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<td>Author(s)</td>
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<td>Citation</td>
<td>Acta medica Nagasakiensia. 1988, 33(1-4), p.102-109</td>
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<tr>
<td>Issue Date</td>
<td>1988-10-25</td>
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<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10069/15715">http://hdl.handle.net/10069/15715</a></td>
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Comparison in the immune response between lung and kidney transplantation

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Received for publication, May 31, 1988

SUMMARY: To know a difference in the immune responses between the lung and the kidney allo-transplantation, the humoral and cell bound antibody levels were compared by means of cytotoxic assay (CA) and Immune hemoagglutination (IA) test in dogs.

1) The humoral antibody levels were increased five to 10 days prior to appearance of rejection following lung transplantation in spite of maintaining relatively low levels in the course although it was different from kidney one.

2) On the other hand, the cell bound antibody levels were not increased in accordance with the rejection response at lung transplantation. Meanwhile, renal transplantation made the cell antibody levels higher with time in the humoral antibody levels, although cell-bound antibody levels were increased on day five to seven with a seven day maximum, thereafter gradually decreased to some extent. It is of considerable interest that a high humoral antibody levels had a close relation to death.

3) The administration of the antilymphocyte serum (ALS) was effective in suppressing the immune response showing the low humoral antibody levels and improved survival period.

4) Skin transplantation prior to lung or kidney transplantation kept high antibody titers on and after day four lung transplantation and on day seven to 10 at kidney. Conclusion was drawn from this study that skin and lung or kidney have the common cross reacting antigenicity to organ transplantation, in particular, the lung has a strong cross reacting antigenicity to the skin rather than the kidney.

INTRODUCTION

It is well known that the lung as well as the spleen has the strong antigenicity concerning organ transplantation as reported by DEODHAR and STWART and these have shown great rejection response when applied for transplantation. TERASAKI reported that the humoral antibody produced by skin allotransplantation selectively attacks the lymphocyte of a donor. On the other hand, there are many reports concerning humoral antibody which plays a key role in activating the immune state, related to organ transplantation. Needless to say, cellular immunity also mainly causes the transplanted organs to be rejected.

The aim of this study is to clarify a difference in the immunologic response with regard to rejection response between lung and kidney transplantation and also a cross reacting antigenicity to a donor organ among the skin, the kidney and the lung. Antilymphocytic serum (ALS) was verified for prevention from rejection response to a donor lung.
MATERIAL AND METHOD

Mongrel dogs weighing from 10 to 20 kg were used for the study. A pair of donor and recipient was selected in almost the same sized dogs, anesthetized with 25 mg/kg of sodium pentobarbital, intubated with a cuffed endotracheal tube and ventilated with room air using a volume respirator (Harvard).

**Lung, kidney & skin transplantation**

Left lung homotransplantation was made with conventional manner as described by many authors.

A donor dog was taken care and survived to provide the target cells from the mesenterial nodes later. After perfusing a donor kidney using a perfusate (500 ml of 10% LWD with saline containing 1 g of 10% HCl-procaine, 10 mg of heparine with 100 cm of gravity during a 20 to 40 min period of time). A kidney was transplanted in the femoral region by vascular anastomoses to the femoral artery and vein in a recipient as well as by ureterocutaneous fistula on the back to check out the daily urine output.

On the other hand, skin transplantation was made to the lateral chest wall using 5 x 5 cm skin graft. The infected and bleeding skin grafts were excluded from this study.

At an interval of seven and 10 days, skin transplantations were repeated and then allo-lung or renal transplantation was attempted.

Thirty mg/kg of penicillin G were used for prevention from postoperative infection.

**Preparation of antilymphocyte serum**

The pulmonary hilar and mesenterial lymph nodes were aseptically taken in 199 Medium, cut into pieces by scissors, filtrated with glass wool and centrifuged at 800 rpm for 10 min, discarded the suspension and rinsed with saline. These were repeated three times and adjusted to 50 x 10^6/mm of lymphocytes, Equal Freud adjuvant was added. 0.2 ml were subcutaneously injected.

At the second time, the lymphocytes with the same as above procedure without adding Freund adjuvant were used one week later. Three times or more, this injection was repeated until reaching the level of 2° of agglutination titer of the leucocyte.

Antidog rabbit serum was taken from the femoral artery and kept at room temperature for two hours. The remained blood was centrifuged and warmed at 56°C for 30 min to make it inactive and stored at -20°C in freezer.

0.5 to 1.0 ml ALS were injected to a recipient five and seven day prior to and three times a week after transplantation.

**Detection of humoral and cell-bound antibody following transplantation**

The levels of the humoral and cell-bound antibody following lung, kidney and skin transplantation were compared by using the methods of cytotoxic assay (CA) and Immunoadherent (IA) titer.

1) CA

The damaged cells were identified by trypan-blue staining and represented as a rate of total cells.

a) target cell: the nodes in the pulmonary hilum and the mesenterium of a donor were taken, kept in 199 Medium for 30 min, filtrated with glass fiber, cooled and discarded the supernatant. Ten ml of 199 Medium were added and centrifuged three times, rinsed the lymphocytes to exclude dead cells and cell debris. After confirming it by trypan-blue staining, the cells were adjusted to 2 x 10^6 or 4 x 10^6/ml.

b) Dilution of antiserum and lymphocyte suspension: Antiserum was diluted with KGVB to 1 x, 2 x, 4 x, 8 x, 16 x, 32 x and 64 x respectively. Sensitized cells into 1 x, 2 x, 4 x and 8 x respectively.

c) Complement (C'): the commercial product (Kyokuto Pharma Co.) was used for complement which corresponds to 150 to 180 CH50/ml and diluted with cooled KGVB to 1 x, 2 x and 4 x respectively.

d) cytotoxic assay: A mixture containing 0.2 ml of 2 x 10^4 target cells, 0.5 ml of dilute antiserum and 0.2 ml of GPC' (3x dillution) were stirred at 37°C for 30 min, centrifuged at 600 rpm for 30 min and discarded a supernatant and suspended with KGVB added 1% tryphn-blue and the cells were harvested within three min, calculating a rate of staining cells.

Over 20% of staining rate were excluded from
this study on account of the mechanical damage to the cells.

**preliminary study on cytotoxic assay**

Target cells: The relationship between target cells and the degree of cell damage was evaluated. Under the condition of the use of three times dilute complement and antiserum (L1 & N2), the greatest damage was obtained in using $2 \times 10^6$ target cells. In $5 \times 10^5$ target cells, the cytotoxic effect was reduced and also when antiserum was diluted by over $2 \times$, the cytotoxic effect was depressed regardless of the number of target cells as generally called "prozone".

Complement dose: The cytotoxic effect was evaluated in relation to the dose of complement on the same condition of target cells. On condition of antiserum (N1) at the 7th and 11th day and $2 \times 10^6$ target cells dilute complement was used as being $1 \times$, $2 \times$, $4 \times$, $8 \times$, $16 \times$ and $32 \times$. The most properly cytotoxic effects required a dilution of over $4 \times$. Above preliminary study indicated that an adequate condition to get the results of the cytotoxic assay. O-type blood is needed for using $2 \times 10^6$ target cells, dilute complement to $2 \times \sim 4 \times$, dilute antiserum to $2 \times \sim 16 \times (5 \times 10^6$/ml).

**Immune adherence hemoagglutinin test (IAMA)**: The target cells were prepared according to the same manner as CA test. Antiserum was diluted with glucose-KGVB to $5 \times$, $10 \times$, $20 \times$, $40 \times$, $80 \times$, and $160 \times$. Complement also was diluted with KGVB to $25 \times$, absorbed three times with O-type human and dog’s erythrocyte at 0°C and centrifuged at 20000 rpm for 30 min. The complemnt titer reaches a level of 150–180 CH50/ml. The O-type human erythrocyte was prepared after taking from O-type human who was tested to be highly sensitive to the IA response, mixing it with equal volume of Alserva-solution, centrifuging three times at 800 rpm for 10 min at 0°C and adjusting to $4 \times 10^6$/ml with EDTA-glucose solution.

0.5 ml of dilute donor antiserum was mixed with 0.2 ml of $5 \times 10^6$ target cells, and ached at 37°C for 20 min. A solution of 0.2 ml of $25 \times$GPC reabsorbed by human O-type and dog’s erythrocyte was stimated at 37°C for 20 min and 0.1 ml of $4 \times 10^6$ human O-type erythrocyte was quickly added and shook for 10 min, stanced at 37°C warm bath for 60 min. Erythrocyte agglutination at the bottom of each tubes was graded. After taking part of the precipitate on the slide glass, adherence of one or two erythrocytes to lymphocytes was microscopically counted as to positive and calculated as percent of total lymphocytes. The higher IA titer was shown in using antiserum at a high concentration of over $20 \times$.

**RESULTS**

Changes in humoral and cell-bound antibody levels following lung homotransplantation

Changes in the level of humoral antibody after lung homotransplantation was measured in six dogs as shown in Fig. 1 and 2. The longest survival was 32 days. CA varied with 21–39% on day three, although IA was 20–38%, indicating the same level as the control. Thereafter, the CA levels gradually increased to 22–50% on day five, 20–70% on day seven, and
20~77% on day 10. On the other hand, the IA values showed 30~50% on day five, 25~55% on day seven and 38~60% on day 10 with slow increase.

In those who survive long, the level of humoral antibody was not raised and there was nothing aware of rejection episode. At the time of rejection, it was generally seen that humoral antibody levels was increased. As a rule, the high levels of humoral antibody was observed on day five to 10. However, even in long survivor of a 32 day duration, the humoral antibody levels at death was abruptly raised to 81% of CA.

Changes in humoral and cell-bound antibody levels following kidney homotransplantation

The survival time of renal transplantation was much better than that of lung and prolonged to 30 days or more with satisfactory urine output.

The CA values prior to transplantation varied from 10 to 18%. The humoral antibody levels of CA following renal transplantation were changed to 25~32% on day 4~5 and 30~62% on day seven without significant increase. However, the function-loss represented by a symptom of oliguria brought high level of humoral antibody which was 80~84% on day nine, 60~88% on day 10~13 and thereafter reached 81~94%.

Changes in the IA titers also showed a similar tendency toward an increase to 25~32% on day five, 30~62% on day seven, 43~63% on day 9~10 and 56~72% on day 10~13. However, there was no accelerated increase in IA titer as seen in CA. However, it takes into consideration that cell-bound antibody levels were not necessarily showed as it was on account of antiserum derived from a different recipient on the same condition for fear of surgical risk to take nodes through the surgical insult of thoracotomy and laparotomy.

Following lung transplantation and renal transplantation, the humoral and cell bound antibody levels were compared with elapsing time and with antisera originated from the lymph nodes that were taken from the different sites of the pulmonary hilum (regional) and the mesenterium (distant) as shown in Fig. 3 and 4. As a result it was indicated that at lung transplantation, the humoral antibody levels measured by IAMA was still more effective in knowing rejection phenomenon than those
done by CA, on the other hand at renal transplantation the cell-bound antibody levels measured by CA were much more sensitive to appearance of rejection phenomenon rather than those by IA.

**Changes in humoral antibody levels at sequential lung or kidney transplantation to skin**

In nine dogs, skin grafts were transplanted three times at intervals of one week, five of the nine dogs received skin grafts from identical donor but the other four had from different donors.

The humoral antibody levels were gradually raised as being 22% (12~28) by CA and 31% (23~40) by IA on day seven of the first skin transplantation, 30% (22~38) by CA and 37% (33~46) by IA on day seven of the second skin transplantation, finally 37% (28~45) by CA and 43% (34~56) by IA on day seven of the third skin transplantation. In the three dogs who survived more than three days following additional lung transplantation, the humoral antibody levels were raised to 69% by CA and 65% by IA on day seven to nine. Thereafter, those were rapidly increased more and more. On day 11, all died with high humoral antibody level. Meanwhile, renal transplantation after performing skin transplantation three times made the humoral antibody levels still more increase rather than renal transplantation alone, that is. 63% (50~83) by CA, 56% (49~61) by IA on day four, 88% (84~92) by CA,

73% (66~82) by IA on day 11 and 76 (70~81) by CA 62 (61~63) by IA on day 16.

**Changes in cell-bound antibody levels at sequential lung or kidney transplantation to skin**

Prior to lung or kidney transplantation, skin grafts were transplanted three times. In such a situation, the cell-bound antibody levels were compared between lung and kidney transplantations. The regional and mesenterial lymphnodes that was necessary for measurement of cell-bound antibody were taken at the sacrifice or immediately before death. At lung transplantation, five dogs died on day five, two dogs on day seven, two, on day eight, one on day 10 and the other one on day 32. The antibody titers of the regional lymph nodes were 52% (37~70) by CA, 53% (41~54) by IA and those of the mesenterial one 46% (32~64) by CA and 47% (41~54) by IA respectively. (Fig. 7)

At kidney transplantation sequent to three times skin transplantation, the longest survival time was 31 days, next to 21 days and 11 days in two dogs. The cell-bound antibody in the regional lymph nodes levels were 50% (22~70)
Fig. 7. Humoral Antibody Response After Lung Allo-transplantation Following Skin Grafting

Fig. 8. Humoral Antibody Response After Renal Allo-transplantation Following Skin Grafting

by CA, 53% (40~68) by IA and those in the mesenterial lymph nodes 37% (22~45) by CA, 33% (25~57) by IA respectively. (Fig. 8)

Changes in humoral antibody levels by using ALS
Following lung transplantation, ALS was used for immunosuppression, and changes in humoral antibody levels were compared to clarify the drug effects. In one of the four dogs the humoral antibody level measured by CA stepwise increased and continued to die on day 15. However, in the other three dogs those either by CA or IA remained almost constant throughout observation, demonstrating the efficacy of the drug administered as shown in Fig. 9.

DISCUSSION
A high prevalence of cardiac and renal transplantation in clinical use has been demonstrated in the United States of America and Europe. However, there is some problem as to lung transplantation with unsatisfactory outcome experienced all over the world despite getting improved results recently. Much has been
reported that it is mainly based on poor healing of bronchial anastomosis, due to interruption of bronchial artery, function deterioration of a donor lung due to denervation and strong immunologic antigenicity to allografts. In the host that underwent renal transplantation, concomitant pulmonary lesions analogous to autoimmune diseases did not so frequently break out as reported by Ripkind in 1964 and by Humburg in 1965.

It is suggested that anti-renal antibody may cross-react to the lung which is quite sensitive to immune reaction.

As compared between renal and lung transplantation, the humoral antibody titers were raised in accordance with advances in rejection response with a maximum of a rise immediately before their deaths.

However, it was characteristic in that rejection causes their death in lung transplantation although it does not so in renal transplantation. It implies that rejection of a donor lung at lung transplantation leads to death, in contrast, rejection of a donor kidney at renal transplantation is not necessarily a sign of death.

It is generally accepted that cellular immunity plays an important role in falling a donor organ into the loss of function by rejection.

In the present study, the cell-bound antibody levels were measured with time following lung or kidney transplantations. Those after lung transplantation were not necessarily high rather than those after renal transplantation.

However, in appearing rejection phenomenon, the humoral antibody levels measured by IA was much more sensitive to knowing a disparity to foresee the rejection rather than those measured by CA. Of interest is the fact that on the contrary at renal transplantation this fact was reversed. It is suggested that not only cellular antibody but humoral one is influential on the rejection response of a donor organ.

Preceding skin transplantation to lung or kidney transplantations made the humoral antibody levels higher. It is indicated that the skin has a common antigenicity to the lung and the kidney. Hardy reported that a recipient sensitized by lung tissues of a donor accelerates a rejection phenomenon to subsequent skin grafts.

Other reports clarified that pretreatment by lung homogenate helped get long survivors after heart transplantation in guinea pig and lung lobe in dog by making a state of immunologic enhancement. Little has been available on the subject of cell-bound antibody regarding organ transplantation. In this study cell-bound antibody was detected from the samples of the regional and mesenterial nodes. On day five, seven and 10 following lung transplantation, cell-bound antibody levels in the hilar and mesenterial nodes were measured by CA and IA methods. The values did not varied except for prior to death. Fujimura pointed out that γG and γG immuno-globulin positive

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**Fig. 7 Humoral Antibody Response After Lung Allograft Transplantation Following Skin Grafting**
cells exist much more densely in the nodes of tracheal bifurcation rather than the mesenteric. At appearing a rejection of a donor lung these were intensified in the spleen and the lymph nodes.

On the other hand, Kitamura reported that cytotoxic activity against the cells derived from the cervical and axillary nodes of the recipient with renal transplantation was increased to 36-46% on day five, thereafter declined to 3% on day 16.

Very little information is available in understanding the mechanism of cellular immunity on the organ transplantation. A few reports confirmed that immunocompetent cells were liberated in the blood stream of the recipient and attacked a donor graft specifically, sticking to the surface of the cell membrane which is part of composing graft, and not specifically exerting as a cytotoxic action.

ALS as an immunosuppressant was of benefit in reducing the humoral antibody levels. However, it was ineffective in some cases to suppress the allograft rejection response. It is our conviction that the humoral antibody level is the most valuable parameter to know the effectiveness of ALS on immunosuppression.

REFERENCE