

**Behavior of immunocompetent cells in the spleen  
and lymphnodes at lung transplantation  
— Especially alteration of Ia positive cell  
population among mononuclear cells —**

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**ABSTRACT :** The Ia-positive ratio of the immunocompetent cells in the spleen and the mesenterial lymphnodes was measured after lung transplantation on dogs treated with cyclosporin A as the immunosuppressive drug and treated without CsA. And Ia positive ratio of those cells in the peripheral blood was also measured after lung transplantation with and without splenectomy.

At rejection, the Ia-positive ratio of lymphocytes and monocytes in the spleen was increased significantly regardless the use of CsA. And when the rejection was inhibited by CsA, Ia-positive ratio of those cells was decreased significantly.

However, when the rejection was inhibited or not, the Ia-positive ratio of monocytes in the peripheral blood has not indicated a significant difference.

Splenectomy after lung transplantation did not influenced the Ia-positive ratio of the peripheral blood immunocompetent cells.

## INTRODUCTION

Clinical applications of organ transplantations have been increased in accordance with advances in immunosuppressive drugs. However there are many problems to be solved about rejection mechanism, monitoring and detective methods for an appearance of immunologic rejection. But it is clear that macrophages and T and B cells play an important role in presenting the rejection response. On the other hand, it is believed that the spleen also plays a key role in exhibiting the immune response, and splenectomy has been considered to be the most valuable method to prevent the eventual rejection.<sup>1-4</sup>) Needless to say, it is certain that splenectomy is not necessarily a potent preventive procedure for rejection,<sup>5,6</sup>) although the spleen fills the key role in preventing allograft

rejection.

The aim of this study is to investigate the response of the lymphatic tissues including the spleen and the mesenterial lymphnodes to lung allograft rejection by using OK Ia-antibody, and also to certify the effect of splenectomy on mononuclear cells in the peripheral blood on the basis of alternation of Ia-positive cell population.

## MATERIAL AND METHOD

### (1) *Lung transplantation technique and procedure*

Experimental studies were carried out in dogs weighed from 8 to 13 kg, supplied from the animal center of Nagasaki university school of medicine, and anesthetized with pentobarbital 25mg/kg iv, intratracheally intubated, ventilated with room air on the condition of 30m!/  
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kg of tidal volume, 12-15 rate/min. with the use of Harvard ventilator. Left thoracotomy was performed through the fifth intercostal space. Immediately after taking out a left lung from recipient, a donor left lung which received general heparinization was reimplanted. The steps of reimplantation anastomosis was made in the following order, the left atrium, left pulmonary artery with continuous suture of 5-0 prolene, and left main bronchus with continuous suture of 4-0 prolene. Postoperative antibiotics of penicillin and aminoglycoside derivative were used to avoid postoperative infection.

The experimental dogs were divided into two groups by immunosuppressive drugs postoperatively given. Group 1 without immunosuppressive drug were 6 dogs, and Group 2 with immunosuppressive drug of cyclosporine A (CsA) of 20mg/kg/day were 9 dogs which contained rejected allograft (R(+)) in 5 and nonrejected (R(-)) in 4.

At the same time, splenectomized and non-splenectomized dogs without immunosuppressive drugs were also prepared for a comparative study with the effect of splenectomy on lung allograft rejection.

(2) *Preparation of the spleen and the mesenteric lymphnodes and separation of mononuclear cells*

The transplanted lungs were followed up by daily chest X-P examination and sacrificed at the time of function loss of a donor lung which

corresponded to the appearance of allograft rejection. A donor lung, spleen and mesenteric lymphnodes were taken and also the spleen and the mesenteric lymphnodes were taken from a donor at the same time as the normal control.

The transplanted lungs were fixed by formalin and stained by HE to examine microscopically.

The spleen was prepared by canulation into the splenic artery and infusion of 500ml of saline by hand. Thereafter, infused spleen and mesenteric lymphnodes were minced in RPMI 1640 medium without FCS (SIGMA Co.), passed through 50  $\mu$ -nylon mesh and the supernatant was prepared. Mononuclear cells were separated by the Ficoll-Hypaque density gradient centrifugation, 1700rpm for 30 minutes.

(3) *Measurement of the Ia-positive ratio in mononuclear cells*

The separated mononuclear cells were washed with PBS and were incubated with OK Ia-antibody (ORTHO Co.) then exposed to FITC-GAM (ORTHO Co.). And the Ia-positive ratio of the fraction of lymphocytes and monocytes were measured by flow-cytometry method. The fraction of lymphocytes and monocytes were shown in Fig. 1 by flow-cytometry (SPECTRUM III, ORTHO Co.). In the suspension of mesenteric lymphnodes, the fraction of monocytes failed to identify from that of lymphocytes. And then the fraction of lymphocytes was measured in the mesenteric lymphnodes.

They were also incubated with a bridging

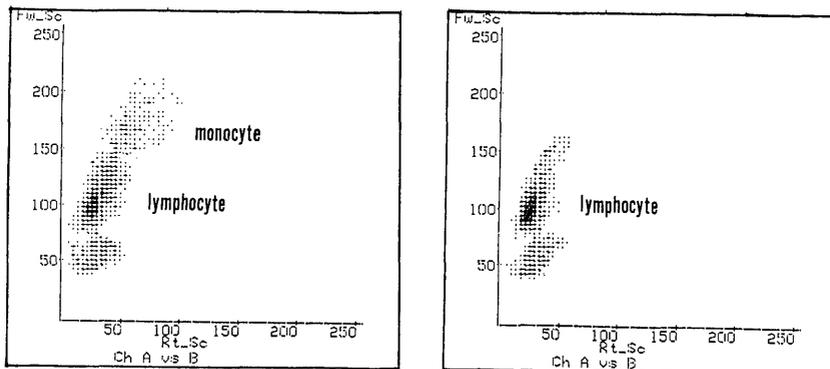


Fig. 1. Cytogram by SPECTRAM III

1a : spleen 1b : mesenteric lymphnode

antibody mouse IgG (SIGMA Co.) and FITC-GAM as a negative control.

(4) *Measurement of the Ia-positive ratio in the peripheral blood mononuclear cells*

The peripheral blood was taken daily from splenectomized and non-splenectomized dogs, and erythrocytes were destroyed by means of low osmic method, and mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation. They were also incubated with OK Ia1-antibody and the Ia-positive ratio was measured by flow-cytometry method after incubation with OK Ia1-antibody.

(5) *Determination of lung allograft rejection*

The lung specimens were stained with HE and the rejection responses were microscopically evaluated according to Shirakusa's classification<sup>7)</sup> that modified Veith's one.<sup>8)</sup> The lungs indicating more than the 2nd degree of the classification were determined as a rejected one.

(6) *Statistical analysis*

The Ia-positive ratio was indicated as the mean  $\pm$  standard error of the mean, and the paired Student's t-test was used for the statistical analysis.

## RESULTS

(1) *Histologic examination of a lung allograft*

In the donor lungs taken from the dogs without the use of immunosuppressive drugs, histologic findings indicated lung damage more than the 2nd degree of SHIRAKUSA's classification. On the other hand, in the donor lungs from the dogs treated with CsA, lung damage more than the 2nd degree was seen in 5 although there was no rejection response in 4. The rejection responses were recognized in all the donor lungs with or without splenectomy. The lungs with histologic evidence of infection were excluded in this study.

(2) *Ia-positive ratio of lymphocytes in the spleen*

The Ia-positive ratio of lymphocytes in the spleen in Group 1 which was not treated with

CsA was  $68.7 \pm 9.7\%$ . It was a significant difference as compared with  $48.5 \pm 6.3\%$  ( $p < 0.01$ ) in the control group. In contrast, in R(+) of Group 2 with the use of CsA, the Ia-positive ratio was  $61.1 \pm 11.7\%$  with significant increase ( $p < 0.05$ ) as compared with that of the control, although in R(-) of Group 2 it was  $30.1 \pm 6.9\%$  with significant reduction ( $p < 0.01$ ). There was a statistical significance between R(+) and R(-) of Group 2. However, there was no significant difference in Ia-positive ratio between R(+) of Group 2 and Group 1 as shown in Fig. 2.

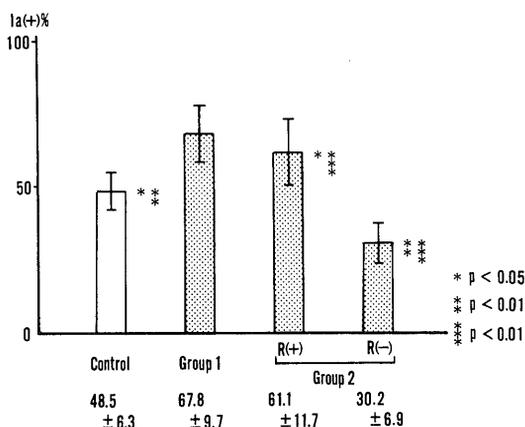


Fig. 2. Ia positive ratio of lymphocytes in spleen

(3) *Ia-positive ratio of monocytes in the spleen*

The Ia-positive ratio of monocytes in the spleen in Group 1 and R(+) of Group 2 were  $75.5 \pm 5.5\%$  and  $80.2 \pm 8.6\%$  respectively with

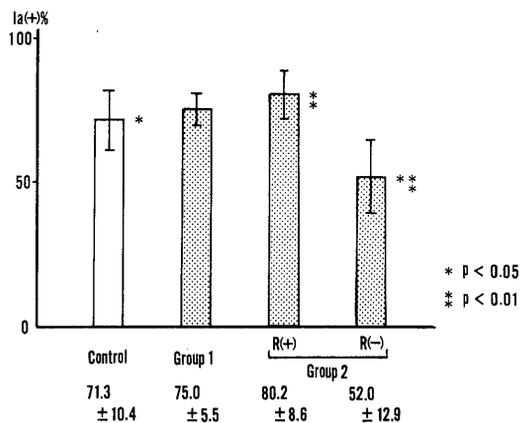


Fig. 3. Ia positive ratio of monocytes in spleen

slight increase as compared with the control without statistical significance. Meanwhile, in R (-) of Group 2, it was  $52.9 \pm 12.9\%$  with significant decrease ( $p < 0.05$ ), and there was a significant difference ( $p < 0.01$ ) between R (+) and R(-) of Group 2, as shown in Fig. 3.

(4) *Ia-positive ratio of lymphocytes in the mesenterial lymphnodes*

The Ia-positive ratio of lymphocytes in the mesenterial lymphnodes was  $91.3 \pm 8.0\%$  in Group 1,  $93.2 \pm 6.4\%$  in R (+) of Group 2, and  $91.7 \pm 2.5\%$  in R (-) of Group 2, although a decrease in R(-) of Group 2 was seen when compared with that in the control without an apparent alteration, as shown in Fig. 4.

(5) *Ia-positive ratio of peripheral blood mononuclear cells in splenectomized and non-splenectomized dogs*

There was no significant change in the Ia-positive ratio of peripheral blood lymphocytes between splenectomized and non-splenectomized dogs as compared with  $89.72 \pm 7.42\%$  of the control. On the other hand, there was a significant decrease in the Ia-positive ratio in monocytes in splenectomized and nonsplen-

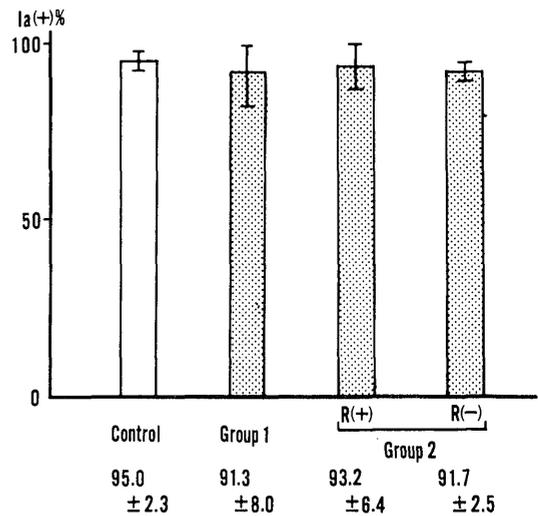


Fig. 4. Ia positive ratio of lymphocytes in mesenteric lymphnodes

ectomized dogs as compared with  $74.92 \pm 7.93\%$  of the control. However, there was no significant difference in the Ia-positive ratio of monocytes between splenectomized and non-splenectomized dogs except for a significant decrease in splenectomized dogs on day 3, as shown in Table 1, 2 and Fig. 5, 6.

Table 1. Behavior of Ia positive ratio of lymphocytes in the peripheral blood after lung transplantation

P. O. D	0	1	2	3	4	5	6	7
A group n=4	89.72	92.55 ±2.19	89.40 ±11.79	89.70 ±11.16	78.71 ±8.55	77.98 ±4.96	83.20 ±13.81	-
B group n=4	±7.42	87.24 ±12.27	84.73 ±12.87	93.80 ±2.69	91.15 ±7.96	83.57 ±11.24	71.87 ±11.56	82.5
		NS	NS	NS	NS	NS	NS	NS

A group : lung transplantation with splenectomy  
B group : lung transplantation without splenectomy

Table 2. Behavior of Ia positive ratio of monocytes in the peripheral blood after lung transplantation

P. O. D	0	1	2	3	4	5	6	7
A group n=4	74.92	56.38 ±3.33	40.10 ±11.89	31.83 ±10.73	63.55 ±2.48	53.47 ±6.41	61.70 ±11.05	-
B group n=4	±7.93	54.08 ±7.40	54.63 ±10.99	61.20 ±11.63	49.25 ±20.91	41.70 ±10.27	42.00 ±6.46	56.8
		NS	NS	p<0.01	NS	NS	NS	NS

A group : lung transplantation with splenectomy  
B group : lung transplantation without splenectomy

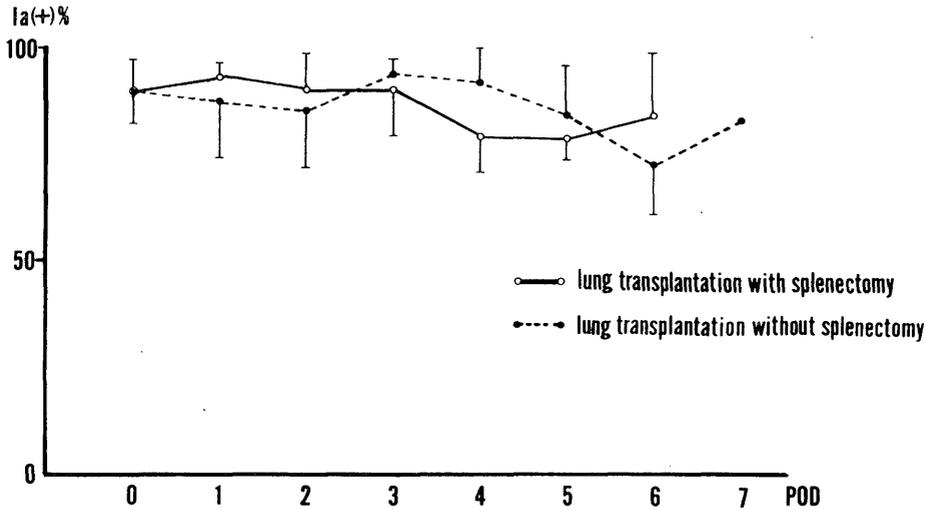


Fig. 5. Behavior of Ia positive ratio of lymphocytes in peripheral blood after lung transplantation

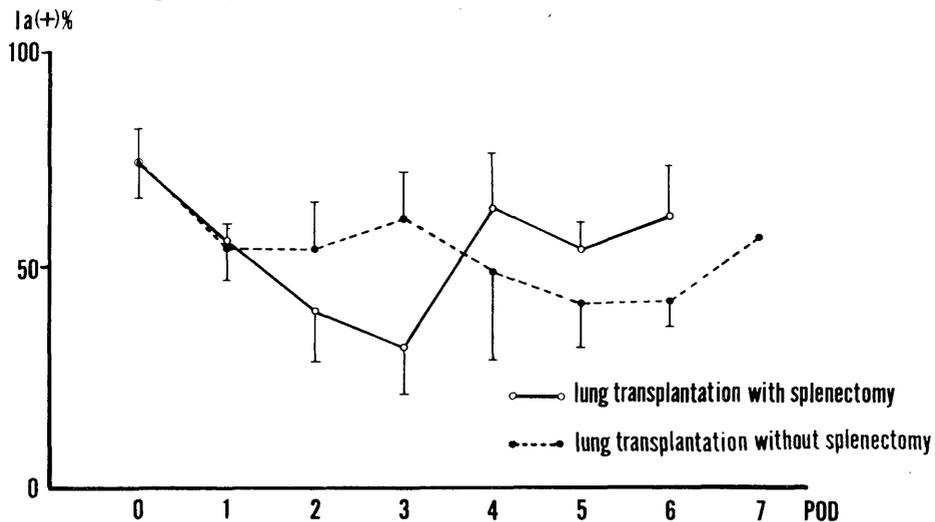


Fig. 6. Behavior of Ia positive ratio of monocytes in peripheral blood after lung transplantation

## DISCUSSION

The rejection response in organ allotransplantation is complex in the genesis and it was not yet clear. It is certain that rejection phenomenon is a sum of various immune responses. It is believed that the sites exhibiting the immune response are the lymph tissues which are divided into central lymph tissues (primary lymph tissues) and peripheral lymph tissues (secondary

lymph tissues). The former corresponds to the thymic gland and bone marrow which play a role in differentiating the stem cells, and the latter is the spleen and lymphnodes which play a role in producing antibody.

Foreign bodies are first trapped in lymphnodes and lymphnodules in the submucosa of the respiratory, digestive and urogenital organs, and also dealt with the spleen for the foreign bodies in the bloodstream. It is well known that lymphnodes and lymphnodules are named

as local defensive organs, and the spleen is regarded as a general defensive organ with a role of the reticuloendothelial system in communication with the vascular system. The recognition of allotransplantation antigen depends upon the two ways.<sup>9)</sup> One is said to be central sensitization that transplantation antigen released from transplanted tissues and carried to the lymphnodes and spleen, thereafter it is dealt with and recognized, the other is called peripheral sensitization that lymphocytes and monocytes from a recipient are transferred through anastomosed vessels and sensitized in the place.

Allotransplantation antigens transferred into a recipient are recognized and treated in a network and advances in rejection process is established.<sup>10,11)</sup> It is known that transplantation antigen is first phagocytosed into macrophages which is antigen-presenting cells. Macrophages come to present a class II antigen on the surface of cell membrane and secrete interleukin-I (IL-I) which activates helper-T (T<sub>H</sub>) cells. T<sub>H</sub> cells recognize allotransplantation and macrophage-presenting antigens, and they are also activated by IL-I and secrete IL-II, B-cell growth factor (BCGF), B-cell differentiation factor (BCDF) and also differentiate themselves. Lymphokine liberated from T<sub>H</sub> cells activated T<sub>K</sub>, T<sub>D</sub>, T<sub>S</sub>, NK cells with a formation of complex networks. At the time presenting antigens correspond to class II antigen in which Ia antigen is the most important one. Many reports confirm that the Ia-positive ratio in the immunocompetent cells of allograft increases,<sup>12, 13)</sup> in spite of some debate about the Ia-positive ratio of the immunocompetent cells in the peripheral blood.<sup>(14, 15)</sup> Ito reported that the Ia-positive ratio of macrophages in bronchoalveolar lavage fluids is increased at the time of lung allograft rejection although that macrophages is reduced in the peripheral blood.<sup>16)</sup> In this study, the Ia-positive ratio of lymphocytes and monocytes in the spleen was increased despite no remarkable change in that of lymphocytes in the mesenterial lymphnodes even at the time of lung allograft rejection. Furthermore, the Ia-positive ratio in the peripheral blood monocytes was significantly reduced in

contrast to no significant change in the Ia-positive ratio in the peripheral blood lymphocytes.

In splenectomized dogs, the same tendency was observed with no significant difference between splenectomized and non-splenectomized dogs. At rejection, the population of Ia-positive cells in the transplanted organs and the spleen is apparently increased although that in the peripheral blood monocytes is reduced and lymphocytes was almost kept constant.

It is suggested that lung allograft rejection provokes activation of immunocompetent cells in the sites of transplanted lungs, followed by the spleen. It is a reflection that immunocompetent cells never carry from the spleen to the lung via blood vessels on the basis of a result no increase in Ia-positive cells in the peripheral blood lymphocytes and monocytes.

It is contemplated that high level and no increase in Ia-positive cells even at rejection of allograft in the mesenterial lymphnodes is associated with constant exposure to external antigens via the digestive tract.

It is concluded that splenectomy is of no benefit to suppress the immune response except for clinically particular conditions<sup>17, 18)</sup>, although some reports confirm the suppression of cellular and humoral immune responses,<sup>19-23)</sup> and some indicate active production of T<sub>S</sub> cells.<sup>24)</sup>

CsA is effective in inhibiting activation of Ia-positive cells in the spleen and it is a potent drug to suppress the immune response in spite of a side effect of nephrotoxicity and hepatotoxicity.<sup>25-27)</sup>

However, it is possible that CsA is not able to suppress the rejection response in the case in which the Ia-positive ratio in the spleen was increased on the condition of the use of CsA at rejection in this study.

## CONCLUSION

- (1) The Ia-positive ratio of the immunocompetent cells in the spleen and the mesenterial lymphnodes was investigated at rejection of lung allografts on dogs.
- (2) At rejection, the Ia-positive ratio of lym-

phocytes and monocytes in the spleen is remarkably increased regardless of with and without the use of CsA.

(3) When the immune response is effectively inhibited by the use of CsA, the Ia-positive ratio of lymphocytes and monocytes in the spleen is significantly reduced

(4) Splenectomy does not greatly influence the Ia-positive ratio of the peripheral blood immunocompetent cells at lung allograft rejection.

(5) It is certified that the spleen does not play an important role in conveying the immunocompetent cells to allograft in organ transplantation in which vascular anastomosis is needed.

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#### REFERENCES

- 1) G. P. J. ALEXANDRE, *et al* : ABO-Incompatible related and unrelated living donor renal allograft ; *Transplantation Proc.*, **18**, 3, 452-455, 1986.
- 2) T. E. STARZL, *et al* : Splenectomy and thymectomy in human renal homotransplantation ; *Proc. Soc. Exp. Biol. Med.*, **113**, 929-932, 1963.
- 3) R. E. GLEASON, *et al* : Analysis of variables in the function of human kidney transplants, ; *Transplantation*, **5**, 343-359, 1967.
- 4) J. C. PIERCE, *et al* : The effect of splenectomy on the survival of first and second renal homotransplants in man ; *Surg. Gynecol. Obstet.*, **127**, 1300-1306, 1968.
- 5) M. BANAJI, *et al* : The effect of splenectomy on engraftment and platelet transfusion requirements in patients with chronic myelogenous leukemia undergoing marrow transplantation ; *Am. J. Hematol.*, **22**, 275-283, 1986.
- 6) G. OPELZ, *et al* : Effect of splenectomy on human renal transplants ; *Transplantation*, **15**, 605-609, 1973.
- 7) T. SHIRAKUSA, K. KAWAHARA : Histologic studies on lung transplantation of canine ; *J. Japan Surg. Soc.*, **87**, 5, 572-580, 1986 (in Japanese)
- 8) S. S. SIEGELMAN, F. J. VEITH, *et al* : Roentgenologic-pathologic evaluation of rejection in allografted lung ; *Am. J. Roentgen*, **112**, 546-558, 1971.
- 9) N. KASHIWAGI : Ishokuniokerumenekioto ; *Med. Clinics of Japan*, **13**, 3, 8-14 (310-316) , 1987 (in Japanese)
- 10) J. J. FARRAR, *et al* : The biochemistry, biology, and role of interleukin 2 in the induction of cytotoxic T cell and antibody-forming B cell responses ; *Immunolo. Rev.*, **63**, 129-166, 1982.
- 11) T. B. STROM, *et al* : The cellular and molecular basis of allograft rejection, -Fact and fancy ; *Transplantation Proc.*, **17**, 801-805, 1985.
- 12) E. M. BENSON, *et al* : Induction of IA antigens in murine renal transplants ; *J. Immunol.*, **134**, 7-9, 1985.
- 13) H. ISHIKURA : Morphological and immunohistochemical study of the infiltrating cells to the rat renal allograft ; *Jap. J. Transplantation*, **19**, 117-130, 1984 (in Japanese)
- 14) A. ROMANIUK, *et al* : Monitoring of lung allograft rejection in the rat ; *Transplantation Proc.*, **18**, 5, 1195, 1986.
- 15) S. MIZUIRI, *et al* : Analysis of lymphocyte subpopulations in allograft and peripheral blood of renal transplanted patients, -Applicability for immunological monitoring ; *Jap. J. Transplantation*, **22**, 373-378, 1987 (in Japanese)
- 16) S. ITO, *et al* : The function of peripheral blood monocytes after lung transplantation ; *Jap. J. Thorac. Dis.*, **25**, 11, 1207-1210, 1987 (in Japanese)
- 17) A. WATANABE : Studies on the cellular immunological response in splenectomized or auto-transplanted mice ; *J. Nippon Med. School*, **52**, 3, 306-314, 1975 (in Japanese)
- 18) H. THARA, *et al* : Clinical study on the effect of splenectomy in renal transplant recipients, - I. Posttransplant leukopenia ; *Jap. J. Transplantation*, **18**, 5, 412-422, 1984 (in Japanese)
- 19) S. SLAVIN, *et al* : Long-term effects of splenectomy on immunocompetent cells of adult

- mice ; *Cellular Immunol.*, **55**, 444-455, 1980.
- 20) A. CONSTANTOPOULOS, *et al* : Defective phagocytosis due to tuftsin deficiency in splenectomized subjects ; *Am. J. Dis. Child*, **125**, 663-665, 1973.
- 21) R. A. BUCSI, *et al* : Splenic replenishment of synergistic ability to bone marrow and thymic cells of neonatally splenectomized CBA mice ; *J. Exp. Med.*, **136**, 761-768, 1972.
- 22) Y. RON, *et al* : Involvement of the spleen in the control of the immunogenic and phagocytic function of thioglycollate-induced macrophages ; *Eur. J. Immunol.*, **11**, 608-611, 1981.
- 23) E. C. DOWNEY, *et al* : Long-term depressed immune function in patients splenectomized for trauma ; *J. Trauma*, **27**, **6**, 661-663, 1987.
- 24) D. SAMPSON, *et al* : The human splenic suppressor cell ; *Transplantation*, **20**, **5**, 362-367, 1975.
- 25) A. J. NORIN, *et al* : Cyclosporin A as the initial immunosuppressive agent for canine lung transplantation ; *Transplantation*, **34**, **6**, 372-375, 1982.
- 26) S. L. KAMHOLZ, *et al* : Single lung transplantation with cyclosporin immunosuppression ; *J. Thorac. Cardiovasc. Surg.*, **86**, 537-542, 1983.
- 27) F. J. VETH, *et al* : Cyclosporin A in experimental lung transplantation ; *Transplantation*, **32**, **6**, 474-481, 1981.