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Relationship between the Decrease of Cytotoxic Antibody with the Elapse of Time and Hyperacute Rejection in Hyperimmunized Rats

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ABSTRACT

Preformed cytotoxic antibody which causes hyperacute rejection is formed on gestation, blood transfusion and infection. It is an important problem whether or not transplantation is possible in the recipients sensitized like this.

We studied the decrease of preformed cytotoxic antibody with the elapse of time in sensitized recipients and its influence on graft survival time, using inbred rats.

Inbred ACI rats and Fischer rats were used as experimental models. Hyperimmunized Fischer rat recipients were prepared by skin grafting and spleen lymphoid cell booster. In order to observe the course of decrease or disappearance of the antibody after sensitization, anti T cell warm cytotoxic antibody (CA-TW) was assayed in rat groups which were hyperimmunized one week, one, three and six months before, respectively. The hearts of ACI rats were transplanted to the groups of hyperimmunized Fischer rat recipients to study the relationship between graft survival time and cytotoxic antibody.

1. Controls: The heart of ACI rat transplanted to nontreated Fischer recipient showed graft survival time of 8.1 ± 1.4 days.

2. Group of rats hyperimmunized one week before: The transplanted ACI heart was hyperacutely rejected 0.55 ± 0.38 hr after grafting in all recipients.

3. Group of rats hyperimmunized one month before: The transplanted ACI heart was hyperacutely rejected in five of the 12 recipients. The graft survival time was 17.2 ± 9.2 hr.

4. Group of rats hyperimmunized three months before: Hyperacute rejection was observed in three of the 12 recipients. The graft survival time was 43.0 ± 28.1 hr.

5. Group of rats hyperimmunized six months before: Hyperacute rejection was not observed. The graft survival time of the transplanted heart was 96.0 ± 37.5 hr. The pattern of rejection varied from accelerate to acute rejection.

6. Spontaneous decrease of preformed cytotoxic antibody after hyperimmunization: Fischer rats were hyperimmunized by skin grafting from ACI rat and five booster shots of spleen lymphoid cell. The change of their antibody titer was examined.

CA-TW of the groups hyperimmunized three and six months before, respectively were significantly lower than that for the group hyperimmunized one week before the transplantation.

7. Relationship between preformed cytotoxic antibody titer and graft survival time of transplanted heart: There was a negative correlation between CA-TW titer and graft survival time; $r=-0.7274$.

To sum up, cytotoxic antibody generated by hyperimmunization was decreased with the

passage of time. It was thought that the decrease of CA-TW closely related to graft survival time. It was revealed that hyperacute rejection no longer occurred after a lapse of six months after sensitization and that the graft was taken for more than 90 hr and then rejected either by accelerate or by acute rejection.

It is a well-known fact that preformed cytotoxic antibody appears in sera of patients waiting for kidney transplant because of active immunization resulting from gestation, blood transfusion, previous renal transplantation and microbism[3,7,17,18,21].

It is thought that the opportunity of these sensitization is increasing since it has became possible for hemodialysis patients to live longer owing to improvement of hemodialysis technique.

In fact, of the patients waiting for the cadaveric kidney available for transplant, there are an increasing number of patients who cannot undergo the transplantation because of danger of hyperacute rejection since preformed cytotoxic antibody is formed in the serum. This fact is now becoming a great issue[3,7].

We have investigated the relationship between hyperacute rejection and preformed cytotoxic antibody which is considered to be the major cause of the rejection. We have elucidated and reported that anti T cell warm cytotoxic antibody (CA-TW) played the principal role[4].

However, there are other unanswered important questions: How the preformed cytotoxic antibody decreases and disappears with the elapse of time after sensitization? Which factor is to be reduced to prevent occurrence of hyperacute rejection? Whether or not the recipient once sensitized will be able to undergo transplantation in the future? As to these problems, only a few clinical investigations have been reported[14,21], and no detailed study using inbred strains of experimental animals has been done.

In order to elucidate these problems, we undertook the investigation below, using hyperimmunized rats.

MATERIAL AND METHOD

A. Experimental Animals

The animals used were Fischer rats and ACI rats weighting 150 to 300g, inbred strains whose major histocompatibility antigens were incompatible[8]. ACI rats were used as donors and Fischer rats as recipients.

B. Preparation pf Hyperimmunized Fischer Recipient

The skin of ACI rat was grafted to Fischer rat. Spleen lymphoid cell of ACI rat was intraperitoneally injected to the Fischer rat five times at intervals of two weeks after skin grafting to prepare hyperimmunized Fischer recipient[4].

C. Heterotopic Heart Transplantation

Heterotopic heart transplantation to the abdomen was performed according to the method of Ono et al[12].

D. Assay of Antibody Titer

Anti T cell antibody titer of hyperimmunized Fischer rat serum was assayed according to the method of Terasaki et al[1,29] as previously reported[4].

The samples not reaching 50% cell lysis were judged as negative for cytotoxic antibody. The titer was calculated by being converted into exponent of 1/2 of minimum observed value.

E. Experimental Model

The following groups of experimental models were prepared using ACI rats as donors and Fischer rats as recipients in order to analyze spontaneous disappearance of cytotoxic antibody after hyperimmunization and its effect on graft survival time of the transplanted heart.

1. Controls

In this group, the hearts of ACI rats were grafted to non-treated Fischer recipients.

2. Group of recipients hyperimmunized one week before (n=12)

3. Group of recipients hyperimmunized one month before (n=11)

4. Group of recipients hyperimmunized three months before (n=12)

5. Group of recipients hyperimmunized six months before (n=10)

CA-TW titers of these groups were assayed. At the same time, the hearts of ACI rats were grafted to Fischer recipients of each group to compare graft survival time.

RESULTS

A. The Effect of the Passage of Time after Hyperimmunization on Graft Survival Time

1. Controls

When the heart of ACI rat was grafted to non-treated Fischer recipient, graft survival time was 8.1 ± 1.4 hr (M. ± S.D., n=10).

2. Group hyperimmunized one week before

The heart graft was hyperacutely rejected in 12 of the 12 recipients from five min to four hr, mean: 0.55 ± 0.38 hr (M. ± S.D., n=12) after blood stream was resumed (Fig. 1).

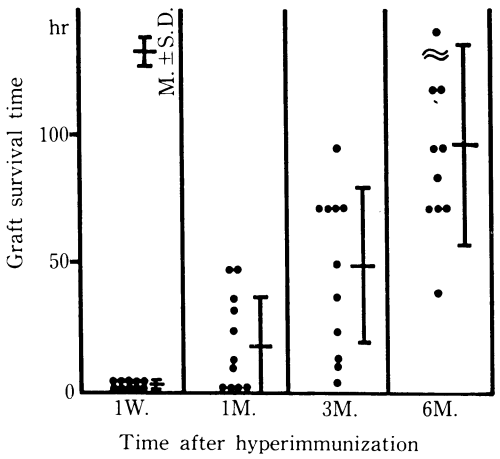

Fig. 1. Correlation between cardiac graft survival time and post hyperimmunization time

3. Group hyperimmunized one month before

Hyperacute rejection was observed in 5 of the 12 recipients. Graft survival time was from five min to 48 hr, the mean being 17.2 ± 9.2 hr (M. ± S.D., n=12). The graft survival time was significantly prolonged compared with the group hyperimmunized one week before ($P<0.05$) (Fig. 1).

4. Group hyperimmunized three months before

Hyperacute rejection was observed in only 3 of the 12 recipients. Graft survival time was from 2 to 96 hr, the mean being 43.0 ± 28.1 hr (M. ± S.D., n=12). The graft survival time was significantly prolonged compared with the group hyperimmunized one week before ($P<0.05$). However, no significant difference was found between this group and the group hyperimmunized one month before (Fig. 1).

5. Group hyperimmunized six months before

None of the ten recipients showed hyperacute rejection. Graft survival time was from 48 to 196 hr, the mean being 96.0 ± 37.5 hr (M ± S.D., n=10). Compared with group hyperimmunized three months before, no significant difference was noted. Compared with the groups hyperimmunized one week or one month before, the graft survival time was significantly prolonged ($P<0.05$) (Fig. 1). On the other hand, this survival time was significantly shorter than 8.1 days for controls, or the group of non-treated Fischer recipients ($P<0.05$).

Table 1. Secular changes in cytotoxic antibody after hyperimmunization

	1 week	1 month	3 month	6 month
CA-TW	8.9 ± 1.2^a	8.2 ± 1.4	$7.2\pm1.0^*$	$5.8\pm0.9^*$

a $2x\pm S.D.$
Titer at leverl of 50% cell lysis.
* Significant difference : ($P<0.05$)

B. Time-course of Spontaneous Decrease of Preformed Cytotoxic Antibody after Hyperimmunization

The time-course of spontaneous decrease of CA-TW is shown in Table 1 and Fig. 2.

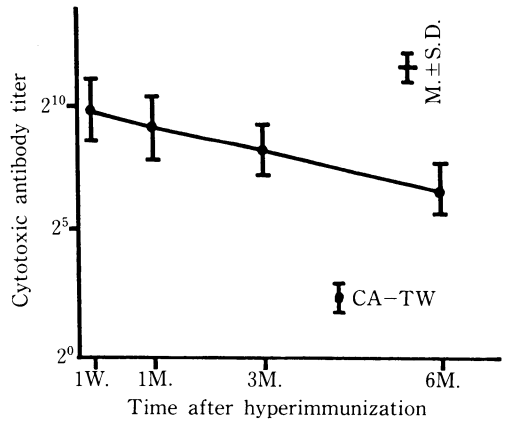

Fig. 2. Secular changes in cytotoxic antibody after hyperimmunization

The titers of CA-TW were $1:2^{8.9\pm1.2}$, $2^{8.2\pm1.4}$, $2^{7.2\pm1.0}$ and $2^{5.8\pm0.9}$ in the groups hyperimmunized one week, one, three and six months before, respectively. The titers of the groups hyperimmunized three and six months before were significantly lower than for the group hyperimmunized one week before ($P<0.05$).

C. Relationship between Preformed Cytotoxic Antibody Titer and Graft Survival Time

There was a high negative correlation between

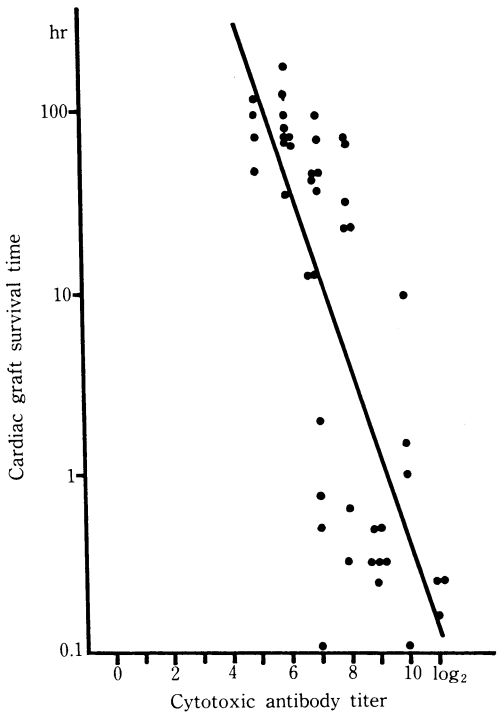

Fig. 3. Correlation between CA-TW titer and cardiac graft survival time after hyperimmunization

CA-TW titer and graft survival time of the transplanted heart. The regression equation was $\log_{10}(Y) = 4.665 - 0.502x \pm 0.774$, $r = -0.7274$, $P < 0.01$ (Fig. 3).

DISCUSSION

Hyperacute rejection in renal transplantation is thought to be caused mainly by rapid antigen-antibody reaction of preformed cytotoxic antibody[3,7,9,17,18,21].

As to the presence of preformed cytotoxic antibody and results of transplantation, many reports have described that the result of transplantation is poorer in the group having cytotoxic antibody for random panel cells before kidney grafting than in the group without cytotoxic antibody[18,21]. When lymphocyte cytotoxicity crossmatch is positive, this is, when antibody for donor exists, transplantation is absolutely contra-indicated since there is a strong possibility of inducing hyperacute rejection[16].

As to how the cytotoxic antibody formed by gestation[9] and blood transfusion[21] changes with the passage of time after sensitization, only Terasaki and Opelz have made clinical investigations and reported on the relation between the changes over time of cytotoxic antibody titers for peripheral blood lymphocytes of random population and results of transplantation[14,16,21].

They reported that cytotoxic antibody for lymphocytes of the random population, once formed in the serum of patients waiting for renal transplant did not disappear and remained for along time in most cases[14,16]. Opelz described that the ratio of the patients with positive cytotoxic antibody for lymphocytes of the random population decreased to 86% two months, 73% six months and 68% one year after sensitization. It was reported that even if cytotoxic antibody had disappeared before transplantation, rejection after transplantation was significantly faster in patients who had once formed cytotoxic antibody than in patients not having the antibody throughout the entire course, and that there was no significant difference from patients having the antibody at the time of the transplantation[14].

It is thought from these reports that kidney transplant should be abandoned in the patients who have once formed cytotoxic antibody.

On the other hand, there was no report describing, with animal experiment, the relation between the changes over time of cytotoxic antibody formed after sensitization and its effect on transplanted graft.

Therefore, we hyperimmunized Fischer rats and transplanted the hearts of ACI rats to the Fischer recipients one week, one, three and six months, respectively after hyperimmunization to observe graft survival time. We also examined how cytotoxic antibody changed with the passage of time after hyperimmunization.

As to the relationship between the antibody changes with the passage of time after hyperimmunization and cardiac graft survival time, it was found that the grafted heart was hyperacutely rejected 0.55 ± 0.38 hr after grafting in all recipients of the group hyperimmunized one week before, whereas hyperacute rejection was induced in only 41% and 25% recipients of the groups hyperimmunized one and three months before, respectively.

Further, in the group hyperimmunized six months before, hyperacute rejection wa not observed at all, graft survival time being 96.0 ± 37.5 hr. The pattern of rejection was from ac-

celerate to acute rejection.

No fundamental study, except our work, on the effect of the antibody changes over time after hyperimmunization on the take of graft has been done. Clinical investigations, however, have been performed by Terasaki et al as mentioned above[14,16,21]. In disagreement with their report, our experiment using animals showed that graft survival time for hyperimmunized recipients, if six months or more had passed after hyperimmunization, came close to that of ordinary acute rejection. Therefore, we think there is a chance of grafting to the hyperimmunized recipients.

Next, we examined how cytotoxic antibody which had been formed by hyperimmunization changed with the passage of time. The titers of preformed cytotoxic antibody were determined over time. CA-TW titers in the groups hyperimmunized three and six months before were significantly decreased compared with that of the group hyperimmunized one week before. However, even in the group hyperimmunized six months before, the titer of remaining CA-TW was on such a level as, clinically speaking, not to permit transplantation. If the antibody for peripheral blood lymphocyte is assumed to consist mainly of CA-TW, this observation is in fair agreement with Terasaki's report on clinical cases that cytotoxic antibody remained in 68% of the cases six months after immunization[20,21].

Our analysis of the relationship between preformed cytotoxic antibody titer after hyperimmunization and graft survival time of transplanted heart revealed a high negative correlation ($r = -0.7274$). As to the relationship between the presence of preformed cytotoxic antibody and results of the survival of graft, experiments using animals by French[6] and Kuwahara[10] and clinical analysis by Terasaki[21] showed that the results of transplantation was unfavorable in recipients positive for CA-TW. Our findings as above mentioned agrees with these reports.

In sum, we have disclosed that cytotoxic antibody formed by hyperimmunization decreased with the elapse of time and that graft survival time was prolonged. As six months after sensitization, hyperacute rejection was no longer induced and the heart graft survived for 90 hr or longer and then rejected in pattern of accelerate or acute rejection.

It was seen that the decrease of CA-TW was closely correlated with the prolongation of graft survival time.

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