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Studies of Alloantibody Effect on Skin Graft Survival in Mice*)

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ABSTRACT

Alloantisera (anti-donor and anti-recipient alloantiserum) were prepared by skin grafting followed four inoculations of 1×10^7 splenocytes.

These alloantisera were cytotoxic ($\times 64$) to target cells specifically.

Anti-recipient alloantiserum was effective on enhancing skin graft survival in mice as same as anti-donor alloantiserum, when administered on the operative day, second postoperative day and fourth postoperative day between 50–200 μl at one time.

INTRODUCTION

For successful clinical transplantation, we have directed our interest to passive enhancement, in particular to alloantibody, which is effective in immunological enhancement.

Many workers have reported that alloantibody is effective in allograft enhancement by anti-donor alloantibody, but only a few reports have been published on allograft enhancement by anti-recipient alloantibody.

This report describes the effect of anti-donor alloantibody and anti-recipient alloantibody on skin graft survival in mice.

MATERIALS AND METHOD

(1) Preparation of alloantibody

Male BALB/c (H-2^d) and C3H/He (H-2^k) were used as donor and recipient. BALB/c anti-C3H/He alloantiserum was prepared by skin grafting followed four inoculations of 1×10^7 splenocytes weekly.

Before use, this alloantiserum was heat-inactivated (56°C, 30 min) and absorbed with RBC of C3H/He in 37°C for 20 min.

C3H/He anti-BALB/c alloantiserum was prepared in the same way as BALB/c anti-C3H/He alloantiserum.

(2) Lymphocyte cytotoxicity test

Lymphocyte cytotoxicity test was conducted in accordance with the NIH standard method.

Splenocytes as lymphocyte sourse were collected through a mesh, RBC in this cell suspension were removed by 0.84% NH_4Cl, and finally cell suspension was concentrated to $300\text{–}500 \times 10^4$/ml.

Mixture of 1 μl of cell suspension and 1 μl of alloantiserum were placed in the well of microtest plate (Terasaki Tray) and left standing for 30 min at room temperature and then 5 μl of complement (fresh rat serum) was added to the well, and left standing for 60 min at room temperature.

They were stained by 5% eosin and fixed by formalin, and then % cell death was counted.

(3) Alloantiserum effect on skin graft survival

BALB/c mice were used as recipient and C3H/He mice as skin donor.

(a) Anti-donor alloantiserum effect on skin graft survival

C3H/He skin was grafted on the back of BALB/c mouse which was injected intraperitoneally with BALB/c anti-C3H/He alloantiserum three times, that is, on the operative day, second postoperative day, and fourth postoperative day. They consisted of three groups which

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were administered 50 μl, 100 μl and 200 μl at one time.

(b) Anti-recipient alloantiserum effect on skin graft survival

C3H/He skin was grafted on the back of BALB/c mouse which was injected intraperitoneally with C3H/He anti-BALB/c alloantiserum three times, that is, on the operative day, second postoperative day, and fourth postoperative day. They consisted of three groups which were administered 50 μl, 100 μl and 200 μl at one time.

RESULTS

(1) Cytotoxic titer of alloantiserum

Cytotoxic titer (%) of C3H/He anti-BALB/c alloantiserum and BALB/c anti-C3H/He alloantiserum for BALB/c and C3H/He splenocytes are shown in Table 1.

Table 1. Cytotoxic titer of alloantiserum

alloantiserum	target cell	× 4	×16	×64	×128	×256
BALB/c anti-C3H/He (anti-donor)	BALB/c	5	5	10	15	10
	C3H/He	90	90	50	10	10
	C57BL/6	10	5	5	5	5
C3H/He anti-BALB/c (anti-recipient)	BALB/c	100	100	90	20	20
	C3H/He	5	5	5	5	5
	C57BL/6	10	5	5	5	5

C3H/He anti-BALB/c alloantiserum was cytotoxic to BALB/c cells at a cytotoxicity of ×64, but was not cytotoxic to C3H/He and C57BL/6 (H-2^b) cells.

BALB/c anti-C3H/He alloantiserum was also cytotoxic to C3H/He cell at a cytotoxicity of ×64, but was not cytotoxic to BALB/c and C57BL/6 cells.

(2) Alloantiserum effect on skin graft survival

(a) Anti-donor alloantiserum effect on skin graft survival

Survival time of C3H/He skin grafts which were transplanted on three groups of BALB/c recipients was examined, that is, the first group was untreated, the second group was treated with BALB/c anti-C3H/He alloantiserum (anti-donor alloantiserum), and the third group was treated with C3H/He anti-BALB/c alloantiserum (anti-recipient alloantiserum).

The mean survival time (MST) of the graft of untreated BALB/c recipients used as control was 12.7±1.6 (S. D.) days.

In the group treated with anti-donor alloantiserum, MST was 17.9±1.9 days in 50 μl group, 18.2±1.9 days in 100 μl group, and 19.8±2.0 days in 200 μl group.

Their graft survival time was significantly longer than that of control group ($P<0.001$), the most prolonged survival was observed in 200 μl group.

(b) Anti-recipient alloantiserum effect on skin graft survival

In the group treated by anti-recipient alloantiserum, MST was 17.5±3.0 days in 50 μl group, 19.2±2.3 days in 100 μl group, and 18.8±4.3 days in 200 μl group. Their graft survival time was significantly longer than that of the control group ($P<0.001$).

Survival was more prolonged in 100 μl group and 200 μl group than in 50 μl group.

The most prolonged time was 28 days in 200 μl group.

DISCUSSION

The immunosuppressants which have been employed during the last two decades in clinical transplantation are non-specific for immunosup-

Table 2. Alloantiserum effect on skin graft survival in mice

Skin graft	treatment	volume	n	MST±SD	survival time (days)
C3H/He (donor) BALB/c (recipient)	(−)		10	12.7±1.6	11,11,11,11,12,13,14,14,15,15
	BALB/c anti-C3H/He (anti-donor)	50 μl	10	17.9±1.9	15,16,17,17,17,18,18,19,21,21
		100 μl	10	18.2±1.9	14,17,17,17,18,19,19,20,20,21
		200 μl	9	19.8±2.0	16,17,19,19,21,21,21,22,22
	C3H/He anti-BALB/c (anti-recipient)	50 μl	11	17.5±3.0	12,15,15,17,17,17,18,18,20,20,24
		100 μl	10	19.2±2.3	16,16,18,18,19,20,20,20,21,24
		200 μl	10	18.8±4.3	13,14,15,17,18,19,19,22,23,28

pressive effects. They suppress not only transplant immunity but also infection protective mechanism and are sometimes lethal due to their side effects.

Therefore, the development of a more effective, safe and specific immunosuppressive therapy has been awaited. Anti-lymphocytic serum (ALS) has been used as a more specific immunosuppressive biological agent[4,5]. ALS, being a heterologous serum, produces antibody in the recipient to decrease the effect of ALS. Symptoms resembling serum sickness develop in the recipient as side effects.

Many reports have been published in the literature on the clinical application of alloantiserum (alloantibody) which has a more specific effect and less side effects with the use of experimental models[1,3,6,9-12].

Among the alloantibodies, French[6], Jeekel[9] and Steines[12] have reported that anti-donor alloantibody is effective in allograft enhancement. However, anti-donor alloantibody is at the same an antibody to the graft and thus there is a risk that this antibody will induce hyperacute rejection[7,8]. This is a serious problem point in the clinical application of this antibody.

On the other hand, in 1976 Davies et al.[2] have reported that anti-recipient alloantibody has no effect on allograft enhancement in his model of skin graft in mice.

In view of the findings that alloantibody is specific as an immunosuppressant and has little side effects and that anti-recipient alloantibody has little danger of inducing hyperacute rejection, we have studied the allograft survival enhancement of this alloantibody.

C3H/He anti-BACB/c alloantiserum and BACB/c anti-C3H/He alloserum were prepared by skin grafting followed by four weekly inoculations of 1×10^7 splenocytes.

These alloantisera were specific for immunogen cells and their cytotoxic titers were both $\times 64$. Before use, this alloantiserum was heat-inactived and absorbed with RBC of the mouse used as immunogen to remove the antibody against RBC.

We examined the enhancing effect of these alloantisera on C3H/He skin transplanted BACB/c recipients when alloantiserum was administered on day 0, 2 and 4 after the transplantation.

In the groups administered anti-donor alloantiserum, a significant enhancing effect on skin graft survival was observed in the groups administered a dose of 50 μl, 100 μl, and 200 μl at one time when compared to the control.

In these experiments, no case of hyperacute rejection was observed in the group that was administered anti-donor alloantiserum, but there is a possibility that hyperacute rejection may be induced in this group when the volume or schedule of alloantiserum administration is changed.

In the group which was administered anti-recipient alloantiserum at a dose of 50 μl, 100 μl, and 200 μl, the skin graft survival was significantly prolonged when compared to that of the control.

These enhancing effects on skin graft survival were all the same for anti-donor alloantiserum and anti-recipient alloantiserum when admin istered in the same volume and schedule.

There has been no report in the literature to the effect that anti-recipient alloantiserum can enhance allograft survival.

Davies et al. have reported that skin graft survival was not enhanced with anti-recipient alloantiserum in mice. It is assumed that our anti-recipient alloantiserum was effective in enhancing skin graft survival, because the administered dose at one time was 5–20 times greater than that given by Davies et al. Both our anti-donor alloantiserum and anti-recipient alloantiserum showed the same enhancing effect on the skin graft survival of mice.

In particular, anti-recipient alloantiserum which does not induce hyperacute rejection is assumed to be more effective, safe and specific immunosuppressant.

Through *in vitro* studies, the mechanism of immunosuppressive effect of anti-recipient alloantiserum will be pursued.

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