Engineering of Bone Marrow Cells With Fas-ligand Protein–Enhances Donor-specific Tolerance to Solid Organs

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ABSTRACT

Effective immunomodulation to induce tolerance to tissue/organ allografts is attained by infusion of donor lymphocytes endowed with killing capacity through ectopic expression of a short-lived Fas-ligand (FasL) protein. The same approach has proven effective in improving hematopoietic stem and progenitor cell engraftment. This study evaluates the possibility of substitution of immune cells for bone marrow cells (BMC) to induce FasL-mediated tolerance to solid organ grafts. Expression of FasL protein on BMC increased the survival of simultaneously grafted vascularized heterotopic cardiac grafts to 90%, as compared to 30% in recipients of naïve BMC. Similar results were obtained for skin allografts implanted into radiation chimeras at 1 week after bone marrow transplantation. Further reduction of preparative conditioning to busulfan resulted in acceptance of donor skin implanted at 2 weeks after transplantation of naïve and FasL-coated BMC, whereas third-party grafts were acutely rejected. The levels of donor chimerism were in the range of 0.7% to 12% at the time of skin grafting, with higher levels in recipients of FasL-coated BMC. It is concluded that FasL-mediated abrogation of alloimmune responses can be effectively attained with BMC. There is no threshold of donor chimerism, but tolerance to solid organs evolves during the process of donor-host mutual acceptance.

SIMULTANEOUS transplantation of solid organs and bone marrow cells (BMC) is an attractive approach to induction of tolerance. In initial stages, pretransplant conditioning provides effective immunosuppression for both grafts, and the ensuing donor hematopoietic chimerism ensures prolonged survival of the organ graft. However, the temporal window between initiation of alloimmune responses and induction of effective chimerism including mutual donor-host tolerization results in acute rejection of the organ grafts before establishment of hematopoietic chimerism. Reduction of transplant toxicity by reduced intensity conditioning imposes further consideration of ways to overcome rejection in the early period without impairing the pace of hematopoietic reconstitution. One of the promising approaches to abrogation of alloimmune responses is the use of death ligands to simulate the physiological process of activation- induced cell death,[1,2] which has been successfully implemented in donor antigen-presenting cells (APC) and lymphocytes.[3–6] Following the observation that the Fas/Fas-ligand (FasL) interaction is involved in the process of hematopoietic cell engraftment, we recognize it does not induce apoptosis but delivers tropic signals to primitive hematopoietic stem and progenitor cells (HSPC).[7,8] We attempted to use these characteristics to foster transplant tolerance. Decoration of HSPC with a short-lived FasL protein resulted in enhanced levels of donor chimerism and reduced responsiveness to donor antigens, attributed to a suppressive effect of the death ligand on host-versus-graft rejection.[9] Lineage-negative HSPC were demonstrated to present donor antigens and mediate donor antigen-specific immune unresponsiveness by delivery of apoptotic signals almost as effective as immune cells[9] which have been used to induce localized[10] and systemic immune unresponsiveness[6] In this study, we assessed whether decoration of whole BMC with a short-lived FasL protein enhances acceptance of simultaneously

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grafted organs, in addition to or because support of hematopoietic cell engraftment.

MATERIALS AND METHODS

Animals and Transplantation

Animals used in this study were C57B1/6J (B6, $H2K^b$, CD45.2), BALB/c (H2kd), and B10.BR-H2k-H2- T18a/SgSnJJrep (C57B1/BR, $H2K^k$) purchased from Jackson Laboratories (Bar Harbor, ME). Fisher344 and Lewis rats were purchased from Harlan (Jerusalem, Israel). Animals were housed in a barrier facility and conditioned using total body irradiation delivered by an X-ray irradiator (RadSource 2000, Suwanee, GA) at a rate of 106 rad/min. Two consecutive doses of 25 μg/g busulfan were administered 2 days before bone marrow transplant (BMT). Donor cells were suspended in 0.2 mL phosphate-buffered saline (PBS) and infused into the lateral tail vein.

Cell Isolation, Manipulation, and Characterization of Chimerism

Whole bone marrow cells (wBMC) were obtained under aseptic conditions and coated with a chimeric streptavidin-FasL protein via biotinylation as previously described.[9–11] Briefly, cells were suspended in 5 μM EZ-Link Sulfo-NHS-LC-Biotin (Pierce, Rockford, Ill) in PBS for 30 minutes at room temperature, washed with PBS, and incubated with streptavidin-FasL chimeric protein (100 ng protein/1 $\times$ 10^6 cells in PBS). Chimerism was determined by phenotyping peripheral blood lymphocytes with PE-anti-$H2K^b$ mAb (clone CTKb, Caltag, Buckingham, UK) for donor cells and FITC-anti-$H2K^d$ mAb for host cells (clone SF-1.1, BD Pharmingen, Eremdodegem, Belgium).[7–9] Measurements were performed with a Vantage SE flow cytometer (Becton Dickinson, Franklin Lakes, NJ), and normalized against control cells stained with isotype control monoclonal antibodies (mAb).

Tissue/Organ Grafting

Heterotopic vascularized heart transplants were performed in rats as previously described,[6,12] by end-to-side anastomosis of aorta-to-aorta and pulmonary artery–to–inferior vena cava. Full-thickness tail skin from BMC-matched and third-party donors was grafted in the interscapular region of anesthetized chimeras.[13] Grafts were inspected on a daily basis for signs of rejection: heart grafts by abdominal palpation and skin grafts by direct visualization. Failure to detect graft pulse for 2 consecutive days and disappearance of the epidermis were considered to represent complete rejection.

RESULTS

Immunomodulation in Vascularized Heart Allografts

To test the efficacy of FasL-mediated immunomodulation by hematopoietic progenitors in vascularized heart grafts, Lewis rats were radiated at a nonmyeloablative dose of 850 rad and grafted with 10^7 whole BMC from Fisher 344 donors. Heterotopic heart transplants were performed after 12 hours. Rats infused with naïve whole BMC rejected the vascularized heart grafts within 12 $\pm$ 1.6 days (n = 6), while rats infused with FasL-coated BMC rejected 4/7 grafts at similar tempo of 14.3 $\pm$ 0.8 days (n = 6) (Fig 1A). Allograft acceptance was further improved to 66% (4/6) by transplantation of 3 $\times$ 10^7 FasL-coated BMC, indicating dose-dependence of the immunomodulatory effect. These data delineate a direct relationship between the number of FasL-modified donor cells and graft acceptance, consistent with counterattack of rejection.

Immunomodulation in Skin Allografts

The encouraging results of vascularized heart grafts motivated the evaluation of a more stringent setting of alloimmune rejection of skin grafts. Balb/c mice were conditioned by sublethal total body irradiation (750 rad) and grafted with 5 $\times$ 10^6 allogeneic BMC ($H2K^b \rightarrow H2K^d$). Under these conditions, simultaneously grafted donor (C57BL/6) skin was acutely rejected. Transplantation of donor skin ($H2K^b$) after 1 week revealed significant differences between 90% graft survival in recipients of FasL-coated BMC and approximately 30% survival in recipients of naïve BMC (Fig 1B), whereas skin grafts without donor BMC were acutely rejected. To dissociate between the veto effect of FasL and hematopoietic chimerism, the transplant conditions were minimized and skin grafting was postponed. Mice were conditioned with 50 μg/g busulfan, grafted with 2 $\times$ 10^6 allogeneic BMC ($H2K^b \rightarrow H2K^d$) and donor skin grafts were placed after 2 weeks. Recipients of both naïve and FasL-coated BMC accepted the skin grafts, whereas they rejected third-party ($K2K^k$) skin at normal tempo (Fig 1C). The mice displayed low levels of chimerism at the time of transplantation, which were higher in recipients of FasL-coated BMC (Figure 1D). It is unlike that this difference in chimerism was the decisive factor in acceptance of the skin grafts considering the wide variability in individual values within the experimental groups. These data attribute a significant anti-rejection effect to FasL over-expressed by donor BMC.

DISCUSSION

Induction of tolerance to solid organ grafts is a challenging procedure that has been not yet implemented on a large scale.[14,15] Simultaneous transplants of allogeneic tissue/organs and BMC are difficult in part because of cumulative toxicity of the two procedures and extreme morbidity caused by preparative conditioning for BMT.[16] A protocol composed of multiple donor lymphocyte infusions using FasL protein to selectively eliminate donor antigen-reactive immunocytes has shown promising results, emphasizing the essential involvement of regulatory T cells (Treg) in maintaining protracted tolerance.[6] Initial observations that hematopoietic progenitors are positively affected by death receptor/ligand interactions in the transplant setting led to this attempt to use the grafted hematopoietic cells for immmunomodulation.[9] Donor bone marrow evidently sensitizes to donor antigens, therefore, they are effective substitutes for donor lymphocyte infusion to achieve FasL-mediated immunomodulation. A dose-dependence of heart survival as a function of the number of grafted BMC and acceptance of secondary skin grafts in radiation chimeras merge to demonstrate the capacity of hematopoietic cells to use FasL to counterattack alloimmune responses. Although the current data corroborate prior demonstration that FasL