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CTLA4-Fas Ligand Gene Transfer Mediated by Adenovirus Induce Long-Time Survival of Murine Cardiac Allografts

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ABSTRACT

Background. Fas ligand gene transfer to induce peripheral allograft tolerance in animal models has shown controversial results. The immunosuppression effects mediated by engineered FasL depend on whether alloreactive T cells are selectively deleted. In the present study, we tested the feasibility of a strategy to induce long-time survival by fusing CTLA4-FasL gene transfer in vivo.

Methods. Cardiac allografts from DA(RT-1^a) rats were transplanted heterotopically into the abdomens of LEW(RT-1^1) rats. Plaque units (5×10^9) of either AdCTLA4-FasL, AdCTLA4Ig, or AdEGFP were administered via the portal vein immediately after cardiac transplantation. The frequencies of helper T lymphocyte precursors (HTLp) and cytotoxic T lymphocyte precursors (CTLp) were determined by a combined single limiting dilution assay on days 5 and 20 after transplantation.

Results. Cardiac allograft survival was significantly prolonged by either AdCTLA4-FasL or AdCTLA4Ig treatment(mean survival times [MST] of 71.0 ± 3.7 and 45.7 ± 2.4, respectively, $n = 6$) compared with untreated hosts or animals treated with AdEGFP(MST of 5.7 ± 0.5 and 5.2 ± 0.4, respectively, $n = 6$). In addition, treatment with AdCTLA4-FasL led to significantly prolonged allograft survival compared with AdCTLA4Ig treatment. Furthermore, the frequencies of HTLp and CTLp on day 20 among rats treated with AdCTLA4-FasL was lower than those on day 5, whereas frequencies of HTLp and CTLp on day 20 were similar with those on day 5 in the other groups.

Conclusion. These results suggest that administration of an adenovirus encoding fusion CTLA4-FasL gene to rat recipients effectively decreased the size of alloreactive T cells and induced long-term survival of cardiac allografts.

APOPTOSIS OF ALLOREACTIVE T CELLS is essential for the induction of some forms of stable peripheral allograft tolerance.[1] Attempt to use FasL to inhibit alloimmune responses has achieved prolonged allograft survival.[2] Conversely, ectopic expression of FasL in many tissues has been shown to have a distinct proinflammatory effect, resulting in allograft destruction.[3,4] These results suggest that if alloreactive T cells were specifically deleted by Fas-mediated apoptosis with few side effects peripheral allograft tolerance may be efficiently induced. A recent study showed that dendritic cells(DC) may be effective vehicles to deliver FasL to alloreactive T cells, leading to donor-specific hyporesponsiveness with less toxicity.[5] But manipulation of DC in vitro limits their value for future clinical use. CTLA4 has the advantage of being a target with high affinity for B7 expressed on DC; it may induce donor-specific T-cell anergy and increase the susceptibility of alloreactive T cells to Fas-mediated apoptosis. Thus, ligation of CTLA4-FasL fusion protein to B7 of DC could take advantage of specific homing patterns of DC to secondary lymphoid organs thereby delivering FasL to Fas on alloreactive T cells then selectively deleting alloreactive T cells. Herein, we report that administration of an adenovirus vector encoding fused CTLA4-FasL gene immediately after transplantation was effective to induce long-term survival of cardiac allografts.

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MATERIALS AND METHODS

Animals

Inbred male DA (RT-1[1]) rats and LEW (RT-1[a]) rats weighing 200 to 250 g were used as donors and recipients, respectively. All animals were housed under specific pathogen-free conditions.

Adenovirus Vector

The plasmid pSRalphaSD7 containing CTLA4Ig cDNA was generously donated by Dr. J.F. Elliot (Alberta University, Canada); the plasmid pShuttleCMV, the adenoviral genome plasmid pAdEasy1 and BJ5183 *Escherichia coli* were gifts from Dr Tong-chuan He, The Howard Hughes Medical Institute, Chevy Chase, Maryland, USA). The recombination adenovirus vectors encoding CTLA4-FasL, FasL, CTLA4Ig, or EGFP were generated previously.[6] Recombinant replication-defective adenoviruses titered using a plaque-forming assay were stored in 10% glycerol (v/v) at −80°C. Either AdCTLA4-FasL, AdCTLA4Ig, or AdEGFP was administered via the portal vein immediately after cardiac transplantation.

Cardiac Transplantation and Experimental Group

Heterotopic cardiac allografts were placed into the abdomen of the recipient, using a technique modified from that of Ono and Lindsay.[7] Graft survival was monitored daily by palpation. Rejection, defined as total cessation of the cardiac beat, was confirmed by direct examination. Cardiac allograft recipients were divided into four groups of six rats each: Group 1 received no treatment; group 2, 5×10^9 pfu of AdEGFP; group 3, 5×10^9 pfu of AdCTLAIg; and group 4, 5×10^9 pfu of AdCTLA4-FasL.

Frequency of HTLp and CTLp Estimation

Limiting dilution mixed lymphocyte cultures (LD-MLC) were used to quantitatively analyze the frequency of HTLp and CTLp as described previously.[8] In brief, a constant number (5×10^4) of gamma-irradiated(2500 rad) DA spleen cells and graded numbers ($5, 4, 3, 2, 1, 0.5, 0.25, 0.125 \times 10^4$) of Lewis responder spleen cells were cultured at days 5 and 20 after cardiac transplantation. Then, the CTLL-2 assay was used for HTLp estimation and a cytotoxity assay for CTLp estimation.

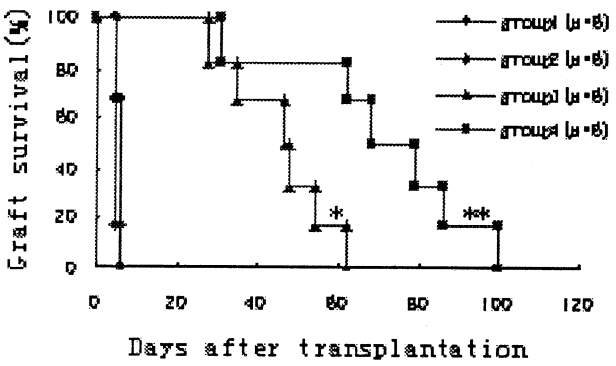

Fig 1. Survival of DA cardiac allografts transplanted in LEW rats that received either no treatment (group 1) or injection of AdEGFP (group 2), AdCTLA4Ig (group 3), or AdCTLA4-FasL (group 4). *$P <$ .001 vs group 1 or group 2, **$P <$ .01 vs group 3.

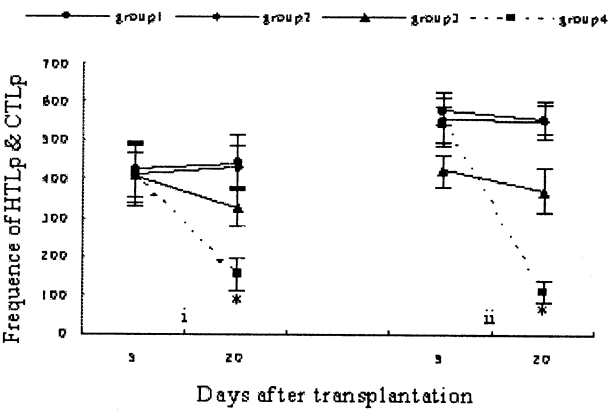

Fig 2. Frequencies of HTLp and CTLp on day 5 or 20 after transplantation in LEW recipient rats that received either no treatment (group 1), or injection of AdEGFP (group 2), AdCTLA4Ig (group 3), or AdCTLA4-FasL (group 4). (i) CTLp frequencies were estimated by cytotoxicity assay. (ii) HTLp frequencies were estimated by IL-2 activity assay. *$P <$ .001 vs day 5.

Statistical Analysis

Results were expressed as mean values ± SD. Continuous variables were compared with one-way ANOVA tests. Cardiac graft survival curves were calculated by the Kaplan-Meier method, with differences between groups compared by the log-rank test. $P < .05$ was considered significant.

RESULTS

The effect of AdCTLA4-FasL on allograft survival was tested in a vascularized heterotopic cardiac transplant model. As shown in Fig 1, the mean survival time (MST) of cardiac allografts in the two control groups(group 1 and group 2) were 5.7 ± 0.5 days and 5.2 ± 0.4 days, respectively. In contrast, the MST of cardiac allografts in group 3 and group 4 were 45.7 ± 2.4 days and 71.0 ± 3.7 days, respectively. Both the difference in MST of cardiac allografts between group 3 and group 1 or group 2 ($P < .001$), the cardiac allograft and in group 4 compared with group 3 ($P < .01$) were significant. These data suggested that AdCTLA4-FasL might be more efficacious to promote acceptance of allografts than AdCTLA4Ig.

Furthermore, to detect the size of alloreactive T cell pool, we use an IL-2 activity assay and a cytotoxicity assay to estimate HTLp and CTLp, respectively. As shown in Fig 2, the frequencies of HTLp and CTLp on day 20 in group 4(410.4 ± 72.4 and 543.7 ± 48.6, respectively) was lower than those on day 5 (152.3 ± 42.6 and 111.7 ± 27.5, respectively). Both differences were statistically significant (each $P < .001$). These findings demonstrate that soluble CTLA4-FasL in vivo produced apoptosis induction of alloreactive T cells. However, the frequencies of HTLp and CTLp on day 20 in the other groups were similar to those on day 5 (each $P > .05$). These observations suggest that other treatments had no effect on T-cell apoptosis.

DISCUSSION

In this study, we observed that administration of AdCTLA4-FasL via the portal vein effectively prolongs the survival of MHC-mismatched cardiac allografts and reduces the size of the alloreactive pool of T cells. Similar to the results of Min et al,[5] the ability of CTLA4-FasL to induce T-cell apoptosis may be due, at least partly, to the use of a membrane-bound form of FasL as well as to the ligation of CTLA4 on B7 of DC. Owing to the function of CTLA4-FasL to reduce the size of the alloreactive T-cell pool in our study, more durable cardiac allograft survival was expected compared with the administration of AdCTLA4. The difference from other regimes was administration AdCTLA4-FasL once immediately after transplantation, thereby avoiding cell manipulation in vitro or repeated exogenous protein injections and thus may be more operational for clinical use.

In conclusion, fusing CTLA4-FasL gene transfer mediated by adenovirus, which disrupts CD28-B7 costimulation and delivers FasL's apoptosis-inducing activity, induced long-term MHC-mismatched rat cardiac allograft survival.

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