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In vivo and in vitro mechanisms of cardiac allograft acceptance in the rat after short treatment with 15-deoxyspergualin

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Abstract. 15-Deoxyspergualin (DSG) has been reported to be a useful immunosuppressive agent already used to inhibit acute rejection in clinical transplantation. In the present study, the survival of heart allograft in rats after a short course of DSG treatment and the mechanisms underlying DSG-induced heart allograft acceptance were studied. Male LEW rats were used as recipients. Male ACI and Wistar rats were used as donors and third-party donors, respectively. Survival of ACI heart grafts in LEW recipients treated with a short course of DSG starting on day 4 after grafting was markedly prolonged, with a mean survival time of 16.6 ± 5.8 days and 29.8 ± 3.0 days at doses of 2.5 mg/kg per day and 5 mg/kg per day, respectively. On day 20 after grafting, the mechanism of inducing allograft survival after DSG treatment at a dose of 5 mg/kg per day was analyzed by testing the activation of spleen cells or serum in several assay systems. Spleen cells from DSG-treated rats with surviving heart allografts showed almost no proliferative response against donor strain stimulator cells compared with controls. The cytotoxic activity towards donor strain target cells of spleen cells from DSG-treated rats with surviving heart allografts was lower than that of spleen cells from rats with rejected heart allografts. Adding various concentrations of spleen cells or serum from DSG-treated LEW rats with surviving ACI heart allografts to the mixed lymphocyte reaction when responder cells from normal LEW rats were exposed to irradiated ACI or Wistar (third-party) stimulator cells, revealed strong suppression in a cell-dose-dependent manner and a serum-dose-dependent manner. Moreover, transfer of 2.0×10^8 spleen cells or 2 ml serum from DSG-treated LEW rats with surviving ACI heart allografts to irradiated grafted host did not prolong the survival either of ACI heart grafts or of third-party Wistar heart grafts. These results suggest that proliferative response and cytotoxic activity are decreased and suppressor cells and suppressor humoral factor(s) are induced by treatment with DSG in rats with surviving allografts.

Key words: Cardiac allograft, in the rat, 15-deoxyspergualin – 15-deoxyspergualin, in cardiac allografts, in the rat – Rat, cardiac allografts, 15-deoxyspergualin

15-Deoxyspergualin {DSG; heptanamide, 7-[(aminoiminomethyl)amino)-N-[2-(4-[(3-aminopropyl)amino]butyl]-amino)-1-hydroxy-2-oxoethyl](±)-trihydrochloride; $C_{17}H_{37}N_7O_3$. 3HCl, MW 496.9}, originally developed for its antibiotic and antitumor activity [6, 30] has shown a potential as a clinically valuable immunosuppressive agent. Several communications have reported that this agent is immunosuppressively active against acute rejection in human renal transplantation [2, 29]. In vitro experiments in human subjects have shown that the principal effect is an inhibition of the later phase of mixed lymphocyte reaction (MLR), mainly by suppression of the expression of interleukin-2 receptors and cytotoxic T cell generation [17]. It was also found that DSG inhibited spontaneous plaque-forming cell (SPFC) generation with alloantigen stimulation [15]. Moreover, many studies in animals have demonstrated that DSG is an effective immunosuppressive agent, capable of inhibiting immunoresponse in rat skin grafts, rat heterotopic heart transplantation and dog renal transplantation [1, 7, 27]. These findings indicate that DSG may facilitate the prolongation of allograft acceptance and led us to further study its mechanisms of action in the inhibition of allograft rejection in the rat model.

In this present study, experiments were therefore designed to show heterotopic heart allograft survival in a rat model after a short course of DSG treatment, and to study the mechanisms of graft acceptance after a short course of DSG treatment.

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Abbreviations: DSG 15-Deoxyspergualin; *MLR* mixed lymphocyte reaction; *CML* cell-mediated lympholysis.

Materials and methods

Animals

Male LEW rats ($RT1^l$) weighing 250–300 g were used as recipients. Male ACI rats ($RT1^a$) weighing 150–200 g and Wistar rats ($RT1^c$) weighing 150–200 g were used as donors and third-party donors, respectively. The animals were obtained from commercial sources (LEW: Charles River, Japan; ACI: Hoshino Experiment Animals, Japan; Wistar: SLC Japan) and kept under specific-pathogen-free conditions in our animal facility.

Immunosuppression

15-Deoxyspergualin was kindly supplied by Nippon Kayaku Co. Ltd (Tokyo, Japan). The drug was dissolved in physiological saline and stored at −70 °C before use.

Heterotopic heart transplantation

Heart transplantations were performed using the modified technique of Ono and Lindsey [21], with the donor aorta and pulmonary artery anastomosed end to side to the recipient's abdominal aorta and inferior vena cava, respectively. The survival of the cardiac allograft was determined by palpation daily. Rejection was considered complete at the time of cessation of a palpable heart beat and confirmed by histological examination.

Preparation of spleen cells

Lymphocytes were obtained from rat spleen. The spleen was isolated and minced, and red cells were then lyzed with buffered hypotonic tris ammonium chloride (0.83% pH 7.21). Cells were washed with RPMI 1640 twice and then suspended in RPMI 1640 complete medium containing 10% fetal calf serum, 30 m*M* Hepes, 2.5 m*M* L-glutamine and 5 μg/ml gentamycin, in various concentrations of cells, for assay.

Mixed lymphocyte reaction (MLR)

One way MLR was performed using spleen cells from DSG-treated LEW rats with surviving ACI heart grafts on day 20 after grafting or from normal LEW rats as responder cells and ACI rats as stimulator cells. 0.5×10^6/ml responder cells were cocultured with 1.0×10^6/ml 2000-rads-irradiated stimulator cells in 96-well tissue culture plates in RPMI 1640 complete medium. The cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 for 4 days and then treated with a 16- to 20-hr [^{3}H]-thymidine pulse. The cells were harvested and [^{3}H]TdR incorporation was measured by a liquid scintillation counter (Packard Tri Carb 4530). The percentage of suppression was calculated using the formula:

$$\%\text{suppression} = \left(1 - \frac{\text{cpm (experimental)} - \text{cpm (negative control)}}{\text{cpm (positive control)} - \text{cpm (negative control)}}\right) \times 100$$

Cell-mediated lympholysis (CML)

Spleen cells from DSG-treated LEW rats with surviving ACI heart grafts, as responder cells, were cocultured with 2000-rads-irradiated normal ACI stimulator cells in RPMI 1640 complete medium at 37 °C in a humidified atmosphere of 5% CO_2 for 6 days. Splenic responder cells from LEW rats with rejected ACI heart allografts were used in the control. After incubation, cells were harvested and used as effector cells for CML. Target cells were prepared by culturing stimulator cells with 50 μg/ml concanavalin A for 2 days. The 4.0×10^6/ml effector cells were cultured with 1.0×10^5/ml ^{51}Cr-labeled target cells for 4 h at 37 °C, in a humidified atmosphere of 5% CO_2. A fixed volume of supernatant was collected from each well, after centrifugation at 1500 g for 10 min. The ^{51}Cr release was counted in a gamma counter (Aloka, JDC-752). The percentage of cytotoxicity was calculated according to the following formula:

$$\%\text{cytotoxicity} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100$$

Suppressor cell and suppressor serum reactivity

Suppressor cell and suppressor serum reactivity in MLR was also assayed by adding 0.5×10^4, 1.0×10^4, or 2.0×10^4 spleen cells or 2.5%, 5.0%, or 10% serum from DSG-treated LEW rats with surviving ACI grafts on day 20 after transplantation to MLR when responder cells from normal LEW rats were exposed to irradiated ACI or Wistar (third-party) stimulator cells. In the control experiment, spleen cells or serum from normal LEW rats were added.

Adoptive transfer by spleen cell or serum

LEW rats were given 250 rads whole-body irradiation, and on the following day were grafted with ACI or Wistar (third-party) hearts. On day 1 of grafting, they received an intravenous injection of 2.0×10^8 spleen cells or 2 ml serum. The spleen cells and serum were obtained from DSG-treated LEW rats with surviving ACI grafts on day 20 after grafting or normal LEW rats. Survival of the graft was the endpoint of this experiment.

Experimental design

Heart allografts were transplanted from ACI to LEW rats following 10 days of DSG treatment, from day 4 to day 13 after transplantation, in two experimental groups: group 1 received DSG intraperitoneally at a dose of 2.5 mg/kg per day (LEW/ACI, $n = 5$) and group 2 received DSG intraperitoneally at a dose of 5.0 mg/kg per day (LEW/ACI, $n = 5$). The result was compared with those in isografts (LEW/LEW, $n = 5$) and untreated allografts (LEW/ACI, $n = 6$). In order to study the mechanisms underlying induction of allograft acceptance, the spleen cells and serum of LEW recipients with surviving ACI heart grafts, on day 20 after grafting, following a short course of treatment with DSG 5.0 mg/kg per day, were used for testing their activation in the following assays: (1) lymphocyte proliferative response, (2) cytotoxic T cell activity, (3) suppressor cell and suppressor serum reactivity in MLR, (4) adoptive transfer assay.

Statistical analysis

The statistical significance of the results was assessed by Student's *t* test.

Results

Induction of allograft acceptance by treatment with DSG

Isografted hearts survived more than 50 days and untreated allografted hearts (LEW/ACI, $n = 6$) were all rejected, with a mean survival time of 6.0 ± 0.7 days. However, survival of ACI heart allografts in LEW recipients treated with DSG 2.5 mg/kg per day or 5 mg/kg per day for 10 days, from day 4 to day 13 postoperatively, were mar-

Table 1. Effect of 15-deoxyspergualin (DSG) on heterotopically transplanted rat heart[a]

Experimental group	Survival (days)	Mean ± SD	P^b value
Isogeneic grafts ($n = 5$)	42, 49, > 50 ($n = 3$) > 50		
Allogeneic grafts			
No immunosuppressant (control, $n = 6$)	5, 6 ($n = 3$), 7 ($n = 2$)	6.1 ± 0.7	$P < 0.05$
DSG 2.5 mg/kg per day ($n = 5$)	10, 12, 14, 16, 21	16.6 ± 5.8	$P < 0.01$
DSG 5.0 mg/kg per day ($n = 5$)	26, 28, 30, 31, 34	29.8 ± 3.0	

[a] DSG was given intraperitoneally for 10 days from day 4 of allografting
[b] Significance determined by Student's t-test

Table 2. Proliferative response of spleen cells from DSG-treated LEW rats with surviving ACI heart graft on day 20 of grafting or from normal LEW rats. SI, Stimulation index

Case	Stimulator cells[a]	Responder cells[b]	cpm[c]	SI
1	LEW	LEW	6382 ± 2263	1
	ACI	LEW	38950 ± 3021	6.1
	ACI	DSG-treated LEW	8656 ± 4247	1.3
2	LEW	LEW	12600 ± 3704	1
	ACI	LEW	47166 ± 3203	5.4
	ACI	DSG-treated LEW	15133 ± 4718	1.2
3	LEW	LEW	16721 ± 776	1
	ACI	LEW	43000 ± 7986	2.6
	ACI	DSG-treated LEW	18833 ± 1625	1.1

[a] 1.0×10^6/ml irradiated (2000 rads) splenic stimulator cells
[b] 0.5×10^6/ml spleen cells from DSG-treated LEW rat with surviving ACI heart graft on day 20 of grafting or normal LEW rat
[c] cpm: mean of a triplicate

kedly prolonged, with mean graft survival times of 16.6 ± 5.8 and 29.8 ± 3.0 days, respectively (Table 1). The histopathological findings also showed severe hemorrhage, edema, and necrosis of myocardial muscle cells in the controls (Fig. 1 a), whereas rats treated with DSG 5 mg/kg per day had only focal cellular infiltration among the myocytes on day 20 after grafting (Fig. 1 b). These results indicate that a short course of DSG treatment prolonged allograft acceptance in rats, even when administration of the drug was started on day 4, and established that the experimental model in which animals were given DSG at a dose of 5 mg/kg per day was suitable for further experiments.

MLR of spleen cells from DSG-treated rats with allograft

To test the proliferative response of spleen cells from DSG-treated LEW rats with surviving ACI heart grafts, spleen cells from DSG-treated recipients or normal LEW rats were used as responder cells and normal ACI spleen cells were used as stimulator cells. The results are shown in Table 2. Responder cells from DSG-treated LEW recipients showed almost no proliferative response against ACI rat stimulator cells compared with normal LEW responder cells.

CML activity in spleen cells from DSG-treated rats with allograft

The cytotoxic activity of spleen cells from DSG-treated LEW rats with surviving ACI heart grafts to donor strain target cells is shown in Table 3. The cytotoxic activity of spleen cells from LEW rats with rejected ACI heart grafts was taken as the control. The results showed that mean cytotoxic activity was 20.2 ± 1.8% in spleen cells from untreated LEW rats with rejected allograft, but 5.7 ± 2.8% in spleen cells from the DSG-treated LEW recipients.

Suppression of MLR by spleen cells or serum from rats with allograft

Studies were performed to assess the capacity of spleen cells and serum obtained from DSG-treated recipients with surviving heart allografts to inhibit the MLR re-

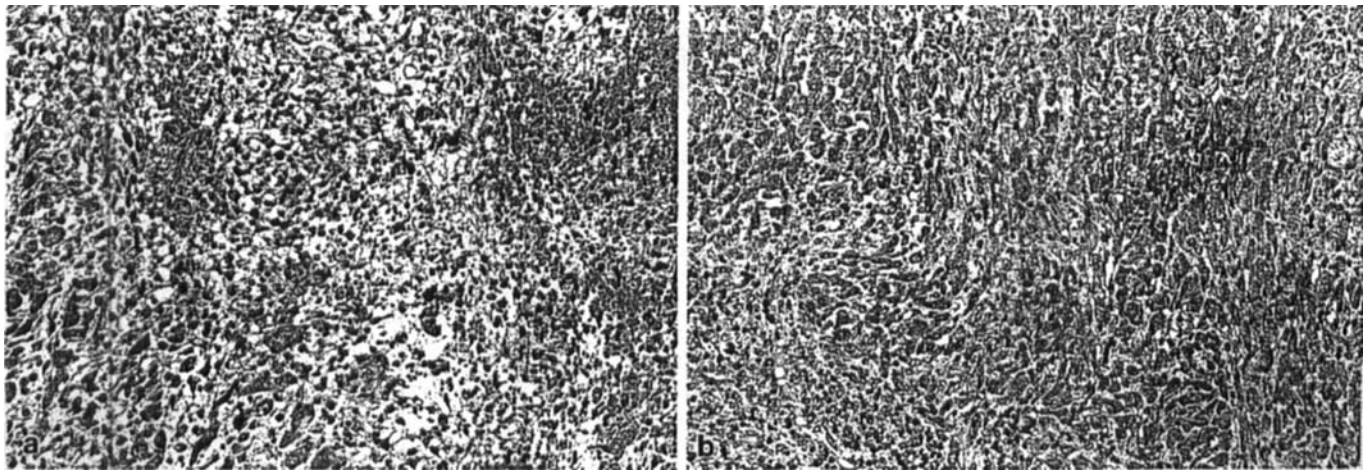

Fig. 1 a, b. Histological sections of heart obtained from **a** untreated LEW rats with rejected ACI heart graft and **b** DSG-treated LEW rats with surviving ACI heart graft at day 20 after transplantation. (H & E. **a**: × 66; **b**: × 66)

Table 3. Cytotoxic activity of spleen cells from DSG-treated LEW rats with surviving ACI heart graft on day 20 of grafting or from LEW rats with rejected ACI heart graft

Case	Effector[a]		Target[b]	Cytotoxicity (%)[c]
	Responder/stimulator			
1	LEWTR[d]	ACI	ACI	19.8
	LEWTS[e]	ACI	ACI	3.1
2	LEWTR	ACI	ACI	18.7
	LEWTS	ACI	ACI	5.4
3	LEWTR	ACI	ACI	22.3
	LEWTS	ACI	ACI	8.8

[a] Responder cells cocultured with 2000-rads-irradiated stimulator cells served as effector cells
[b] Target cells were prepared by culturing stimulator cells with concanavalin A (50 μg/ml) for 2 days
[c] Cell-mediated lympholysis activity was assessed at a 40:1 effector/target ratio
[d] Cells from LEW rat with rejected ACI heart graft
[e] Cells from DSG-treated LEW rat with surviving ACI heart graft

sponse. Spleen cells from normal LEW rats were used as responder cells and normal ACI or Wistar (third-party) spleen cells were used as stimulator cells. Tables 4 and 5 show the results of representative experiments in which 0.5×10^4, 1.0×10^4, or 2×10^4 of spleen cells or 2.5%, 5%, or 10% serum obtained from DSG-treated LEW rats with surviving ACI heart grafts or from normal LEW rats were added to MLR at the initiation of cultures. Both cells (Table 4) and serum (Table 5) from DSG-treated rats markedly suppressed MLR when the responder cells from normal LEW rats were exposed to irradiated ACI or Wistar (third-party) stimulator cells. The addition of diluted cells or serum to MLR also resulted in cell-dose-dependent and serum-dose-dependent suppression. There were no significant differences in the suppressive rate between donor strain and third-party MLR. In contrast, when cells or serum from normal LEW rat were added, no inhibition was observed either in the donor strain or in the third-party MLR response.

Effect of spleen cells or serum transfer from rats with allograft

Alloreactivity of the spleen cells or serum from DSG-treated LEW rats with surviving ACI heart grafts was further analyzed by adoptive cell transfer experiments. Control LEW rats receiving irradiation alone or irradiation plus transfer of normal LEW spleen cells rejected ACI grafts (median graft survival 6.1 ± 2.1 days and 7.0 ± 2.1 days) and Wistar (third-party) grafts (median graft survival 6.1 ± 1.1 days and 6.3 ± 1.2 days). Transfer of 2×10^8 spleen cells from DSG-treated LEW rats with surviving ACI grafts did not significantly prolong the graft survival time of the ACI or Wistar (third-party) hearts transplanted into 250-rads-irradiated LEW rats, although in one rat graft survival was prolonged to 10 days after grafting (Table 6). Transfer of 2 ml serum from DSG-treated LEW rats with surviving ACI grafts also did not alter the graft survival time of the ACI or Wistar (third-party) hearts transplanted into irradiated LEW rats as compared with the control (Table 7).

Table 4. Inhibition of mixed lymphocyte reaction (MLR) by adding spleen cells from DSG-treated rats with surviving ACI heart graft on day 20 of grafting or from normal LEW rats

MLR Responder[a] stimulator[b]	Spleen cells added (normal LEW rat) (no. of cells)	Inhibition (%)	Spleen cells added (DSG-treated LEW recipient rat) (no. of cells)	Inhibition (%)
LEW/ACI	0.5×10^4	-83.4 ± 28.0	0.5×10^4	34.5 ± 17.4
LEW/ACI	1.0×10^4	-101.0 ± 35.6	1.0×10^4	58.9 ± 16.8
LEW/ACI	2.0×10^4	-112.2 ± 64.3	2.0×10^4	86.2 ± 13.0
LEW/Wistar	0.5×10^4	-18.9 ± 32.0	0.5×10^4	22.8 ± 10.0
LEW/Wistar	1.0×10^4	-8.8 ± 17.6	1.0×10^4	50.2 ± 19.4
LEW/Wistar	2.0×10^4	-25.5 ± 28.1	2.0×10^4	76.0 ± 14.6

[a] 0.5×10^6/ml purified normal LEW spleen cells served as responder cells
[b] 1.0×10^6/ml irradiated (2000 rads) ACI or Wistar splenic stimulator cells

Table 5. Inhibition of MLR by adding serum from DSG-treated LEW rats with surviving ACI heart graft on day 20 of grafting or from normal LEW rats

MLR responder[a] stimulator[b]	Serum added (normal LEW rat) serum dose (%)	Inhibition (%)	Serum added (DSG-treated LEW recipient rat) serum dose (%)	Inhibition (%)
LEW/ACI	2.5	2.2 ± 66.2	2.5	13.6 ± 15.3
LEW/ACI	5.0	-22.5 ± 96.4	5.0	37.9 ± 6.1
LEW/ACI	10	-27.3 ± 37.8	10	67.7 ± 7.5
LEW/Wistar	2.5	8.2 ± 47.6	2.5	17.7 ± 13.2
LEW/Wistar	5.0	-0.4 ± 64.3	5.0	35.8 ± 7.6
LEW/Wistar	10	7.0 ± 27.9	10	62.9 ± 14.8

[a] 0.5×10^6/ml purified normal LEW spleen cells as responder cells
[b] 1.0×10^6/ml irradiated (2000 rads) ACI or Wistar splenic stimulator cells

Table 6. Survival of ACI and Wistar heart allografts in irradiated LEW rats after adoptive transfer of spleen cells from normal or DSG-treated LEW rats with surviving ACI heart grafts on day 20 of grafting. NS, Not significant

Heart donor	Cell transfer[a]		Graft survival (days)	Mean ± SD	P^b value
	Lymphocyte donor	No. of cells			
ACI	–	–	5, 6 ($n = 3$), 7 ($n = 2$)	6.1 ± 0.7	
ACI	Normal LEW	2.0×10^8	5, 6 ($n = 2$), 7, 8	6.4 ± 1.1	NS
ACI	DSG-treated LEW with surviving ACI graft	2.0×10^8	5, 6, 7, 8, 10	7.2 ± 1.9	NS
Wistar	Normal LEW	2.0×10^8	14, 15 ($n = 2$), 16, 17	15.4 ± 1.1	
Wistar	DSG-treated LEW with surviving ACI graft	2.0×10^8	14, 15 ($n = 2$), 17, 18	15.8 ± 1.6	NS

[a] Spleen cells transferred to the unmodified LEW rat with ACI or Wistar heart graft on day 1 of grafting
[b] Significance determined by Student's t-test

Table 7. Survival of ACI and Wistar heart allografts in irradiated LEW rats after adoptive transfer of serum from normal or DSG-treated LEW rat with surviving ACI heart graft on day 20 of grafting. NS, Not significant

Heart donor	Serum donor[a]	Dose	Graft survival (day)	Mean ± SD	P^b value
ACI	–	–	5, 6 ($n = 3$), 7 ($n = 2$)	6.1 ± 0.7	
ACI	Normal LEW	2 ml	6 ($n = 3$), 7, 8	6.6 ± 0.8	NS
ACI	DSG-treated LEW with surviving ACI heart graft	2 ml	5, 6 ($n = 2$), 7, 8	6.4 ± 1.1	NS
Wistar	Normal LEW	2 ml	14, 15 ($n = 2$), 16, 17	15.4 ± 1.1	
Wistar	DSG-treated LEW with surviving ACI heart graft	2 ml	15, 16 ($n = 2$), 17 ($n = 2$)	16.2 ± 0.8	NS

[a] Serum transferred to the unmodified LEW rat with ACI or Wistar heart graft on the day 1 of grafting
[b] Significance determined by Student's t-test

Discussion

Suzuki et al. [28] reported that a short course of DSG treatment could induce long-term graft survival in ACI recipients with WKAH heart graft. We therefore conducted studies to investigate whether DSG has this immunosuppressive property in the ACI-to-LEW strain combination, a strain combination considered to have a strong histocompatibility difference. In the present study, DSG treatment from day 4 to day 13 in the LEW recipients with ACI heart grafts resulted in a mean graft survival of 16.6 ± 5.8 days at a dose of 2.5 mg/kg per day and 29.8 ± 3.0 days at a dose of 5 mg/kg per day. Although survival was not prolonged indefinitely, the standard deviation of the graft survival period was low and a high reproducibility of the acceptance state on day 20 could be maintained. The reason for this result, which differed from the data reported by Suzuki et al., was not clear, but it can be assumed that a strong histocompatibility mismatching between two rat strains [3, 8], was involved in our experiment.

One finding in our present study was that DSG has the ability to induce prolongation of graft survival even when administration is started on day 4 after grafting. This finding is similar to that of the clinical study in renal transplantation by Amemiya et al. [2], that DSG is most effective in rescue against ongoing rejection. These results suggest that DSG may selectively suppress the activated T lymphocytes during the acute phase of graft rejection. This is an important difference between DSG and other immunosuppressive agents, such as cyclosporin A and FK-506, which have been reported to have no suppressive effect on activated T cells [19].

With regard to the mechanism of effect, we examined the MLR response and cytotoxic activity of spleen cells from DSG-treated LEW rats with surviving ACI heart grafts. The results showed that both MLR response and CML activity were significantly reduced in spleen cells from the DSG-treated recipients. This effect of DSG treatment strongly suggests that DSG may inhibit lymphocyte response and cytotoxic T cell activity in the spleen in the rats with allografts and consequently allow the prolongation of allograft survival. These results coincide with our previous human in vitro study of MeDSG, which showed suppression of MLR and CML [17]. Moreover, our data also demonstrated that the CML response in spleen cells of DSG-treated rats with surviving allografts is lower than that in spleen cells of rats with rejected allografts towards donor strain target cells. The findings indicated that cytotoxic activity was most closely associated with allograft survival state in the rat [16].

The mechanism of inducing allograft survival after a short course of DSG treatment was further studied by adding spleen cells or serum to the MLR assay. The results showed that MLRs in LEW spleen cells both to donor party (ACI) and to third-party (Wistar) stimulator spleen cells were inhibited in cell-dose-dependent or serum-dose-dependent manner. Various mechanisms of graft survival have been postulated, such as modulation of graft antigen expression [11, 18, 24], depletion of clonally active cells following immunosuppression and exposure to the graft [13, 14], antigen/antibody blockade of effector cells [25, 26], activation of suppressor cells [4, 5, 9, 10, 12, 22] and production of suppressor humoral factor(s) [20, 23]. Our results strongly suggest that DSG may fail to affect suppressor cells and suppressor serum factor(s); these cells and serum factor(s) may progressively develop and play a partial role in continuing allograft survival states. In addition, the inhibition of MLR response between donor and third-party stimulator cells showed no significant difference when spleen cells or serum from DSG-treated recipients were added. This result indicates that the

property of inhibition on day 20 after grafting was a non-specific suppression.

To determine whether spleen cells or serum taken from a short-course DSG-treated rats with surviving allografts would be able to prolong the survival of the donor strain (ACI) or third-party (Wistar) heart grafted into irradiated LEW rats, the cell and serum transfer assay systems were established. The results showed that neither spleen cells nor serum significantly prolonged the mean graft survival time in LEW recipients either in the donor-strain group or in the third-party group, compared with the control group. The reasons for these results are not yet understood, but we believe that the suppressive effect of spleen cells or serum from DSG-treated rats with surviving allografts in the early phase by one dose transfer at a single time point may not be sufficient to withstand the graft rejection.

In conclusion, it was shown that a short course of DSG treatment could induce allograft acceptance. This is mainly due to a decrease in lymphocyte response and cytotoxic activity and partly due to an induction of suppressor cells and suppressor humoral factor(s), especially in the early phase following allografting.

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