Methods: T cells from C57Bl/6 wild type (WT) (H-2b) or CD30−/− mice (H-2b) were co-cultured with mitomycin C-treated Balb/C (H-2d) splenocytes as allogeneic stimulators. Cells were harvested after 1-5d of culture and proliferation and cytokine production was assessed (3H-thymidine incorporation, ELISA, ELISPOT). In addition, CD30L-expressing 293 cells were mixed with CD30+ activated T cells and proliferation and cytokine production were assessed. To examine the role of CD30 in vivo, we chose a heterotopic cardiac transplant model utilizing donor hearts from bm3 mice transplanted into WT (H-2b) or CD30−/− (H-2b) mice. The natural bm3 class I gene mutation results in two amino residue site differences in the H-2Kb molecule.

Results: Proliferation of CD30−/− T cells was reduced compared with WT T cells at every timepoint following allogeneic stimulation. IFN-γ and IL-13 production were reduced in CD30−/− T cells on d 3, 4 and 5 following in vitro stimulation, while no differences were observed in IL-2 or IL-10 production. CD30−/− T cells displayed a lower frequency of IFN-γ production when WT. Addition of CD30L-expressing cells to activated WT CD30+ T cells resulted in increased proliferation, IFN-γ and IL-13 production. C57Bl/6 recipients of bm3 grafts demonstrated gradual and persistent loss of graft function over 100 days. Histological analysis revealed interstitial inflammatory infiltrates, edema and minimal fibrosis. In contrast, CD30−/− recipients of bm3 grafts maintained significantly better graft function over 100 days and displayed focal perivascular inflammation, more fibrosis and reduced numbers of apoptotic cells in the graft. Grant coronary artery disease (% affected vessels, stage of GCA, % luminal narrowing, I/M ratio) and circulating alloantibody was also reduced in CD30−/− recipients. Conclusions: These results indicate that CD30 can modulate T cell proliferation as well as cytokine production in response to alloantigen. Moreover, these data demonstrate a role for CD30 costimulation in long term graft outcome.

Abstract#2482 Poster Board #-Session: P357-III THE IMMUNOSUPPRESSIVE DRUG FK778 INDUCES REGULATORY ACTIVITY IN STIMULATED HUMAN CD4+CD25− T CELLS. Ellen Kreijveld, 1 Hans JPM Koenen, 1 Luuk B Hilbrands, 1 Hans van Hoof, 1 Irma Joosten. 1Dept Blood Transfusion and Transplantation Immunology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; 2Dept Nephrology, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands; 3Dept Internal Medicine, University Hospital Maastricht, Maastricht, Netherlands.

The induction of transplantation tolerance involves a T-cell mediated process of immune regulation. In clinical transplantation, the use of immunosuppressive drugs that promote or facilitate this process would be highly desirable. Here, we investigated the tolerance-promoting potential of the immunosuppressive drug FK778, currently under development for clinical therapy. Using a human allogeneic in vitro model we showed that, upon TCR triggering, FK778 induced a regulatory phenotype in CD4+CD25− T cells. Purified CD4+CD25− T cells primed in the presence of FK778, showed hyporesponsiveness upon restimulation with alloantigen in the absence of the drug. This anergic state was reversible by exogenous IL-2 and was induced independent of naturally occurring CD4+CD25− regulatory T cells. The FK778 induced anergic cells showed suppressor activity, were CD25+CD45RO−CD27−CD62L− and expressed CTLA-4, GITR and FoxP3. The cells revealed enhanced phosphorylation of STAT3. In conclusion, the new drug FK778 shows tolerizing potential through the induction of a regulatory T cell subset in CD4+CD25− T cells.

Abstract#2483 Poster Board #-Session: P358-III HEME OXYGENASE-1 CONTROLS CD4+ T CELL PROLIFERATION THROUGH A CD25-ASSOCIATED MECHANISM. Gabriel Glockzin, 1 Hans J Schlitt, 1 Tung-Yu Tsui, 1 Department of Surgery, University of Regensburg Medical Centre, Regensburg, Germany.

Background: To tailor the immune suppression individually, understanding the mechanism of T cell activation is of great interest to minimize the advance effects of immunosuppressive agents after organ transplantation. In this study, we investigated the potentiality of heme oxygenase-1, a homeostatic protein, in T cell activation.

Methods and results: The expression of HO-1 was induced during the human CD4 cell activation upon CD3/CD28 stimulation in a dose-dependent manner. The expression levels of HO-1 were associated with the proliferation levels of CFSE-labelled CD4 cells and with higher levels of Bel-αL and CD25 expression. Yeast two-hybrid and luciferase reporter assays showed that HO-1 could interact with CREB protein, the common transcription factor of Bel-αL and CD25 expression, and could enhance CREB-mediated transcriptional activities. Phenotype analysis of HO-1 deficient mice showed that there were no significant differences in the amount of CD4+CD25+ and CD8+CD25+ in the spleen or lymph nodes as compared to their wild-type littermate. Interestingly, HO-1-deficient CD4+ cells were resistant to Con-A or CD3/CD28 stimulation. The proliferation capabilities of CFSE-labelled HO-1-deficient CD4+ cells were significantly lower than wild-type cells. Although the expression of Bel-αL and CD25 correlated strictly with the proliferation profile, the production of IL-2 was not affected by the HO-1 status of stimulated CD4+ cells. In addition, increase of HO-1 activity by TAT-mediated HO-1 protein transduction, a functional synthetic protein, was able to restore the expression levels of CD25 and the proliferation capabilities of HO-1-deficient CD4+ cells.

Conclusions: Our data suggest that HO-1 plays a pivotal role in CD4+ T cell proliferation through enhancement of CREB activities on the transcription of CD25.

Abstract#2484 Poster Board #-Session: P359-III SELECTIVE CD28 BLOCKADE, WITH MR1 OR CSA, INDUCES CD4+CD25+FOXP3+ TREG CELLS IN MURINE CARDIAC ALLOGRAFTS. Tianluo Zhang, 1 Stephanie Fresnay, 1 Kecheng Liu, 1 Richard O’Hara, 1 Richard N Pierson, 1 Agnes M Azimzadeh. 1Surgery, University of Maryland, Baltimore, MD; 2Wyeth Research, Cambridge, MA.

Aims: Previously we showed that administration of a non-activating anti-CD28 single chain F-variable (scFv) Ab (αCD28) in conjunction with αCD154 (MR1) or CSA induces long-term allograft survival and donor-specific tolerance in mouse cardiac transplantation. Here we investigated whether αCD28 targeting therapies is associated with upregulation of Treg cells in the spleen and in the graft.

Methods: BALB/c recipients of C57Bl/6 or BALB/c heterotopic cardiac transplants were untreated or received combined treatment of αCD28+MR1 or αCD28+CsA (scFv Ab, 200µg, d0-13; MR1, 250µg, d0; CsA, 400µg, d0-3). Donor and third-party skin grafting was performed at day 100. Graft infiltrating cells and splenocytes were harvested at D 10-12, and analyzed by FACS. On POD 100, intragraft gene expression was quantified by real-time RT-PCR (n=2/group).

Results: Graft survival >100d was seen in 8 of 11 mice with αCD28+MR1 and 9 of 12 mice with αCD28+CsA, vs. 9 days without treatment. At 100 days, recipients with surviving cardiac allografts demonstrated prolonged survival of donor skin (median 12 d with CsA, 41 d with MR1), but not third party (MST <8 days in both groups). On POD 10-12, FACS analysis revealed that the proportion of CD4+FoxP3+ in graft infiltrating cells was increased in anti-CD28 treated mice (αCD28+MR1: 5%; αCD28+CsA: 3.4%) as compared to untreated (0.9%) or naive (0.3%) controls; in contrast, the CD4+ profile was similar in the spleen of all transplant groups. On POD 100, real-time RT-PCR revealed that in αCD28+MR1- and αCD28+CsA-treated mice, intragraft foxp3 at was at 57 times higher than in isograft controls; CTLA-4, CD25, IFNy, IL-2, IL-4, IL-10, and TGFB-1 were also increased, from 1.8 to 33 times higher than in isograft controls.

Conclusions: Presence of CD4+CD25+foxp3+ Treg cells in the graft during tolerance induction, and persistent expression of foxp3 and CTLA4 genes within an accepted allograft, suggest that emergence and persistence of Treg cells in the graft may be a critical immunomodulatory mechanism following perioperative anti-CD28 induction therapy. We postulate that these cells mediate durable peripheral donor-specific tolerance in situ, whereas, with this regimen, modulation of systemic anti-donor immunity is only sufficient to prolong donor skin survival.