ELSEVIER

Retroviral Gene Transfer of a Donor Class I MHC Gene to Recipient Bone Marrow Cells Induces Tolerance to Alloantigens in Vivo

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GENE THERAPY has been used to treat a variety of genetic disorders. This approach may also be used to transduce recipient cells with donor antigen(s) as a means of pretransplant alloantigen delivery to prolong graft survival.[1] This will eliminate the need for the availibity of donor cells before transplantation and the associated risk of graft-versus-host disease.[2]

MATERIALS AND METHODS

Bone Marrow Cells

Bone marrow cells (BMCs) were harvested from recipient strain mice CBA.Ca ($H2^k$) after intravenous injection of 150 mg/Kg of 5-Fluorouracil (5-FU) to deplete the more mature clongenic cells and to recruit primitive stem cells into cycle.[3] Flow cytometry was used to determine the optimal time for 5-FU pretreatment: BMCs were stained for stem cells using c-kit[4] as positive and Cd4, Cd8, B220, Mac-1 and GR-1 as negative markers and with propidium iodide to determine the proportion of cells in cycle.

Retroviral Transduction and Transplantation

The mouse class I MHC gene, K^b, was inserted into a replication defective LNSX retroviral vector.[6] CBA BMCs were cultured with this retroviral vector (K^bYF) for different periods of time and at different concentrations to determine the optimal conditions for gene transduction. Transduced recipient type CBA BMCs were injected into CBA mice together with 2 doses of anti-Cd4 monoclonal antibody[7] 28 days before transplantation of a heterotopic cardiac graft from a fully allogeneic donor, C57BL/10 ($H2^b$), expressing full complement of allogeneic major and minor histocompatibility antigens including the class I molecule K^b.

RESULTS

Bone Marrow Cells

Intravenous administration of 5-FU increased the bone marrow stem cell population from 0.6% to 1.5% after 12 days and the proportion of cells in cycle from 18% to a peak of 34% after 6 days. 5-FU treatment 7 days before bone marrow harvest was chosen as a compromise, as retroviral vectors only transduce cells in cycle.[5]

Retroviral Transduction and Transplantation

When 5×10^5 transduced recipient BMCs that had been cultured for 2 days with the retroviral vector K^bYF were used, long term graft survival (LTGS) was achieved in 40% and 50% of recipients in 2 separate experiments respectively. This increased to 60% when 5×10^6 transduced BMCs were used. Interestingly, shortening the time during which BMCs were incubated with K^bYF in vitro to 4 h resulted in 100% LTGS in recipients treated with transduced BMCs.

CONCLUSIONS

Treatment of the recipient with syngeneic BMCs transduced with a single donor class I MHC molecule before transplantation can induce long term survival of a fully allogeneic cardiac allograft, demonstrating that gene therapy can be used to induce operational tolerance to alloantigens in vivo.

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