

PNAS

www.pnas.org

Supplementary Information for

Treg-mediated prolonged survival of skin allografts: tolerance via ignorance

Nina Pilat, Mario Wiletel, Anna Weijler, Romy Steiner, Benedikt Mahr, Joanna Warren, Theresa Corpuz, Thomas Wekerle, Kylie Webster, Jonathan Sprent

Jonathan Sprent

Email: j.sprent@garvan.or.au

This PDF file includes:

Figs. S1 to S3

Fig. S1

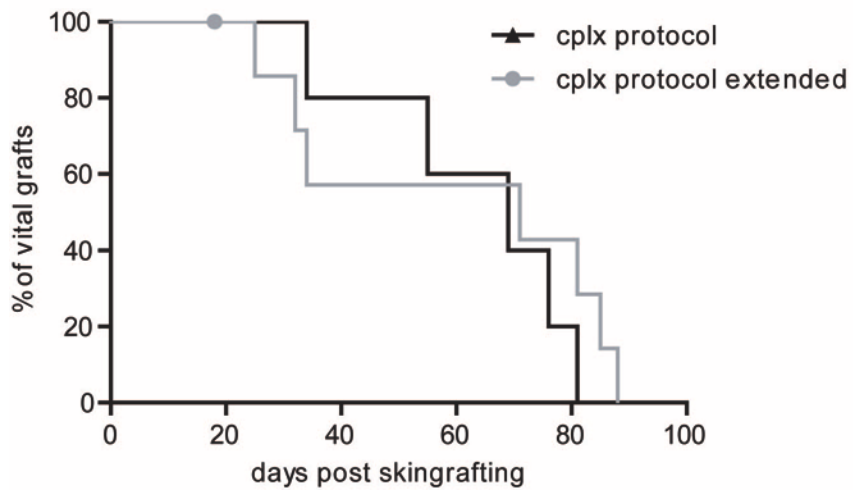


Fig. S1. Extension of IL-2 cplx/rapa treatment doesn't prolong graft survival.

Groups of mice were grafted with fully mismatched BALB/c and treated with IL-2 cplx protocol (30 d LD IL-2 cplx/rapa + a-IL-6; n = 5, MST = 69 d), or prolonged IL-2 cplx protocol (70 d rapa + a-IL-6; n = 8, MST = 71 d) p = n.s. log-rank test.

Fig. S2

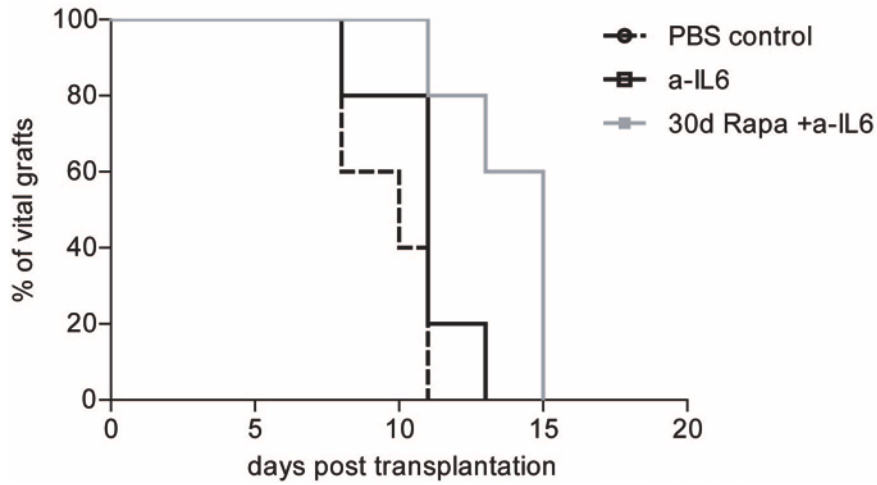


Fig. S2. Rapamycin and anti-IL-6 cannot prevent acute rejection.

Groups of mice were grafted with fully mismatched BALB/c and treated with a-IL-6 (a-IL-6; n = 5, MST = 11 d), a combination of rapamycin and a-IL-6 (30 d rapa +a-IL-6; n = 5, MST = 15 d) or PBS (control; n = 5, MST = 10 d) p = 0.079 log-rank test.

Fig. S3

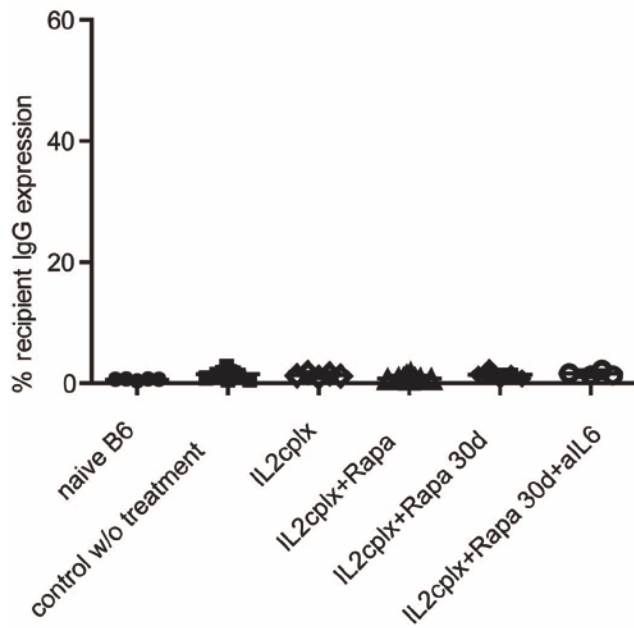


Fig. S3 Control recipient IgG was absent in all groups.

Sera of mice were collected ~2 weeks post skin graft rejection and analyzed for the presence of recipient IgG (naïve B6 n = 5; PBS control n = 11; 3d cplx = 5; 3d cplx/rapa n = 10; 30 d LD cplx/rapa n = 5; 30 d LD cplx/rapa + a-IL-6 n = 4).

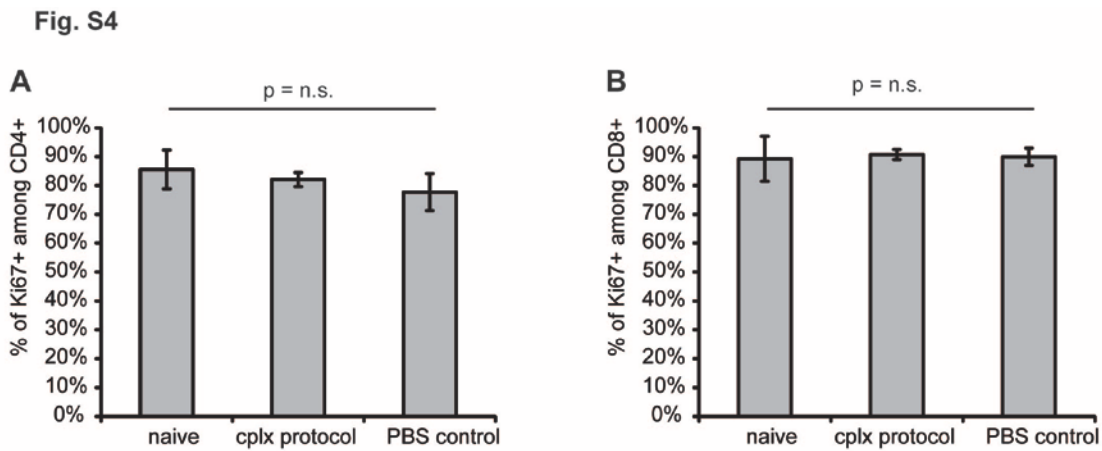


Fig. S4. IL-2 cplx based tolerance protocol does not impair polyclonal T cell responses.

Polyclonal T cell proliferative capacity of operationally tolerant mice was measured *in vitro* on day 50 (3 weeks after complete stop of treatment) in (A) CD4 and (B) CD8 T cells by Ki67 expression after 96h of incubation with polyclonal stimulation with plate-bound anti-CD3/anti-CD28 (naïve (no skinraft) = 2; PBS control = 3, cplx = 4; two tailed t-test with unequal variances).