Hiroshima Journal of Medical Sciences
Vol. 33, No. 2, 223~231, June, 1984
HIJM 33-29

The High Dose Therapy of Anti-Human Lymphoblast Globulin in Living Related Renal Transplantation*)

Kiyohiko DOHI, Yasuhiko FUKUDA, Toshimasa ASAHARA, Hiroshi YAHATA, Eiji ONO, Masaharu TAKENAKA, Seiji MARUBAYASHI, Tamon OMOTEHARA, Yasuji TABE, Takaaki ETO and Haruo EZAKI

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

(Received Merch 23, 1984)

Key words: AHLG, Lymphocyte subpopulation, Kidney transplantation

ABSTRACTS

Large doses of antihuman lymphoblast globulin (AHLG) were used prophylactically in three recipients of renal allografts from living related donors. These recipients received 1,500 mg/day of AHLG for 14 days begining on the second day before transplantation in addition to the standard immunosuppression. Serum AHLG levels, serum anti-AHLG antibodies and T cell subsets were checked serially. The results were as follows:

1) No side effects and or complications of AHLG were encountered in these recipients.
2) None of patients treated with AHLG had any rejection episodes within three months after transplantation but after that several rejections occurred in two patients.
3) Maximum serum AHLG concentrations, reached at 10-12 days after transplantation, ranged from 252 mg/dl to 465 mg/dl. Serum AHLG levels were detected at least one month after final injection of AHLG.
4) No patients made detectable antibodies to horse IgG.
5) The values of T cell counts, % T cell and T cell subsets (Leu 3a/2a) varied considerable from patient to patient, in two cases were kept moderately low by AHLG treatment. We concluded that the effect of AHLG on T cell subsets was not distinctive.

INTORODUCTION

Since 15 years or more ago antihuman lymphocyts serum has been employed clinically for renal allografts[27]. However, among the reports available some state that it is apparently effective to extend renal graft survival[25,26,28], and some state that it is completely not effective in view of graft suvival rate[10,31]. The most apparent reason of that is different ways of producing antilymphocyte serum and different immunogen[18,19,20,31].

On the other hand difference in dosage, such as dose, time and term of application, can hardly be ignored. In view of the records from clinical cases[17,20] and the results of animal experimental studies[32] the more the graft survival improves when the longer and larger administration is given. However, actually there is few antilymphocyte serum available with less side effect upon massive amdinistration.

The authors used to dose comparatively large amount of antilymphocyte serum prophylactically, i.e., at 500-1,000 mg/day, and succeeded to completely inhibit acute rejections for a month after transplantation[6]. Thus, this time for the purpose of obtaining further prolonged control of rejection such as a massive dose as 1,500 mg/day of antilymphocyte serum was dosed on three cases for 14 days beginning from two

*) 土肥雪彦, 福田康彦, 浅原利正, 八幡 浩, 小野栄治, 竹中正治, 丸林誠二, 表原多文, 田部康次, 江藤高陽, 江崎治夫：生体腎移植における抗リンパ芽球グロブリン大量療法

days prior to the transplantation, the results of which are reported below.

PATIENTS AND METHODS

1. Patients

Three recipients of renal allografts from living related donors (LD 44, 46 and 48) at the Second Department of Surgery, Hiroshima University School of Medicine, from September 1981 to January 1982 were used.

2. Immunosuppressive dosage

The antilymphocyte serum for this study is an anti-human lymphoblast globulin *(hereinafter abbreviated as AHLG) produced by chronic immunization of horses with human cultured lymphoblast, and purified to IgG fraction. It contains 50 mg/ml of horse globulin, the titers of which are shown in Table 1. Upon confirmation of negativity with the intracutaneous and conjunctival tests, it was dosed at 1,500 mg/day for 14 days everyday starting from two days prior to the transplantation by i. v. drip. Other combined prophylactic immunosuppressive agents were given according to the protocol as stated in Table 2.

Table 1. AHLG titer

Lymphocytotoxity	1 : 5000
Rosette Inhibition	1 : 8000
Hemagglutinin	1 : 2
Antiplatelet	1 : <1

Table 2. Prophylactic immunosuppression for renal transpntation at Hiroshima University School of Medicine

Steroid (MP)
- Pre-op dosage···50–150 mg for 2 day (Medrol).
- 1 g Solu-Medrol on the day of transplantation and first two post-op days.
- Third post-op day···3 mg/kg/day (Medrol).
- Reduce level rapidly to 1 mg/kg/day in 2 weeks post-op day.
- Reduce level slowly to achieve a maintenance dose of 0.25 mg/kg/day

Azathioprine (AZ)
- Pre-op dosage···2 mg/kg/day for 3 days (Imuran).
- On the of transplantation···100 mg (Endoxan).
- Maintain at 2–3 mg/kg/day.

Bredinin (BR)
- Pre-op dosage···3 mg/kg/day for 3 days.
- Second post-op day···2 mg/kg/day. Maintain this dosage.

Anti-Human Lymphocyte Globulin (AHLG)
- 20–30 mg/kg/day for, 14 days begining on the second day before transplantation.

Local Irradiation
- Third, fifth and seventh post-op day at a dose of 150 rads/day.

3. Measurement of serum AHLG concentration and anti-horse lymphoblast globulin antibody

Serum AHLG concentration has been measured according to the SRID method, and serum anti-horse IgG antibody is expressed with hemagglutinin titer and inhibition titer according to the PHA method.

4. Measurement of T cells and T cell subsets

Mononuclear fraction was collected from heparinized peripheral blood by Ficoll-Conray's centrifugation, and Rossette forming cells with sheep red cells were made as T cells[33]. T cell subsets were measured using fluorescein-conjugated anti-Leu-3a and anti-Leu-2a, which are monoclonal antibodies of Becton Dickinson, according to the direct fluorescein method, and expressed with the value of Leu-3a divided by Leu-2a (Leu 3a/2a)[30].

RESULTS

1. Case reports

a. LD 44

The 34 year male patient received renal allograft from his 66 year old father as HLA C matched fonor. Since the donor was of old age and at the same time the kidney was bearing fairly large cyst, serum creatinine was higher than 2.0 mg/dl in the course without rejection although sufficient urination was observed immediately after transplantation. He was discharged on the 32nd day after the operation with serum creatinine of 2.3 mg/dl (Fig. 1). AHLG was dosed at 1,500 mg/day for 14 days, 21 g in total, without no side effect at all.

After discharge renal functions were stable, but 7 months after the transplantation a comparatively gradual increases of serum creatinine was observed, and upon renal biopsy it was diagnosed as chronic rejection. Without reaction to the bolus therapy with Solu-Medrol, now the course is uneventful with serum creatinine of 6–7 mg/dl.

* Pressimmune^{R}, Hoechst, Behling Institute, Lot No. 220025

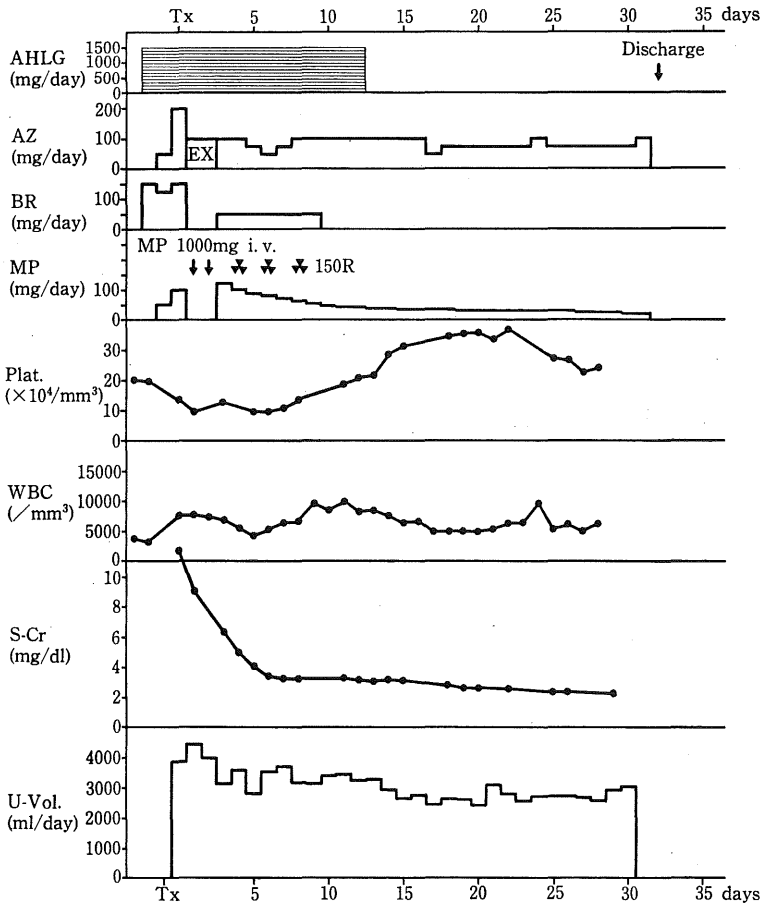

Fig. 1. Clinical course of LD 44

b. LD 46

This is a case of 29 year female with renal transplantation from her father as HLA D matched donor. As shown in Fig. 2 the course after transplantation was very favorable, and on the 32nd day after the operation she was discharged with serum creatinine of 1.0 mg/dl. Meanwhile, AHLG was given throughout the course at 1,500 mg/day excepting the day of transplantation and the postoperative 12th day at 1,000 mg/day. No side effect derived from AHLG was noted.

On the 88th day after transplantation she had pneumonia complicated with hepatic dysfunction, that was improved during one month's admission period. During that time the renal function was kept favorable and at present, 9 months after transplantation, she has completely returned to her routine life.

c. LD 48

The 29 year male patient with renal transplantation from his brother as HLA D matched donor. In this case, too, since immediately after transplantation sufficient urination was observed and serum creatinine was reduced orderly. Without experiencing any rejection he was discharged on the 33rd day after transplantation with serum creatinine of 0.8 mg/dl (Fig. 3). During that time AHLG was dosed at 1,500 mg/day excepting the day of transplantation at 1,000 mg/day. No side effect was noted at all.

After discharge the course propressed all right, but three months after transplantation acute rejection occurred, which once improved by an increased oral dose of steroid and the bolus therapy with Solu-Medrol, however, again serum creatinine increased, on which another anti-rejection therapy with Solu-Medrol was not successful, and four times plasmapheresis in total (11 liters in total of plasma exchanged) was ineffective, and now the course progresses with

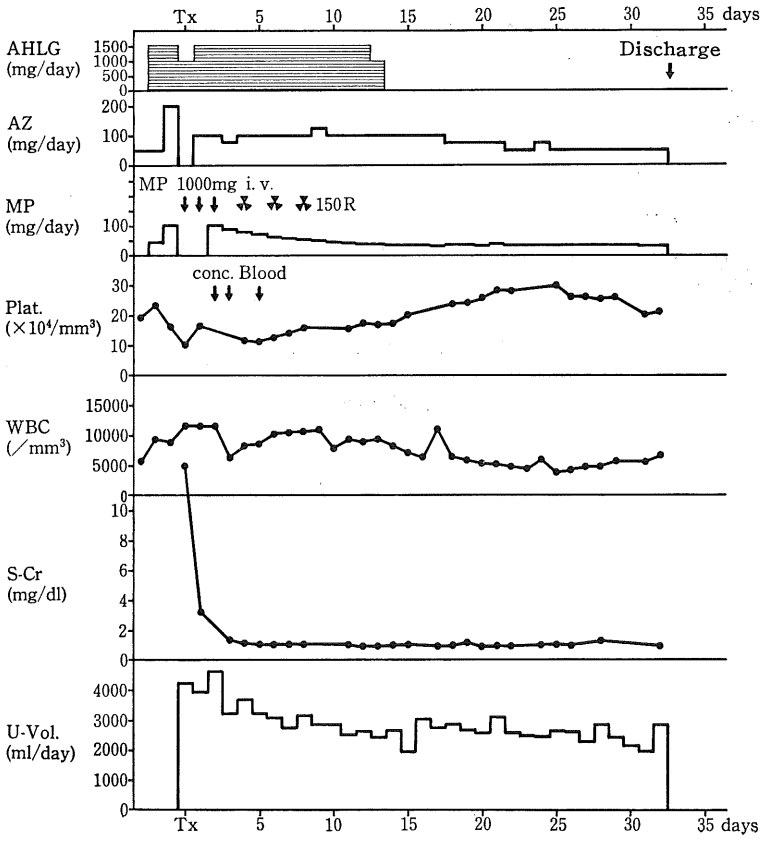

Fig. 2. Clinical course of LD 46

serum creatinine of 5.0 mg/dl.

2. Suppressive effect of AHLG on acute rejection

Those three cases stated above experienced no rejection at all for three months after transplantation, which practically confirmed the effect of AHLG. However, after three months, two cases out of the three had resistant rejections, specially LD 44 had a chronic rejection in the 7th month after transplantation.

3. Side effect of AHLG

In spite of large doses of AHLG at 1,500 mg/day for 14 days, all of three cases experienced no side effect at all. Specially it was proved to be a favorable antilymphocyte serum without showing marked reduction of platelet nor bleeding tendency derived from it as seen frequently.

4. Rise and fall of serum AHLG concentration and anti-horse IgG antibody titer

The serum AHLG concentration increased rapidly for a week after starting administration and then gradually continued to rise arriving at the peak on or around the final administration point (Fig. 4). In case of LD 44 after suspension of dosis at least for one month AHLG was detectable in blood. Meanwhile, AHLG concentration of these three cases at the peak was 257, 321 and 471 mg/dl respectively, and the half life in blood obtained from the attenuation curve was 9 ± 2.0 days.

Fig. 4 shows anti-horse IgG antibody expressed with hemagglutinin- and inhibition titers, in which both of them indicate nearly the same movement. LD 44 shows a value as high as 32-fold of that before administration of AHLG, which further increased to 64-fold from the 14th to 20th day of administration. This however is hardly determined as antibody positive. Two cases of LD 46 and LD 48

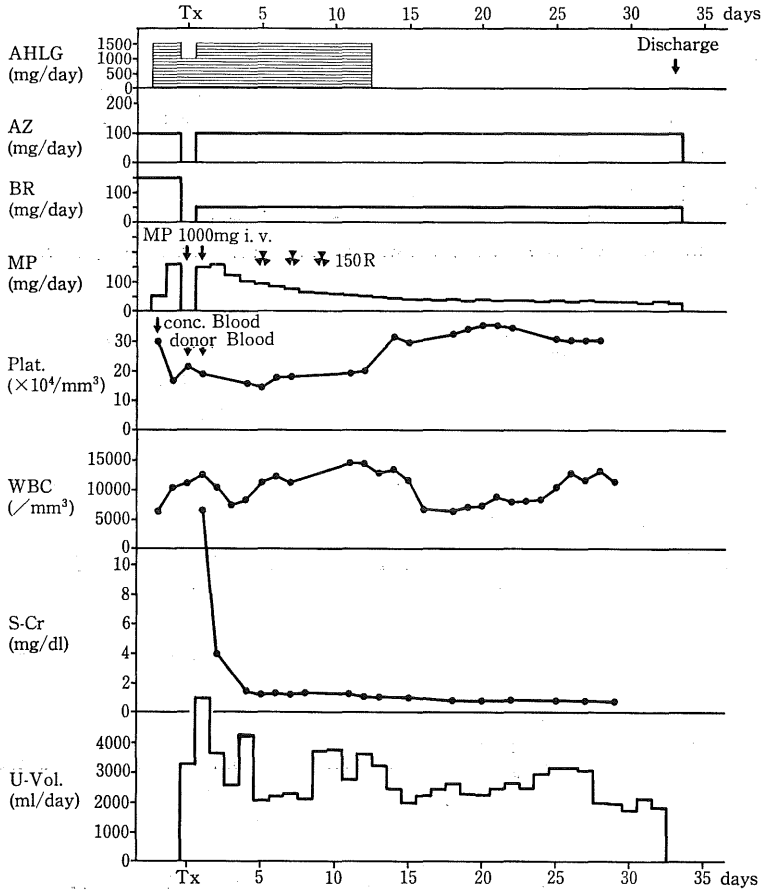

Fig. 3. Clinical course of LD 48

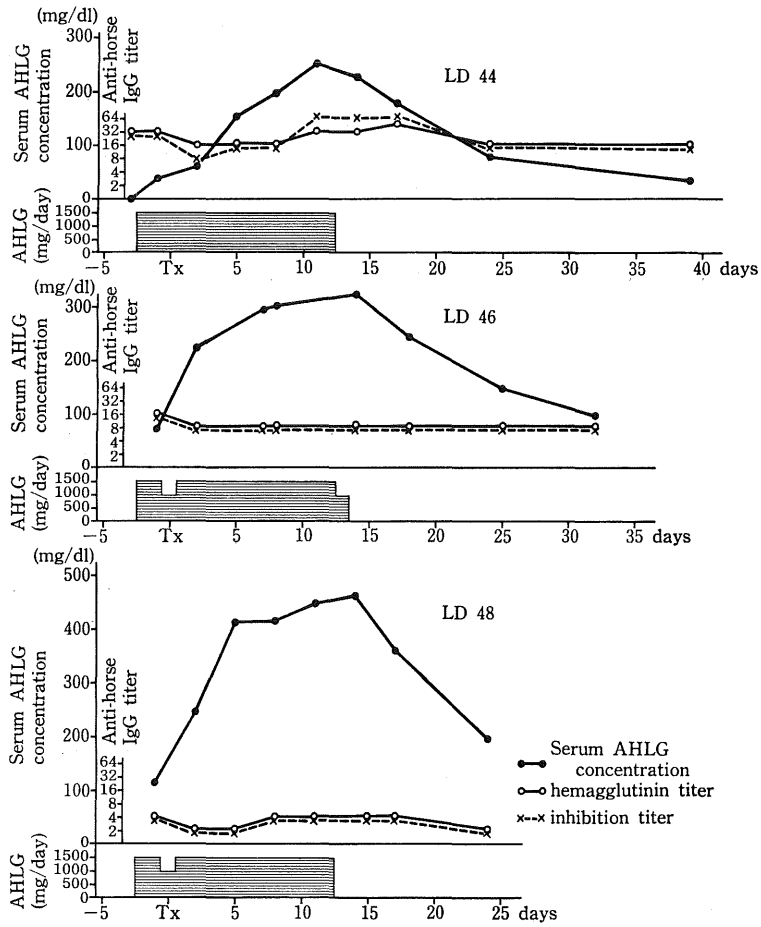

Fig. 4. Variation of serum AHLG concentration and serum antihouse IgG antibody titer

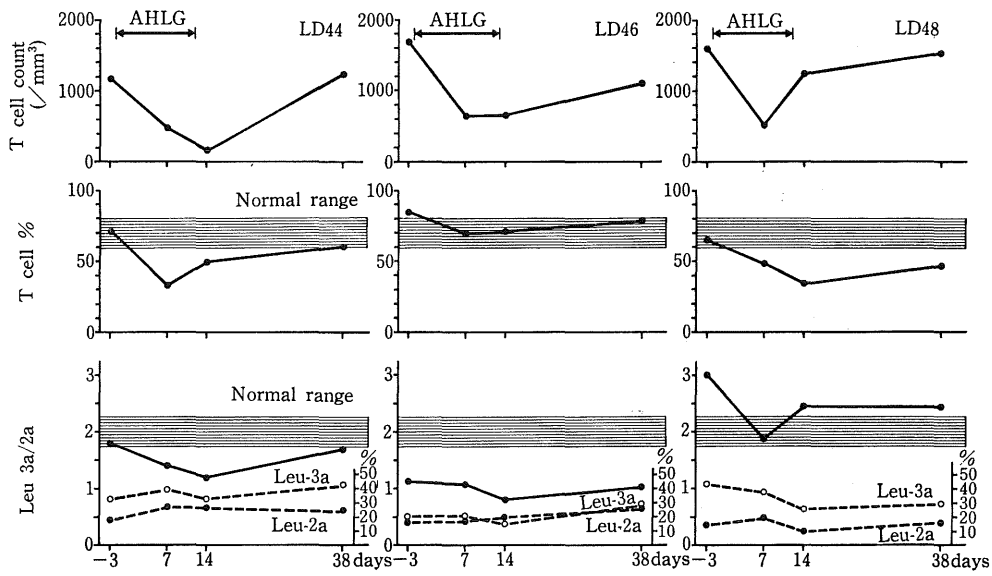

Fig. 5. Variation of T cells and T cell subsets

showed low values the throughout the course.

5. Variation of T cells and T cell subsets (Fig. 5)

Two cases of LD 44 and LD 48 showed reduction of T cells in number and percentage respectively as soon as administration of immunosuppressive agent was started, which was at the lowest in 1-2 weeks after transplantation and then gradually increased to nearly the former value on the 38th day after transplantation. Leu 3a/2a varies in parallel to the change of T cells, which suggests that the main reason of T cell reduction by the immunosuppressive agent is possibly due to reduction of Leu 3a positive T lymphocytes.

The pattern of LD 46, being different from other two cases, shows a decrease of T cell number by administration of the immunosuppressive agent, but % T cell and 3a/2a remain unchanged throughout the course. Accordingly in this case it can be said that influence of immunosuppressive agent occurs in the type of equally reducing T cells and B cells. It is interesting that % T cell was kept as high as 60% or over throughout the course, while 3a/2a on the contrary was moving on a low level.

DISCUSSION

Immunosuppressive effect of antilymphocyte serum is largely influenced by amount, time and term of administration[16], and as much as possible administration is required[17]. However, actually on account of various side effects sufficient amount cannot be applied. Antilymphocyte serum produced by chronic immunization of horses with human cultured lymphoblast is to be used in Japan most frequently, since red cell, platelet, granulocyte, etc., are not included in immunoantigens, is an antilymphocyte serum with less side effect[1,19]. Some report[24] states that with such so-called antilymphoblast globulin sufficient efficacy can only be achieved by massive administration, and Najarian et al.[20,21] have obtained a good result upon dosis of 20-30 mg/kg/day for two weeks. However, in Japan a dose of AHLG is limited to only 5-15 mg/kg/day[22] in consideration of the side effect and insufficient availability, up to 1,000 mg/day maximum[23,35]. The authors had already expeienced the comparatively large doses of AHLG at 10-20 mg/kg/day (500-1,000 mg/day), and this time succeeded in dosing at 1,500 mg/day for 14 continuous days without any side effect on three cases with renal transplantation.

As known already antilymphocyte serum are heterologous globulin that is highly antigenetic[2,11,14]. Such heterologous protein produces antibody any various allergic reactions as the major side effect of dosing antilymphocyte serum[27,29], antibody has not been produced in these three cases, which endorses the fact that AHLG causes less side effect.

As to the reports related to AHLG in Japan, it has been reported by Sagawa[22] that under prophylactic administration at 15 mg/kg/day for two weeks, rejection can completely by inhibited within one week after transplantation. The authors too have reported that with a comparatively large dose of 20 mg/kg/day rejection within one month after transplantation can be inhibited[6]. On the contrary there is a report stating that at a mean dose of 15 mg/kg/day about 50% of rejection was noted within two weeks[8]. Recently Yamada[35] had reported that by a dose of AHLG at 1,000 mg/day rejection was completely inhibited within three months after transplantation, thus this time the authors planned to prevent rejections for further prolonged term with a massive dose of 1,500 mg/day. Although three cases taken up as object showed no rejection at all within three months after transplantation, two cases experienced the resistant rejections after that. Thus, it was meaningless to increase dosis from 1,000 to 1,500 mg/day, and it is necessary, therefore, to study further on time and term of administration in future.

It is considered that as far as serum AHLG concentration is kept sufficiently, rejection is hardly caused[12,35], from which it seems that not only dosis and term but presence of serum antibody to antilymphocyte[3,19] and personal difference of the patients are influential. Howard[12] states that in a dosis of antilymphoblast serum at 30 mg/kg/day for 14 days from the maximal serum concentration of 800 μg/ml onward some differences are observed on suppression of rejection in the early posttransplantation period, while in those three cases in this report either of them showed a very high serum concentration of 2,500 μg/ml maximum, which enables to understand sufficient suppressive effect on rejection in the early posttransplantation period. However, there remains a

problem how rejection after disappearance of antilymphocyte serum out of blood can be inhibited, for which there is no successful method available, and many reports state that being same as the conclusion of the authors antilymphocyte serum reduces occurrences of rejections in the early posttransplantation period and improves seriousness, but cannot turn the graft survival to the better side[4,9,18,31], However, it does not mean that dosis of antilymphocyte serum is useless, but inhibition of rejection in early posttransplantation period redures onset of various complications being useful to improve the survival rate with the patients, which can be easily assumed. It is an interesting question that cultured lymphoblast being of B cell, how Antilymphoblast serum works to T cell which plays the leading part of rejection. First, as to the point whether Antilymphoblast serum reduces T cells or not, those three cases in the report showed different degrees of reduction respectively. Such reduction was not so much different from that of T cells in cases without using Antilymphoblast serum[7,34], thus it cannot be considered that Antilymphoblast serum strongly reduces T cell numbers. Anti-thymocyte globulin (ATG) is said to reduce T cells markedly[15], from which it can be assumed that ATG and Antilymphoblast serum are different in their mode of action.

Whether Antilymphoblast serum acted to reduce a certain T cell subsets or not was studied using a monoclonal antibody, but it was hardly concluded that Antilymphoblast serum reduces a certain T cell subset, as the value of Leu 3a/2a was different in such three cases respectively. LD 44 and LD 48 showed variation of Leu-3a positive cells in parallel to increase and decrease of T cells, while LD 46 showed low Leu 3a/2a value in spite of high % T cell. It draws attention that this case only has not brought any rejection up to date. In consideration of the report stating that no rejection occurs when OKT4 positive cell, which is a helper/inducer subset corresponding to Leu 3a, is comparatively low[5], it might be a sign of favorable prognosis that Leu 3a/2a is kept low.

REFERENCE

1. **Barnes, A. D., Dickerson, C. and Hawker, R. J.** 1972. The production of antilymphoblast globulin for clinical use. Behling Institute Miteilungen 51 : 66-68.
2. **Betler, W. T., Rossen, R. D. and Hersh, E. M.** 1969. Prevention of rapid immune elimination of antilymphocytic globulin in transplant patients. Nature 224 : 856-860.
3. **Bieber, C. P., Lydick, E. and Griepp, R. B.** 1975. Radioimmune assay of heterologous serum γ-globulin patients receiving rabbit antihuman thymocyte globulin. Transplantation 20 : 393-398.
4. **Chatterjee, S. N.** 1973. Antirejection drugs and procedures. Amer. J. Surg. 126 : 678-682.
5. **Cosimi, A. B., Colvin, R. B. and Burton, R. C.** 1981. Immunologic monitoring with monoclonal antibodies to human T cell subsets. Transplant. Proc. 13 : 1589-1593.
6. **Dohi, K., Asahara, T., Tatsukawa, Y. and Ezaki, H.** 1978. Clinical experience of the high dose therapy of intravenous antihuman lymphocyte globulin in living related renal transplantation. Clinical Immunology 10 : 421-435.
7. **Fukuda, Y., Yahata, H. Tabe, Y., Dohi, K. and Ezaki, H.** 1979. Criteria for assessing immunosuppressive effect by immunological monitoring. Jap. J. Clinical Transplant Immunology 2: 73-79.
8. **Fukunishi, T., Ishibashi, M. and Ichikawa, Y.** 1977. Immunosuppressive effect of AHLG in kidney transplantation. Jap. J. Transplantation 12 : 177-183.
9. **Groth. C. G.** 1981. The value of ATG in clinical renal transplantation Recent european experience. Transplant. Proc. 13 : 460-461.
10. **Hamburger, J., Grosinir, J. and Dormont, J.** 1972. Renal transplantation. Theory and Practice. p. 100-101. lst ed. Williams and Wilkins.
11. **Hardy, M, A., Quint, J. and Monaco, A. P.** 1970. Studies on heterologous antilymphocyte serum in mice. V. Effect of prior administration of normal or antilymphocyte rabbit γ-globulin. Transplantation 9 : 487-496.
12. **Howard, R. J., Condie, R. M. and Sutherlans, D. E. R.** 1977. The effect of serum ALG concentration on results following renal transplantation. Ann. Surg. 186 : 619-624.
13. **Iwasaki, Y., Poter, K. A. and Amend, J. R.** 1967. The preperation and testing of horse antidog and antihuman antilymphoid plasma or serum and its protein fraction. S. G. O. 124 : 24.
14. **Lance, E. M. and Dresser, D.** 1967. Antigenecity in mice of antilymphocyte gamma globulin. Nature 215 : 488-490.
15. **Light, J. A., Alijani, M. R. and Biggers, J. A.** 1981. Antilymphocyte globulin (ALG) reverses "Irreversible" allograft rejection. Transplant. Proc. 13 : 475-481.
16. **Livey, R. H. and Medawar, P. B.** 1966. Nature and mode of action of antilymphoid antiserum. Proc. Natl. Acad. Sci. U. S. A. 56 : 1130-1137.

17. **Monaco, A. P., Wood, M. L. and Russel, P. S.** 1967. Some effects of purified heterologous antihuman lymphocyte serum in man. Transplantation. **5** : **supple** : 1106-1114.
18. **Monaco, A. P. and Codish, S. D.** 1976. Survey of the current status of the clinical uses of antilymphocyte serum. S. G. O. **142** : 417-426.
19. **Najarian, J. S., Simmons, R. L. and Gewurz, H.** 1969. Antiserum to cultured human lymphoblasts. Preparation, purification and immunosuppressive properties in man Ann. Surg. **170** : 617-732.
20. **Najarian, L. S., Simmons, R. L. and Gewurz, H.** 1970. Antihuman lymphoblast globulin. Fed. Proc. **29** : 197-201.
21. **Najarian, J. S. and Simmons, R. L.** 1972. Transplantation, p. 460-462 Lea and Febiger.
22. **Sagawa, S., Arima, M. and Akiyama, T.** 1976. Suppressive effect on rejection of antilymphocyte globulin in kidney transplantation. Jap. J. Transplantation **11** : 47-55.
23. **Saito, Y., Komdo, A. and Ishikawa, Y.** 1980. A study for the kidney transplant recipients administrated antihuman lymphocyte globulin (AHLBULIN). Jap. J. Transplantation **15** : 265-281.
24. **Sel , K., Woody, J. and Smith, J.** 1973. Evaluation of human cultured lymphoblasts as a source of antigen for production of immunosuppressive antilymphocyte serum. Transplant. Proc. **5** : 541-547.
25. **Simmons, R. L., Moberg, A. W. and Gewurz, H.** 1970. Immunosuppressive assay of antilymphoblast globulin in man: Effect of dose, histocompatibility and serologic response to horse gamma globulin. Surgery **68** : 62-68.
26. **Sheil, A. G., Kelly, G. E. and Mears, D.** 1973. Antilymphocyte globulin in patients with renal allografts from cadaveric donors. Lancet **1** : 227-228.
27. **Starzl, T. E., Marchioro, T. L. and Porter, K. A.** 1967. The use of heterologous antilymphoid agents in canine renal and liver homotransplantation and in human renal homotransplantation. S. G. O. **124** : 301-308.
28. **Starzl, T. E., Marchioro, T. L. and Porter, K. A.** 1968. Heterologous antilymphocyte globulin, histocompatibility matching and human renal homotransplantation. S. G. O. **126** : 1023-1035.
29. **Starzl, T. E., Brettschneider, L. and Penn, I** 1969. A trial with heterologous antilymphocyte globulin in man. Transplant. Proc. **1** : 448-454.
30. **Thomas, F., Mendez-Picon, G. and Thomas, J.** 1978. Effective monitoring and modulation of recipient immune reactivity to prevent rejection in early posttransplant period. Transplant. Proc. **10** : 537-541.
31. **Turcotte, J. G., Feduska, N. J. and Haines, R. F.** 1973. Antilymphocyte globulin in renal transplant recipients. A clinical trial. Arch. Surg. **106** : 484-488.
32. **Woodruff, M. F. A. and Anderson, N. A.** 1963 Effect of lymphocyte depletion by thoracic duct fistula and administration of antilymphatic serum on the survival of skin homografts in rats. Nature **200** : 702.
33. **Yahata, Y. Dohi, K., Omotehara, T. and Ezaki, H.** 1977. Human lymphocyte T and B cell subpopulation. Effects of different techinques on the lymphocytes adhered to sheep cells. J. Hiroshima Med. Assoc. **30** : 471-476.
34. **Yahata, H., Dohi, K., Fukuda, Y. and Ezaki, H.** 1977. Cellular immune response in long term kidney transplant patients. T and B cell supopulation of peripheral blood lymphocytes. Jap. J. Transplantation **12** : 240-243.
35. **Yamada, Y., Takahashi, I. and Kuzuhara, K.** 1981. Experience of administration of antihuman lymphocyte globulin (AHLG) in kidney transplantation. Jap. J. transplantation. **16** : 267-275.