

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Alexaner SI, Smith N, Hu M, et al. Chimerism and tolerance in a recipient of a deceased-donor liver transplant. *N Engl J Med* 2008;358:369-74.

Online Supplement

Materials and Methods:

Cells were stained and sorted on a FACSAria. FISH analysis was done on sorted cells in metaphase using probes CEPX to the X centromere and DYZ1 (E>Rubin) to Y heterochromatin (VYSIS, Downer's Grove, IL) in a certified cytogenetics laboratory.

10^7 mononuclear cells from donor (at time of organ harvest) and recipient post-transplant were frozen in DMSO and fetal calf serum and stored in liquid nitrogen. Cells were thawed at 37°C and viability was confirmed by trypan-blue exclusion. Third-party mononuclear cells were provided by a dialysis patient and were isolated by ficol-gradient separation. 4 color flow cytometry was performed using anti-CD3, anti-CD4, anti-CD8, antiCD45RO anti-CD45RA directly conjugated antibodies (BDbiosciences San Jose, CA) on a FACSCalibur and results analyzed using Cellquest (Becton Dickinson Mountain View, CA).

Mixed lymphocyte culture was performed as previously described; 2.8×10^5 in responder cells were cultured with irradiated 2.8×10^5 stimulator cells in $200\mu\text{l}$ in 96-well flat-bottom plates for 5 days. ^3H thymidine was added on day 4. The cells were harvested 18 hours after ^3H thymidine was added.³

TRECS were quantitated on DNA extracted from peripheral blood lymphocytes using QIAamp DNA Mini-Kit (Qiagen,Doncaster,Australia). SYBR-green real time polymerase chain reaction (PCR) was used for TRECs on Rotor-Gene6000 (Corbett Life Sciences,Sydney,Australia). Real-time PCR was performed in triplicate on each sample. SYBRgreen QPCR master-mix was used (Stratagene,LaJolla CA). Primers were TRECforward-primer: CACCTCTGGGCTACGTGCTAG and reverse-primer

GAACACATGCTGAGGTTTAAAGAGAAT. GAPDH primers were forward-primer ACAGCGACACCCATCCTC and reverse-primer CATACCAGGAAATGAGCTTGACAA. Relative quantitation of TRECs to GAPDH was performed using comparative CT method as described.⁴