1- and 3-year actuarial patient survival of 95% and 85% ($P < .03$) and 1- and 3-year actuarial graft survival of 92% and 82% (P = ns). Late peripheral blood chimerism has been demonstrable in 91% of the bone marrow recipients and 51% of the control patients ($P < .00001$).[38]

The incidence of acute rejection, delayed graft function, cytomegalovirus infection, and the quality of graft function have been similar in the bone marrow and the control groups. Decreasing donor-specific reactivity has been comparable and has been seen in 43% of the bone marrow and 41% of the control patients. GVHD has not been observed.

CONCLUSIONS

Chimerism has been augmented in all reported series, although at a low level. Long-term outcomes are not yet available, although in some series chronic rejection has remained a problem. The ultimate effect of bone marrow augmentation will await further studies and more follow-up of current patients. It seems apparent, however, that bone marrow augmentation in transplantation is clinically feasible, reasonably safe, and may well play an important role in immunosuppressive strategies of the future.

With appreciation of the commonality of infectious and transplantation tolerance,[3] it may be possible to develop better strategies to augment chimerism. Although it is clear that chimerism is responsible for tolerance by the principal early mechanism of clonal exhaustion/deletion, the role of the immune indifference discussed by Starzl and Zinkernagel is less clear and may be equally subject to biologic immune modulation.

It is probable, as discussed elsewhere,[37,39] that the current cumbersome and expensive techniques of adjunct bone marrow infusion can be replaced by systemic treatment with hematolymphopoietic growth factors (eg, G-CSF, GM-CSF, or Fit ligand).[40] If so, all the lessons currently being learned with the infusion techniques, including appropriate timing, should be applicable to the pharmacological approach to enhancement of chimerism.[34,37]

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