

# An Experimental Model for Hyperacute Rejection in Inbred Rat Cardiac Transplantation : Correlation Cardiac Graft Survival Time and Anti T-Cell Warm Cytotoxic Antibody Titer<sup>\*</sup>

Kiyohiko DOHI, Yasuji TABE and Haruo EZAKI

*The Second Department of Surgery, Hiroshima University School of Medicine, 1-2-3,  
Kasumi, Minami-ku, Hiroshima 734, Japan*

(Received March 24, 1984)

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*Key words: Hyperacute rejection, Active immunization, Cardiac transplantation, Hyper immunization, Anti T-cell warm cytotoxic antibody*

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## ABSTRACT

Using genetically homogeneous inbred ACI and Fischer rats, the author has conducted active immunization with the skin and lymphoid cells from ACI rat to Fischer rat and succeeded in making an experimental model that unfailingly causes hyperacute rejection, which has heretofore been considered to be extremely difficult.

Semipermanent survival was gained with syngeneic graft. With allograft, acute rejection of the cardiac graft took place in  $8.1 \pm 1.4$  days. On heart transplantation from ACI to Fischer recipients sensitized by skin graft from ACI and two times active immunization with spleen lymphoid cells from ACI, accelerate rejection occurred in  $60.8 \pm 32.7$  hr.

On transplantation of ACI heart in Fischer recipients subjected to skin graft and five times or more hyperimmunization with spleen lymphoid cells, hyperacute rejection of the cardiac graft occurred in all cases in  $2.2 \pm 3.5$  hr.

As regards active immunization and anti-T cell warm cytotoxic antibody formation, skin graft followed by five times active immunization gave a titer of  $2^{8.11 \pm 1.5}$  and the antibody formation reached a plateau.

Anti-T cell warm cytotoxic antibody and survival time of the cardiac graft showed the following correlation.

$$\log_{10}(y) = -0.054x + 2.113 \quad (x < 3.622)$$

$$\log_{10}(y) = -0.4742x + 3.4778 \quad (x > 3.622)$$

$C = 3.622$  was recognized.

The anti-T cell warm cytotoxic antibodies were found to be decreased with a significant difference ( $P < 0.01$ ) after hyperacute rejection.

## INTRODUCTION

Hyperacute rejection in clinical renal transplants is an abrupt form of rejection that appears in from several min to 24 hr after transplantation<sup>4,16)</sup>, and the recipient's preformed cytotoxic antibodies are considered to play the main role in this<sup>14,22)</sup>.

Reportedly, hyperacute rejection occurs in 0.9

% of all transplantation cases, and the risk of this occurring at a high frequency is great especially in transplants where the preformed lymphocyte cytotoxic antibody crossmatching test between the donor and the recipient is positive, so that organ transplantation is considered to be absolutely contraindicated in such cases<sup>21)</sup>. Opportunities for renal transplants have increased with the growth of cadaveric

<sup>\*</sup> 土肥雪彦, 田部康次, 江崎治夫: ラット心移植における hyperacute rejection モデルの作製

kidney transplantation, but patients on whom transplantation cannot be performed because they are positive for preformed cytotoxic antibody are increasing, and this has become a problem<sup>2,7)</sup>.

As treatments for hyperacute rejection, globulin removal by means of selective plasmapheresis<sup>19)</sup>, antibody adsorption<sup>15)</sup>, and administration of EDTA, heparin, and citrate have been tried<sup>18)</sup>, but no noteworthy results have been obtained.

The delay in the establishment of treatment for hyperacute rejection has been due to the great difficulty of causing hyperacute rejections in the experimental model of allografts<sup>6)</sup>.

It is also attributable to the fact that hardly any basic studies on hyperacute rejection using inbred animals have been made to date because of the very great difficulty of causing this, especially with such animals that are genetically homogeneous.

The main purpose of making this study is to first establish an experimental model of hyperacute rejection, then analyze its pathogenesis, and, based on the knowledge obtained, develop methods of prevention and treatment of hyperacute rejection.

## EXPERIMENTAL ANIMALS AND METHODS

### A. Experimental animals

Two strains of inbred rats<sup>9)</sup>, Fischer rats (RTI<sup>1</sup>) and ACI rats (RTI<sup>a</sup>), 8~12 weeks old and weighing 200~300 g, were used.

### B. Experimental methods

#### (1) Anesthesia

As anesthetics, 100 mg/kg·wt of pentobarbital and 0.1 mg/kg·wt of atropine were injected into the rats intraperitoneally.

#### (2) Heterotopic cardiac transplantation in rats<sup>1,20)</sup>

Heterotopic cardiac transplantation was done according to Ono's method. In abstract, after performing laparotomy on the recipient, side to end anastomosis of the aorta and pulmonary artery of the graft to the abdominal aorta and the inferior vena cava below the renal artery of the recipient was performed.

#### (3) Immunization of rats and preparation of hyperimmunized recipients

ACI rat skin was grafted on Fischer rats and, two weeks after the skin graft, active immunization was done by intraperitoneal injection of  $5 \times 10^7$  spleen lymphoid cells obtained from ACI rats 2 times, 5 times or 8 times at intervals of two weeks, and the active immunized Fischer rats were subjected to experiment one week after the final immunization<sup>21)</sup>.

### C. Measurement of state of immunization of rats

#### (1) Separation of lymphocytes from peripheral blood

Heparinized peripheral blood of rat (50 units/ml) was collected and stratified on Ficol-conray (specific gravity 1078), and this was then centrifuged for 20 min at 1800 rpm. The lymphocyte layer, after being harvested, was washed twice in Hanks fluid. Then, destroying the red blood cells with 0.84% NH<sub>4</sub>Cl and removing their debris, the lymphocytes were resuspended in Hanks fluid.

The lymphocyte suspension suspended in FCS-McCoy fluid was allowed to seep into a nylon wool column, and then incubating this for 30 min at 37°C in 5% CO<sub>2</sub> with air, the nonadherent cells were considered to be the T cell-rich fraction<sup>9)</sup>.

#### (2) Cytotoxicity test

The test for anti-T cell warm cytotoxic antibody (hereinafter abbreviated CA-TW) was made according to the method of Terasaki<sup>5,22)</sup>.

One  $\mu$ l of the lymphocyte suspension adjusted to contain 3000 cells/ $\mu$ l was added to each well into which antisera had been dispensed and this was incubated for 30 min at 37°C. Then, adding 2  $\mu$ l of fresh guinea pig sera (diluted eightfold in Hanks fluid) as complement, this was incubated for one hr at temperature, and after eosin staining, it was fixed with formalin. The antisera titer was expressed in terms of the diluted concentration causing cell lysis in 50% of the target lymphocytes.

### D. Experimental groups

#### (1) Syngeneic graft groups

- a) Group in which Fischer heart was grafted in Fischer recipient
- b) Group in which ACI heart was

grafted in ACI recipient Using 20 randomly sampled inbred Fischer rats, heterotopic grafting was done with Fischer rats as donors and Fischer rats as recipients. Cardiac grafts were made similarly between ACI rats.

- (2) Acute rejection group  
a) Group in which ACI heart was grafted in Fischer recipient

When the heart of an ACI rat was grafted in a Fischer rat having different major histocompatibility antigens, acute rejection of the allograft occurred.

- (3) Accelerate rejection and hyperacute rejection groups

- a) Skin graft immunized group

This is a group of Fischer rats sensitized by ACI skin graft.

- b) Group skin grafted and active immunized with spleen lymphoid cells

This is a group of Fischer rats given ACI skin graft and then subjected to active immunization with ACI spleen lymphoid cells 2 times, 5 times or 8 times at intervals of two weeks.

## RESULTS

### A. Cardiac graft survival time

- (1) Syngeneic graft groups

- a) Group in which Fischer heart was grafted in Fischer recipient

Semipermanent cardiac graft survival of 100 days or more was gained using untreated Fischer rats and grafting Fischer rat heart in Fischer rat recipients.

- b) Group in which ACI heart was grafted in ACI recipients

Semipermanent cardiac graft survival of 120 days or more was gained.

- (2) Acute rejection group

- a) Group in which ACI heart was grafted in Fischer recipient

Cardiac transplant of allografts using ACI rats as donors and Fischer rats as recipients gave cardiac graft survival time of a minimum of 6 days and a maximum of 10 days, or  $8.1 \pm 1.4$  days (M.  $\pm$  S. D.,  $n=10$ ) on the average. (Fig. 1)

- (3) Accelerate rejection and hyperacute rejection group

- a) Skin graft immunized group

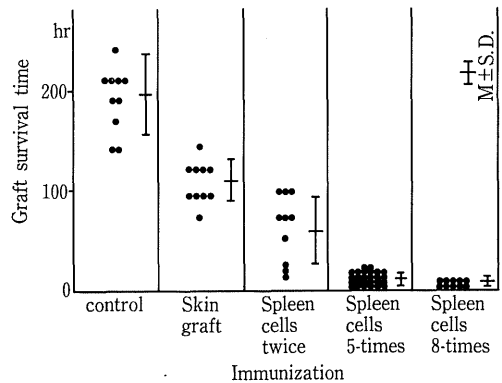


Fig. 1. Correlation between active immunization and cardiac graft survival time

When ACI heart was transplanted in Fischer recipients in the second week after skin grafting, the cardiac graft was rejected in from 72 hr to 144 hr, or in  $108 \pm 20.3$  hr ( $n=10$ ) on the average. Compared with the control group, cardiac survival time was shorter with a significant difference ( $P < 0.01$ ). (Fig. 1)

- b) Group skin grafted and 2 times active immunized with spleen lymphoid cells

The cardiac graft became hard in so-called accelerate rejection in from 12 to 96 hr after blood reflow. Graft survival of  $60.8 \pm 32.7$  hr ( $n=10$ ) was gained. Compared with the control group and the skin graft immunized group, the survival time of the cardiac graft was shorter with a significant difference ( $P < 0.01$ ). (Fig. 1)

- c) Group skin grafted and 5 times active immunized with spleen lymphoid cells

Hyperacute rejection of the cardiac graft occurred in all cases in from 5 min to 12 hr after blood reflow, or in  $2.2 \pm 3.5$  hr ( $n=27$ ) on the average. Compared with the group 2 times active immunized, the survival time of the cardiac graft was shorter with a significant difference ( $P < 0.01$ ). (Fig. 1)

- d) Group skin grafted and 8 times active immunized with spleen lymphoid cells.

Hyperacute rejection of the cardiac graft occurred in all cases in from 8 min to 8 hr after blood reflow, or in  $2.0 \pm 3.1$  hr ( $n=10$ ) on the average. Compared with the group 5 times active im-

munized, no significant difference ( $P > 0.05$ ) was observed by t test. (Fig. 1)

**B. Study of state of immunization of hyperimmunized rats.**

- (1) State of formation of CA-TW by active immunization

CA-TW was found to be formed by skin graft alone and their titer was found to rise sharply with active immunization, rising as high as  $2^{8.11 \pm 1.5}$  and approaching a plateau with 5 times active immunization. (Fig. 2)

- (2) Relation between CA-TW titer and cardiac graft survival time

The relation between cardiac graft survi-

val time and CA-TW in 57 cases subjected to skin graft and active immunization with spleen lymphoid cells was as shown in Fig. 3.

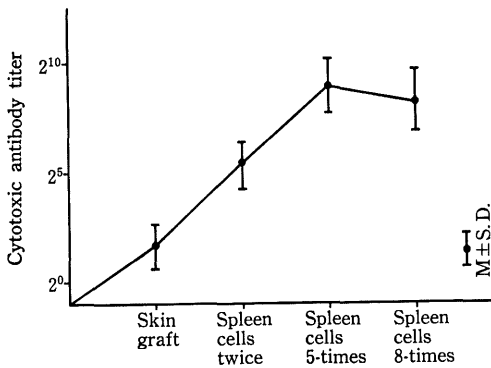
The relation between CA-TW titer and cardiac graft survival time was found to be.

$$\log_{10}(y) = -0.054x + 2.113 \quad (x < 3.622)$$

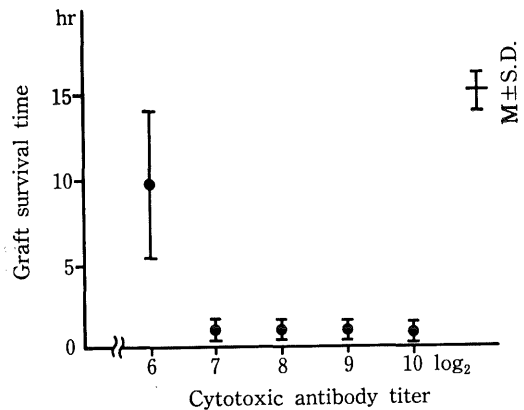
$$\log_{10}(y) = -0.474x + 3.477 \quad (x > 3.622)$$

$C = 3.622$  and  $R^2 = 0.7683$  were recognized.

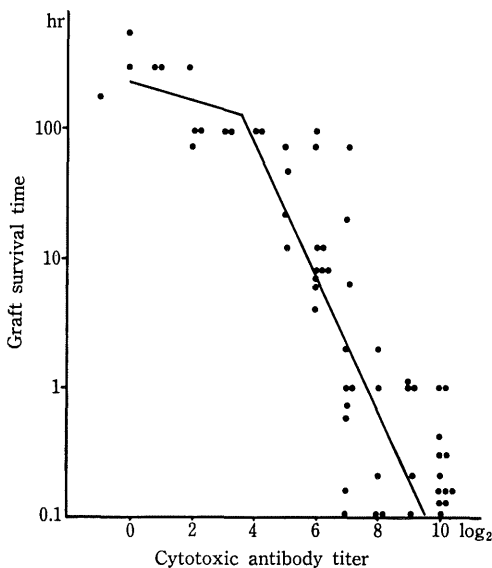
Next, taking just the groups skin grafted and 5 times or 8 times active immunized with spleen lymphoid cells, in which hyperacute rejection occurred in all cases,



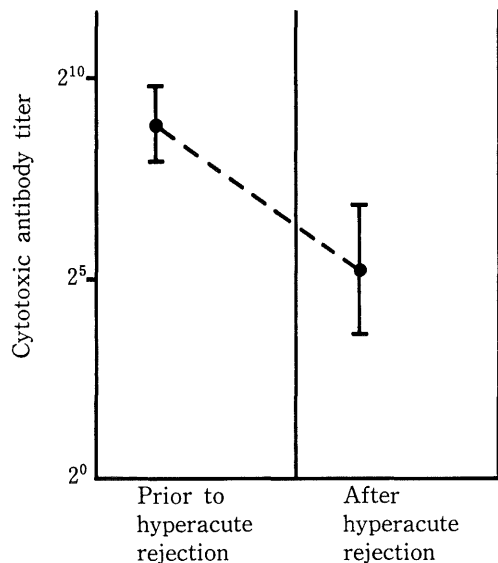
**Fig. 2.** Correlation between active immunization and CA-TW formation



**Fig. 4.** CA-TW titer and cardiac graft survival time at time of hyperimmunization



**Fig. 3.** Correlation between C-TW titer and cardiac graft survival time



**Fig. 5.** Changes in CA-TW titer prior to and after hyperacute rejection

the CA-TW titers and survival times were as shown in Fig. 4. Whereas the cardiac graft survival time was  $8.12 \pm 2.7$  hr (M.  $\pm$  S. D.,  $n=8$ ) in the  $2^6$  titer group, it was  $0.56 \pm 0.55$  hr (M.  $\pm$  S. D.,  $n=28$ ) in the  $2^7$  and over group, the *t* test showing it to be shorter with a significant difference in this group compared with the former group. (Fig. 4)

(3) Changes in CA-TW before and after hyperacute rejection

The CA-TW titer before hyperacute rejection was  $2^{8.9 \pm 0.73}$  and that after hyperacute rejection was  $2^{4.9 \pm 1.30}$ , the *t* test showing the titer in the latter case to be lower with a significant difference ( $P < 0.01$ ). (Fig. 5)

## DISCUSSION

It may be mentioned as a cause for the delay in the establishment of effective countermeasures for hyperacute rejection that, although hyperacute rejection can easily be caused by organ transplantation between hetero-animals<sup>9,18,19</sup>, it is extremely difficult to prepare an experimental model that will unfaillingly cause hyperacute rejection in allografts, especially using inbred experimental animals<sup>6</sup>, and basic studies have not been sufficiently made.

The author therefore has prepared an experimental model using inbred rats which will unfaillingly cause hyperacute rejection and, using this experimental model, studied how anti-T cell warm cytotoxic antibodies are involved in hyperacute rejection. As relevant experiments, there are the regrafting experiment made with dogs by Dempster in 1953<sup>4</sup>, the pre-sensitization of rabbits with repeated skin grafts made by Ueno in 1972<sup>24</sup>, the pre-sensitization of monkeys by repeated skin grafts made by Kobayashi in 1972<sup>15</sup>, and the immunization of dogs with skin grafts and lymphocytes by Kuwahara<sup>17</sup>. These investigators have reported that hyperacute rejection sometimes occurs when the recipient is pre-sensitized with skin graft and injection of lymphocytes from the donor and grafting is done at the time the titer of the cytotoxic antibody to the donor has risen.

French reported in 1972 that hyperacute rejection does not occur in rats because of their weak complement activity<sup>6</sup>. This failure to prepare an experimental model for hyperacute

rejection may probably be attributed to the low pre-transplantation cytotoxic titer of less than  $2^5$  times and insufficient active immunization.

The author's data also shows that, whereas the graft survival time was 489 min in the group whose cytotoxic titer was  $2^6$  times or less, it was 33 min in the  $2^7$  times or over group, demonstrating certain occurrence of hyperacute rejection, and the author is given to consider this to be the reason why certain occurrence of hyperacute rejection could not be gained by French's method.

Later, in 1975, Guttman<sup>9-12</sup> reported that hyperacute rejection occurs when cardiac transplantation is made in recipients pre-sensitized with three repeated skin grafts. By this method, possibly because the immunization was weaker compared with the author's method, it took 24 hr or more for the rejection to occur in some cases and the deviation of graft survival time was great, hence, it is a little questionable whether this can be said an experimental model that will unfaillingly cause hyperacute rejection.

The author grafted an ACI heart in a Fischer recipient after it was immunized with ACI-derived skin graft and spleen lymphoid cells. With active immunization of up to 2 times, the cardiac graft was rejected in  $60.8 \pm 37.7$  hr and only accelerate rejection occurred. In order to cause hyperacute rejection with certainty, skin graft followed by 5 times or more active immunization was necessary. In these groups, hyperacute rejection of the cardiac graft was made with certainty in  $2.2 \pm 3.5$  hr.

Based on the author's results, there presumably exists in the earlier experimental method a problem in the method of sensitization of the recipient, an inappropriateness in the selection of antigen, and an insufficiency in the frequency of immunization.

What belongs to the IgG fraction and has previously been called preformed cytotoxic antibody to peripheral blood lymphocytes is considered to be mostly CA-TW<sup>21</sup>. Transplantation in recipients showing positive on cross-matching, in other words, in those having cytotoxic antibodies to donor T cells, is considered to be absolutely contraindicated because of the great risk of hyperacute rejection<sup>23</sup>.

The author therefore has studied the relation between CA-TW formation and active immunization and hyperacute rejection in rats.

CA-TW is produced just by active immunization with skin grafting, and its titer rises sharply as active immunization with lymphoid cells is repeated. The cytotoxic titer reaches a plateau by 5 times active immunization with lymphoid cells, and it contrarily shows a tendency to decrease with any more immunization. The mechanism bringing this phenomenon about is not known, but it may possibly be that because excessive formation of CA-TW has already occurred, a feedback mechanism is acting to prevent any further hyperplasia.

Between the titer of CA-TW formed by skin grafting and immunization with spleen lymphoid cells and the cardiac graft survival time, there exists the following relation:

$$\log_{10}(y) = -0.054x + 2.113 \quad (x < 3.622)$$

$$\log_{10}(y) = -0.4742x + 3.4778 \quad (x > 3.622)$$

With 3.622 as the dividing point, the graft survival time decreases precipitously as the titer rises and the cardiac graft is rejected by the accelerate and hyperacute rejection mode.

In view of this finding of the author, the view presented in Terasaki's report that grafting in recipients with anti-donor CA-TW should be avoided because of the risk of hyperacute rejection can be considered to be reasonable<sup>23)</sup>. Further, it is a merit of this author's experiment that, whereas heretofore reports have only stated that transplantation results are unsatisfactory in recipients with anti-donor CA-TW, it has made prediction of the result possible by calculation of the cardiac graft survival time based on cytotoxic antibody titer. Changes in the CA-TW titer in the recipient's sera before and after hyperacute rejection have been studied in order to investigate whether or not CA-TW is directly involved in hyperacute rejection.

As reported that preformed cytotoxic antibodies to lymphocytes are absorbed into the transplanted graft when hyperacute rejection occurs, it has been found in the author's experiment that CA-TW is decreased with a significant difference after hyperacute rejection compared with before the transplantation, and it is believed that they developed antigen-antibody reaction with the cardiac graft and were adsorbed into it.

The author's hyperacute rejection model and these findings are believed to be very useful also in making clear hyperacute rejection in clinical cases.

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