

Induction of Tolerance to Limb Transplants: A Clinically Feasible Protocol Using Mixed Allogeneic Chimerism

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Mixed allogeneic chimerism (MAC) induced tolerance to composite tissue allografts (CTAs) like hand transplants, could obviate the need for long term immunosuppression, and in doing so revolutionize reconstructive surgery. Experimentally, MAC induced tolerance is produced by first infusing donor bone marrow (BM) into “conditioned” hosts then the donor limb is transplanted. The delay period required between BM infusion and limb transplantation makes clinical application of MAC impractical.

Purpose:

The purpose of this study was to develop a protocol whereby this delay period was eliminated to make MAC clinically applicable to induce tolerance to CTAs.

Methods:

ACI rat limbs were transplanted to fully mismatched Wistar Furth (WF) rats. Group I were controls (n=8); chimeras (ACI + WF) were prepared by infusing ACI + T-cell depleted BM into WF rats irradiated with 950 cGy. Sixty days after BM infusion these chimeras received ACI limbs irradiated with 1050 cGy. In Group II (n=10) WF rats irradiated with 950 cGy received T-cell depleted ACI BM and irradiated ACI limbs (1050 cGy) on the same day. Flow cytometry was performed monthly to detect donor chimerism. Animals were assessed daily for evidence of rejection/graft versus host disease (GvHD) for 5 months after which they were electively euthanized.

Results:

Mean donor chimerism levels were 92±1% in Group I and 93±0.5% in Group II. Multilineage chimerism showed high levels of α -TCR positive T-cell chimerism. MLR assays in both groups revealed donor specific hyporesponsiveness with vigorous third party reactivity. Animals in both groups showed no signs of rejection or GvHD during the 5-month follow-up period. All rats were housed in a non-barrier facility, yet none succumbed to opportunistic infections throughout the experiment.

Conclusions:

Limb transplantation followed by BM transplantation induced both high levels of donor chimerism and tolerance in our CTA hind limb model. For the first time, this study shows that infusion of donor BM into conditioned hosts immediately after limb transplantation, in a clinically feasible manner, results in stable MAC with robust tolerance and survival for at least 5 months without rejection or GvHD. This has important implications for tolerance protocols that are currently being tested clinically. Studies are underway to replace radiation based conditioning of the recipient with co-stimulatory blockade or peri-transplant low-dose combination immunosuppression.