

Expression of a dominant T-cell receptor can reduce toxicity and enhance tumor protection of allogeneic T-cell therapy

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SUPPLEMENTARY MATERIALS

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SUPPLEMENTARY METHODS:

Retroviral transduction and culture of transduced primary T cells. PhEco packaging cells were transfected using Fugene HD with the pCL-Eco construct and either F5-, F5-TCR-CD19, OTII-TCR or iCre-GFP construct, according to manufacturer's instructions. Splenocytes were harvested from C57BL/6 (H2^b), BALB/c (H2^d) or DBA/J1 (H2^q) female mice and activated with CD3/CD28 beads + 300U/ml IL2 (Chiron) for 24 hrs according to manufacturer's instructions. Where stated, splenocytes were stained for V β 8.1,2-PE and V β 8.3-PE and sorted with anti-PE MACs beads (Miltenyi Biotec) prior to activation. For some experiments splenocytes were depleted of the endogenous V β 11 expressing T cell population by using anti-V β 11-PE Ab and anti-PE MACs beads (Miltenyi Biotech), according to manufacturer's instructions. For some tumor challenge experiments splenocytes were sorted with CD8+ MACs beads (Miltenyi Biotech) prior to activation. After 24 hours, the viral supernatant was harvested from the transfected PhEco cells and used to transduce 6×10^6 activated splenocytes on retronectin-coated (Takara-Bio Inc.) non tissue culture treated 6-well plates. On day 1 post transduction T cells were cultured in fresh medium in the presence of 100U/ml IL2 (Chiron) and the medium was changed again on day 3 post transduction containing 10U/ml IL2 (Roche), 5ng/ml IL7 (R&D Systems) and 5ng/ml IL15 (R&D Systems). Transduced T cells were FACS sorted on day 5 post transduction.

Flow cytometry and FACS cell sorting. The following monoclonal antibodies were used for flow cytometry: anti-murine V β 8.1,2, V β 8.3, V β 11, V β 5, CD3, CD8, CD4, CD19 and Thy1.1. Propidium Iodide staining was used to gate out non-viable lymphocytes. Intracellular cytokine staining was carried out using Fix and Perm and murine anti-IFN γ mAb. All reagents in this section were obtained from BD Biosciences. Data acquisition was performed on a LSR II or Fortessa flow cytometer (BD Biosciences) and analyzed using FACSDiva Version 6.1.3 software (BD Biosciences) or FlowJo Version 7.6.5 software (Tree Star).

For adoptive transfer experiments transduced T cells were stained with either anti-murine V β 11 mAb alone or in combination with NP-pentamer and sorted for T cells binding high levels of V β 11 mAb or V β 11 mAb and NP-pentamer. GFP control transduced T cells were sorted for high GFP expression. FACS sorting was performed on a FACSAria (BD Biosciences).

Generation of dendritic cells and peptide pulsing. Bone marrow was harvested from femurs and tibias of C57BL/6 donor mice and plated out at a concentration of 2×10^6 /ml on a 24 well plate in RPMI medium in the presence of 40ng/ml GM-CSF (Peprotech) to generate bone marrow derived dendritic cells. On day 7 of DC culture, cells were harvested and pulsed with 0.5 μ M NP peptide for 30 min at 37°C prior to transfer into recipient mice.

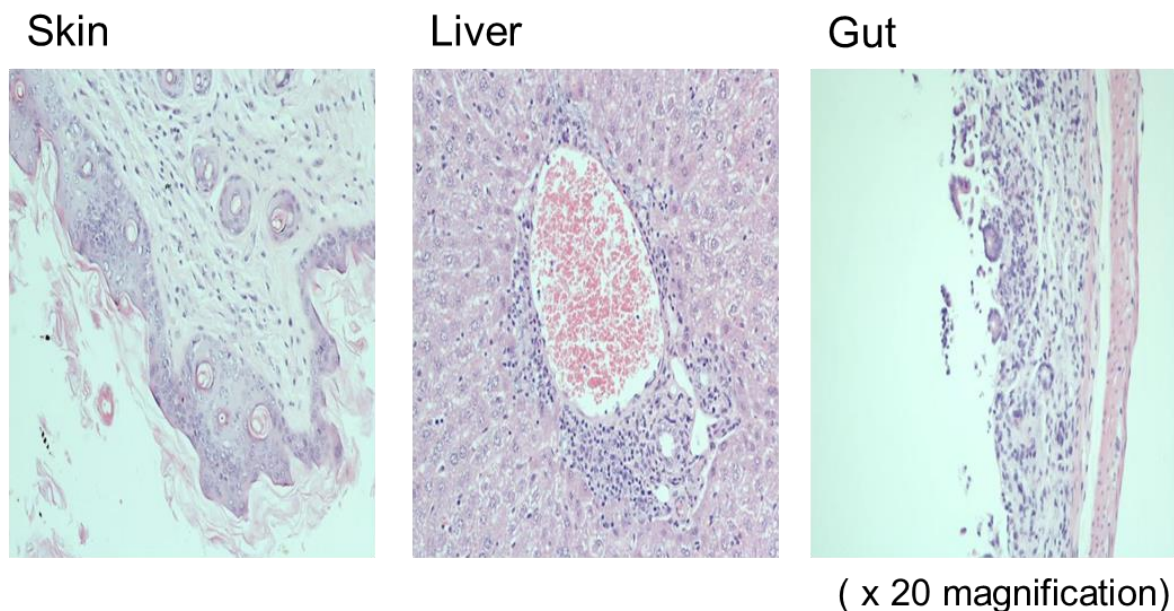
Interferon gamma production. F5-TCR transduced T cells were mixed at a 1:2 ratio with either parental EL4-NP tumor cells or ex vivo isolated EL4-NP tumor cells from recipient mice that had been treated with F5-TCR T cells. Cells were cultured for 4 hours at 37°C and Brefeldin A (Sigma) was added at 5mg/ml for the final 2 hours. IFN γ production was detected as described above.

Assessment of GvHD. Body weights were measured on the day of first irradiation dose and then 2-3 times per week. Weight loss greater than 10% of initial body weight counts as 1. Animals were also scored for clinical evidence of GvHD by assessment of changes in skin, posture, activity, piloerection, inflammation of the

eyes and diarrhoea. Each parameter was quantified by scoring as follows: 0=normal, 1=mild, 2=moderate, 3=severe. Total clinical GvHD scores were calculated as the sum of the scores from each parameter.

Statistical analysis. *P* values comparing changes in body weight were calculated with the unpaired Student's *t*-test using GraphPad Prism software. Survival curves were compared using a log-rank (Mantel-Cox) test. *P* values < 0.05 were considered statistically significant.

SUPPLEMENTARY FIGURE 1:



Supplementary Figure 1: Mice treated with F5-TCR transduced DBA/J T cells had to be sacrificed on day 26 post T cell transfer due to developing toxicity. Skin, liver and gut were H&E stained and histological GvHD was confirmed. Shown are representative sections of skin, liver and gut from one mouse (*n*=6).