Hematopoietic cell transplantation for tolerance induction

Norma S. Kenyon & Camillo Ricordi

Diabetes Research Institute, University of Miami School of Medicine, Miami, Florida

Received and accepted 18 December 1995

A major focus of the entire transplant community has been to delineate anti-rejection strategies which, alone or via multifaceted approaches, result in the development of donor specific tolerance, thus obviating the need for chronic, generalized immunosuppression of the recipient. Many approaches which have proven successful in rodent models of solid organ or cellular transplantation have not translated well to large animals or humans. This may be due, in part, to a need for multifaceted immunointerventions when transplanting cells or organs between members of outbred, large animal populations.

Historically, both therapeutic manipulation of the recipient and immunomodulation (or immunoisolation) of the graft itself have been explored as potential avenues to acceptance of transplanted cells and/or organs. Intrathymic administration of donor cells or antigens, monoclonal antibody therapy and treatment of cellular grafts to deplete or modulate the function of antigen presenting cells [1] are among the many approaches which have resulted in consistent prolongation of graft survival in rodent models. Although many monoclonal antibody strategies which have shown great promise for the induction of donor specific tolerance in rodents have not been effective in large animals, such studies have led to the widespread use of OKT3 and ATG (anti-thymocyte globulin), in combination with immunosuppressive agents (CSA, FK506, steroids, etc.) for the purposes of induction and/or anti-rejection therapy in humans [2]. With regards to immunomodulation, utilization of this approach for islet cell transplantation has resulted in modest prolongation of allograft survival in dogs treated with decreased levels of CSA [3, 4], although the results have been inconsistent, and this type of approach has not been widely studied for human transplantation.

Hematopoietic reconstitution of cytoablated mice, via transplantation of bone marrow from an allogeneic donor, results in the production of a chimeric animal which, depending on the irradiation/transplant protocol, possesses varying degrees of both host and donor derived hematopoietic cells. Even prior to the introduction of organ transplantation in humans, it was demonstrated that the induction of specific immunological tolerance could be achieved in inbred rodent strains by infusing allogeneic bone marrow or bone marrow derived cell suspensions, with subsequent lifelong acceptance of donor specific skin grafts [5, 6]. With the development of techniques for vascularized anastomoses, organ transplantation in rodents became possible [7, 8], and it was demonstrated that specific immunological tolerance to organ allografts could also be achieved via infusion of bone marrow derived cells [9, 10]. The perceived requirement for cytoablation of the recipient, however, to provide a niche for donor bone marrow cells, has severely limited the application of such an approach to the fields of solid organ and cellular transplantation. Chemotherapy and irradiation are generally not indicated nor desirable for the types of diseases which result in a patient being placed on an organ waiting list.

Infusion of bone marrow derived cells, in the form of donor specific blood transfusions, has been shown to have a favorable effect on human allograft survival [11], although the mechanism responsible for the beneficial effect remains controversial. It has been proposed that enhancement of allograft survival, with either blood transfusion or bone marrow infusion, is a result of the establishment of low grade chimerism [12]. Sharing of Class II antigens between donor and recipient appears to augment the effect, possibly due to the sparing of stem cells from immune attack, with sub-

sequent engraftment and survival of the donor derived cells [12].

Thomas and her colleagues have developed a primate model in which infusion of donor bone marrow (into non-cytoablated recipients), which has been depleted of the Class II bright cells, results in the prolongation of renal allograft survival [13, 14]. A veto mechanism has been proposed to account for the observed effect [15], and the critical cell has been reported to be Class II dim/negative and CD8 positive [15]. Interestingly, this cell shares phenotypic characteristics with the recently described facilitating cell of Ildstad and colleagues [16].

Attempts to tolerize humans with donor bone marrow infusions, however, have been skeptically viewed by clinicians. Using a protocol similar to those which he had described for mice and dogs [17, 18], which employed anti-thymocyte globulin as induction therapy, Monaco and his colleagues performed simultaneous kidney/bone marrow transplants in humans, with mixed results [19]. In the discussion of his findings, Monaco emphasized the critical importance of cell dose, as well as the number and timing of infusions, on allograft survival. Barber and his colleagues utilized PCR to detect the presence of microchimerism in patients who received donor bone marrow infusions in conjunction with a kidney transplant [20]. A significant correlation ($p = 0.01$) between the presence of chimerism (as detected by PCR) and the absence of rejection episodes was observed [20]. The majority of patients (91.3%) who received donor bone marrow were also rejection free; in contrast, 85.7% of patients with undetectable chimerism experienced at least one rejection episode [20].

The recent demonstration that chimerism can be achieved in non-cytoablated animals via multiple infusions of donor bone marrow [21, 22], however, has led to renewed interest in the potential of this approach, especially in light of results from studies of long term human transplant recipients who have discontinued their immunosuppressive medications, yet have maintained excellent graft function 27 to 29 years posttransplant [23]. Analysis of recipient tissues has revealed the presence of microchimerism, in the form of donor derived dendritic cells (DC), thus leading to the concept that the establishment of chimerism may be a prerequisite for the induction and maintenance of donor specific tolerance. Since DC are the most potent antigen presenting cells in the body, and have long been thought to be responsible for direct stimulation of the rejection response, it was postulated that non-antigen

presenting, progenitor DC may somehow be critical to the tolerogenic process [24–26]. These findings led to a series of clinical trials at the University of Pittsburgh, designed to enhance the potential for the establishment of chimerism via infusion of donor vertebral body marrow.

Initially, two recipients of simultaneous liver – bone marrow transplants were conditioned with total lymphoid irradiation. One of the patients experienced a rejection episode, and the second one developed severe graft versus host disease (GVHD), which was resolved by infusion of cryopreserved autologous marrow [27, 28]. All subsequent patients were transplanted in the absence of radiation conditioning, using FK506 and steroids as immunosuppressive agents. Whole bone marrow was infused, in the absence of antibody based induction therapy, into recipients of kidneys, livers, hearts, and intestinal allografts [29]. The key findings of this study were that donor bone marrow infusion was safe, and enhancement of chimerism was achieved [29]. It was not possible to establish any clear correlation between the degree of chimerism and the presence of rejection and/or GVHD [29]. It therefore appeared that one donor bone marrow infusion, at the time of transplant, was not of substantial benefit to patients. The dose and timing of the bone marrow infusion, coupled with the type and timing of immunosuppression, may not have been optimal for the establishment of donor specific tolerance.

At the University of Miami, we have begun large animal trials of donor vertebral body marrow cell (DBMC) infusion in the dog. Our results indicated that, similar to the results obtained in the Pittsburgh trial, peri-transplant DBMC infusion with standard immunosuppression did not produce any beneficial effect. In fact, an increase in the incidence of rejection episodes was observed, as compared to control dogs which did not receive DBMC. A short course of induction immunosuppression (days –3 to –1) with a monoclonal antibody specific for canine T cells, however, allowed for allograft survival in all animals that received DBMC and CSA (20 mg/kg, days –5 to +30) treatment [30].

These observations, together with recent reports regarding the importance of DBMC dose and timing on the ability of DBMC to engraft [31], prompted us to reconsider clinical application of this approach. We therefore initiated a trial to test the effect of dose, timing, and inductive immunosuppression on human liver and kidney allograft survival. Since July of 1994, we have performed 63 liver allografts (32 with DBMC)

and 30 kidney allografts (8 with DBMC).

We now have results which clearly indicate that 2 infusions of donor vertebral body marrow ($5 \times 10^8/\text{kg}$ at day 0 and at day 11) can improve liver allograft survival [31]. The incidence of rejection was reduced following 2 infusions of DBMC. In contrast, patients who received only one DBMC infusion, with or without OKT3 induction therapy, had a higher incidence of rejection than the control group. Despite the presence of rejection, allograft survival at four month follow-up was significantly better in recipients of either one DBMC (89%) or two DBMC (100%) infusions, as compared to controls (74%) [31]. No significant difference between the groups was observed in either the dose of steroids, the dose and level of FK506, or the degree of HLA matching. As was the case in Pittsburgh, we were unable to establish a correlation between the presence or absence of rejection and the degree of chimerism. Longer follow-up time will be required in order to assess the stability, lineage specificity, and effect of chimerism on the incidence of chronic rejection. Reduction in the levels of immunosuppression will be initiated one year posttransplant, in order to determine if donor specific tolerance has been achieved.

While we have not yet attained the donor specific tolerance which has been observed in rodent models, the encouraging results emerging from clinical trials of donor bone marrow infusion provide an impetus to continue to explore this approach in humans.

References

- Charlton B, Auchincloss Jr H, Fathman CG (1994) Mechanisms of transplantation tolerance. *Annu Rev Immunol*: 707–734.
- Waldmann TA (1992) Immune receptors: Targets for therapy of leukemia/lymphoma, autoimmune diseases and for the prevention of allograft rejection. *Annu Rev Immunol* 10: 675–704.
- Kenyon NS, Strasser S and Alejandro R (1990) Ultraviolet light immunomodulation of canine islets for prolongation of allograft survival. *Diabetes* 39 (3): 305–311.
- Alejandro R, Latif Z, Noel J *et al.* (1987) Effect of anti-Ia antibodies, culture and cyclosporin on prolongation of canine islet allograft survival. *Diabetes* 36 (3): 269–273.
- Billingham RE, Brent L and Medawar PB (1953) Actively acquired tolerance of foreign cells. *Nature* 172: 603–606.
- Billingham RE and Brent L (1954) Quantitative studies of tissue transplantation immunity. II. The origins, strength and duration of actively and adoptively acquired immunity. *Proc R Soc B* 143: 58–80.
- Lee S and Fisher B (1961) Portocaval shunt in the rat. *Surgery* 50: 668–673.
- Fabre JW, Lim SH and Morris PJ (1971) Renal transplantation in the rat: Details of a technique. *Aust NZ J Surgery* 41: 69–75.
- Stuart FP, Saitoh T and Fitch FW (1968) Rejection of renal allografts: Specific immunologic suppression. *Science* 160: 1463–1465.
- French ME and Batchelor JR (1969) Immunological enhancement of rat kidney grafts. *Lancet* 2: 1103–1106.
- Opelz G, Sengar DPS, Mickey MR *et al.* (1973) Effect of blood transfusions on subsequent kidney transplants. *Transplant Proc* 5: 253–259.
- De Waal LP and Van Twuyver E (1991) Blood transfusions and allograft survival: Is mixed chimerism the solution for tolerance induction in clinical transplantation? *Crit Rev Immunol* 10: 417–425.
- Thomas JM, Carver FM, Cunningham PR *et al.* (1991) Kidney allograft tolerance in primates without chronic immunosuppression – the role of veto cells. *Transplantation* 51: 198–207.
- Thomas JM, Carver FM, Kasten-Jolly J *et al.* (1994) Further studies of veto activity in rhesus monkey bone marrow in relation to allograft tolerance and chimerism. *Transplantation* 57: 101–115.
- Thomas JM, Verbanac KM and Thomas FT (1991) The veto mechanism in transplant tolerance. *Transplantation Rev* 5: 209–229.
- Kaufman CL, Colson YL, Wren SM *et al.* (1994) Phenotypic characterization of a novel bone marrow-derived cell that facilitates engraftment of allogeneic bone marrow stem cells. *Blood* 84 (8): 2436–2446.
- Monaco AP, Wood ML and Russel PS (1966) Studies on heterologous anti-lymphocyte serum in mice: III. Immunologic tolerance and chimerism produced across the H-2 locus with adult thymectomy and anti-lymphocyte serum. *Ann NY Acad Sci* 129: 190–206.
- Hartner WC, De Fazio SR, Maki T *et al.* (1986) Prolongation of renal allograft survival in antilymphocyte-serum-treated dogs by postoperative injection of density-gradient-fractionated donor bone marrow. *Transplantation* 42 (6): 593–597.
- Monaco AP, Clark AW, Wood ML *et al.* (1976) Possible active enhancement of a human cadaver renal allograft with antilymphocytic serum (ALS) and donor bone marrow: Case report of an initial attempt. *Surgery* 79: 384–392.
- McDaniel DO, Naftilan J, Hulvey K *et al.* (1994) Peripheral blood chimerism in renal allograft recipients transfused with donor bone marrow. *Transplantation* 57: 852–856.
- Stewart FM, Crittenden RB, Lowry PA *et al.* (1993) Long-term engraftment of normal and post-5-fluorouracil murine marrow into normal mice. *Blood* 81: 2566–2571.
- Harrison DE (1993) Competitive repopulation in unirradiated normal recipients. *Blood* 81: 2473–2474.
- Starzl TE, Demetris AJ, Trucco M *et al.* (1993) Chimerism and donor specific nonreactivity 27 to 29 years after kidney allotransplantation. *Transplantation* 55: 1272–1277.
- Starzl TE, Demetris AJ, Murase N *et al.* (1993) Donor cell chimerism permitted by immunosuppressive drugs: a probable basis of organ transplant acceptance and tolerance. *Immunology Today* 14: 326–332.
- Ricordi C, Ildstad ST, Demetris AJ *et al.* (1992) Donor dendritic cells repopulation in recipients after rat-to-mouse bone marrow transplantation. *Lancet* 339: 1610–1611.
- Ricordi C, Ildstad ST and Starzl Te (1992) Induction of pancreatic islet graft acceptance: The role of antigen presenting cells. *Transplantation Science* 2: 34–38.

27. Ricordi C, Tzakis AG, Demetris AJ *et al.* (1993) Reversal of graft-versus-host disease with infusion of stored autologous bone marrow cells following combined liver-bone marrow allotransplantation in man. *Transplantation Science* 3: 76–77.
28. Ricordi C, Tzakis Ag, Zeevi A *et al.* (1994) Reversal of graft-versus-host disease with infusion of autologous bone marrow. *Cell Transplant* 3: 187–192.
29. Fontes P, Rao A, Demetris AJ *et al.* (1994) Augmentation with bone marrow of donor leukocyte migration for kidney, liver, heart, and pancreas islet transplantation. *Lancet* 344: 151–155.
30. Brendel M, Kong SS, Schachner RD *et al.* (1995) The effect of donor specific vertebral body derived bone marrow cell infusion on islet allograft survival in the dog without cytoablative conditioning of the recipient. Presented at the International Symposium on Tolerance Induction, Breckenridge, Colorado, Jan 22–25.
31. Ricordi C, Karatzas T, Selvaggi G *et al.* (1995) Multiple bone marrow infusions to enhance acceptance of allografts from the same donor. *Ann NY Acad Sci*: in press.

Address for Correspondence: Camillo Ricordi, Diabetes Research Institute, University of Miami School of Medicine, 1450 N.W. 10th Avenue, Miami, Florida 33136.