Increase in T. G–Cell Ratio in Renal Transplant Recipients and the Relationship to Complications[*]

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ABSTRACT

The changes in lymphocyte subpopulations in renal transplant recipients were determined. Prior to immunosuppressive therapy, the subpopulation levels in the recipients were similar to those of healthy adults, but after administration of therapy, the T-cell ratio (%T) decreased, while the IgG–Fc receptor-bearing T-cell ratio (%T. G) increased. However, review of these changes in individual cases showed that among those with the same degree of decrease in %T, the %T. G increased only slightly in some, but markedly in others, thus, indicating that there was not necessarily an inverse correlation between the decrease in %T and increase in %T. G. Further, it is noted that when %T. G was markedly increased, the patient was susceptible to bacterial infection.

INTRODUCTION

The peripheral blood T–cell count decreases following the use of immunosuppressive therapy in renal transplant recipients, but it has been reported that the degree of decrease varies by patient[6], acute rejection tends to occur when there is an increase in T-cell count[1], and infection tends to develop when there is a decrease in T-cell count[3]. However, there are problematic points yet to be elucidated. Moretta et al.[5] have reported that human T–cells can be further classified into subsets depending on the type of Fc receptors, and that the IgG–Fc receptor bearing T-cells (T. G.-cells) can suppress immunoglobulin synthesis of B–cells. Therefore, it can be expected that better monitoring can be achieved by measuring not only the T–cells, but the T. G–cells as well at the same time. The authors obtained interesting results during their measurement of changes in T-cells and T. G–cells in the peripheral blood of renal transplant recipients. These will be reported.

MATERIALS AND METHODS

Twenty-five patients who had received living related renal transplantation during the period from February 1977 to April 1981, and who had been followed in the department from prior to administration of immunosuppressive therapy were studied. There were 8 episodes of bacterial infection involving 5 patients within 60 days after transplantation. During this period, death occurred in 1 case, the transplanted kidney was removed in 1, but the remaining 3 recovered without any damage to their renal function. Also during the same period, 17 episodes of acute rejection involving 12 patients occurred necessitating removal of the transplanted kidney in 2. Thus, follow-up to the 60th day after transplantation was possible in 21 patients. Acute rejection was defined as an increase in serum creatinine in excess of 0.4 mg/dl which persisted for 3 days or more.

Immunosuppressive therapy was commenced from 36 hr prior to transplantation in all cases beginning with the administration of Medrol 0.4 mg/kg q.i.d., followed by Imuran 2 mg/kg/day from the day prior to surgery. Anti-human lymphocyte globulin (AHLG, Behring, West Germany or MidoriJuji, Japan) was given in doses of 5–20 mg/kg/day from the day prior to transplantation until 14 days after surgery. From the day of surgery, Solumedrol in a dose of 1000 mg/day was administered for 3 days. After transplantation, the dose of Medrol was gradually reduced from the initial dose of 1.6 mg/kg/day, and from the 90th the patients were placed on a maintenance dose of less than 0.5 mg/kg/day. The Imuran dose was kept at about 2 mg/kg/day under close monitoring of the WBC count.

The lymphocyte fraction (PBL) was sought by Conray-Ficoll (S.G. 1,078) gradient centrifugation of heparinized peripheral blood drawn from the patients prior to administration of their daily immunosuppressive drug. This was washed twice by RPMI 1640 (GIBCO, U.S.A.) and adjusted to 4×10^6/ml for determination of the lymphocyte subpopulations.

The T-cells were determined by adding 2×10^5/0.05 ml of PBL and 2×10^7/0.05 ml of sheep erythrocytes (E) to a round bottom glass tube, which were centrifuged for 5 min at 160 g and incubated overnight in an ice bath. The contents were gently resuspended, stained with brilliant cresyl blue and examined under a microscope. Two-hundred lymphocytes were counted and those with more than four E's adhering to it (E-RFC) were defined as T-cells.

The T.G-cells were determined by adding 0.05 ml of 1% chicken erythrocytes (EchAg) coated with rabbit antichicken erythrocyte IgG to 2×10^5/0.05 ml of PBL in a round bottom glass tube. Tese were left at room temperature for 30 min after which they were incubated in an ice bath for 90 min. Of the supernate, 0.05 ml was drained and E 2×10^7/0.05 ml was added and mixed. This was centrifuged for 5 min at 100 g, incubated in an ice bath overnight and observed under a microscope. Lymphocytes with more than two E's and more than two EchAg adhering to them (E. EchAg-RFC) were defined as T.G and entered into the following formula to calculate the proportion within the T-cell.

$$\%\text{T. G} = \frac{\text{E. EchAg-RFC}}{\text{E-RFC} + \text{E. EchAg-RFC}} \times 100$$

The B-cells were determined by adding F.I.T.C. conjugated rabbit anti-human Ig (IgG + IgA + IgM) serum (Behring, West Germany) to 5×10^5 of PBL cells pellets, which were incubated in an ice bath for 50 min. This was washed 3 times with PBS to which 0.02% sodium azide had been added, after which it was observed under a fluorescent microscope. Lymphocytes releasing granular fluorescence were defined as B-cells. All determinations were made in duplicates, and when complications were detected within 6 days after determination, this time was recorded as the time of onset of complication.

Statistical analysis was performed by Student's T test.

RESULTS

The %T value of healthy adults (n : 310) was $71.5 \pm 8.0\%$, the %T.G was $4.7 \pm 2.7\%$ and the %B was $11.6 \pm 3.9\%$. The %T value of transplant recipients prior to immunosuppressive therapy was $69.7 \pm 12.2\%$, which was similar to that of healthy adults. On the day prior to transplantation, the value dropped to $58.5 \pm 11.1\%$ (p<0.001) and 4 days after transplantation decreased further to $45.9 \pm 16.5\%$ (p< 0.001). Subsequently, it gradually increased, and 60 days after transplantation, the value recovered to almost the same level as the value prior to immunosuppressive therapy. The %T.G value prior to therapy was $5.5 \pm 2.8\%$, which did not differ significantly with that of the healthy adults. On the day prior to transplantation, a slight increase trend was noted, and on the 4th day after surgery the value increased markedly to $11.5 \pm 5.2\%$ (p<0.001) as compared to that prior to suppressive therapy. Subsequently, it decreased, and 60 days after transplantation, the value returned to almost the same level as the pre-therapy value (Fig. 1). In other words, immunosuppressive therapy caused a decrease in %T, but the drop in T-cells was mainly due to loss of non T.G-cells. The T.G-cells are comparatively resistant to immunosuppressive drugs, and thus it is considered that they remained in the peripheral blood and were responsible for the increase in %T.G. Further, as the %T.G showed a marked increase 4 days after pulse therapy with Solumedrol, but dropped
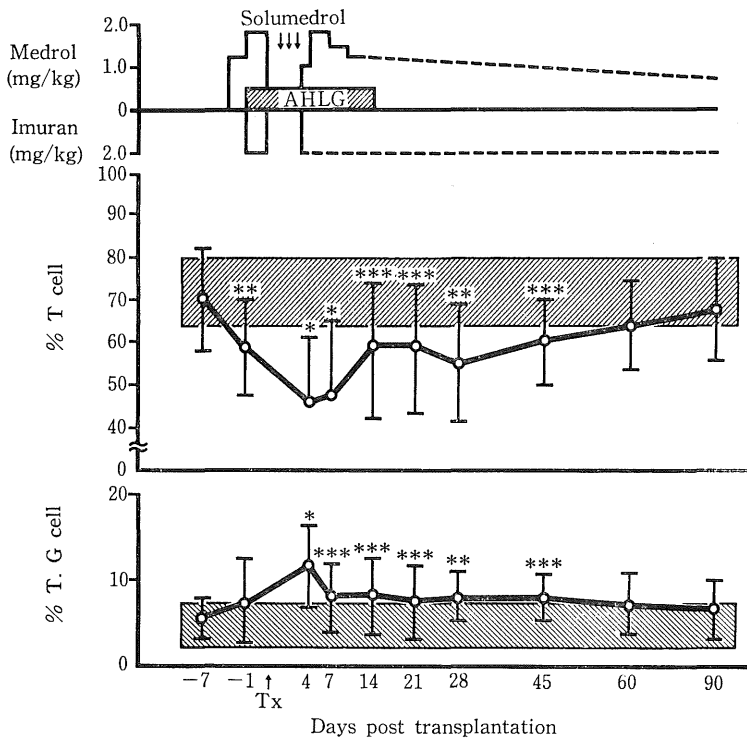
Fig. 1. Changes in %T cells and %T.G cells prior to and after renal transplantation (21 patients)
　　　　　* p<0.001　Prior to immunosuppressive therapy
　　　　** p<0.01　　〃　〃　　　　　〃　　　　　〃
　　　*** p<0.05　　〃　〃　　　　　〃　　　　　〃
-------------　doses of Medrol and Imuran vary by patient
　　　　⚬　indicates "mean±standard deviation" (Mean±S. D.)
　　　▨　indicates %T cell value (Mean± S. D. of healthy persons)
　　　▩　indicates %T. G cell value (〃　　〃　　〃　　〃　　〃　　)
　　Tx indicates day of transplantation

considerably on the 7th day despite the fact AHLG and Imuran were being administered, led us to believe that the increase in %T. G was due primarily to the massive doses of steroids administered.

It was noticed that the increase in %T. G coincided with the timing of decrease in %T, and thus individual cases were studied to ascertain whether there was an inverse correlation between the amounts of decrease in %T and increase in %T. G.

Fig. 2 shows the %T. G patterns in patients whose %T tracing were almost the same. The AHLG lots for cases LD30 and LD37 were defferent, but the doses of Imuran and Medrol administered up to the 18th day after transplantation were the same. Both the LD30 and

LD37 cases showed approximately the same amount of decrease in %T, but the LD30 case showed a marked increase in %T. G whereas the increase of the LD37 was slight (Fig. 2a). The same AHLG lot was used for the LD27 and LD28 cases, and the Imurn and Medrol doses were the same up to the 16th day after transplantation. Both the LD27 and LD28 cases showed approximately the same amount of decrease up to the 14th day after transplantation. However, whereas there was a marked increase in %T. G in the LD27 case, there was only a slight increase in the LD28 case (Fig. 2b). Differences in the amount of decrease in %T and increase in %T. G were also observed in other cases as well. These results led us to the interpretation that even when the same
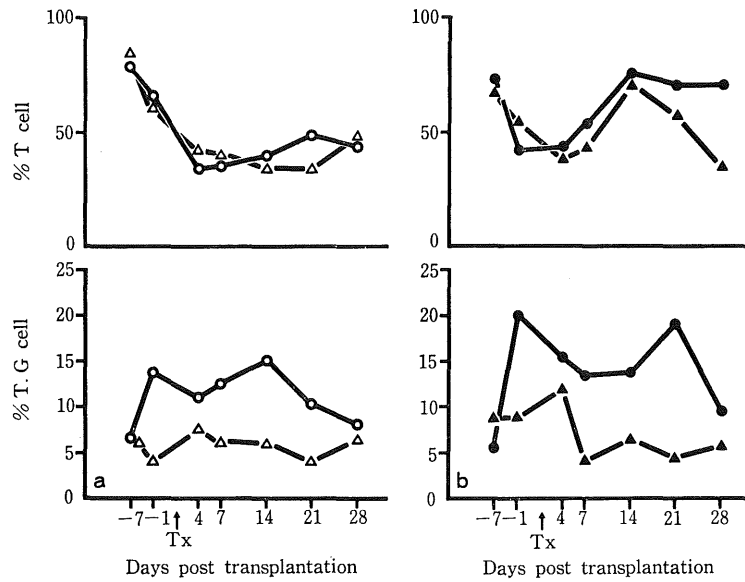
Fig. 2. %T. G cell variations in patients whose %T cell variations were approximately the same
　　　　　O‐O　LD30
　　　　　△‐△　LD37
　　　　　●‐●　LD27
　　　　　▲‐▲　LD28

Table 1. Lymphocyte subpopulations in renal transplant recipients with and without comlications within 60 days post-transplantation

Patient group	Lymphocyte[a] counts	T cells %	T cell[a] counts	T. G cells %	T. G cell[a] counts	B cells %	B cell[a] counts
Without complications	736±509[b]	55.7±15.1	422±339	7.8±4.1	32±36	16.9±5.7	117±95
(n : 132)	(94−2430)[c]	(20.0−88.5)	(25−1614)	(0.5−19.8)	(2−239)	(4.3−36.8)	(13−547)
Acute rejection	510±364[b]	64.6±17.0**	359±287	6.1±3.1	18±11	14.0±3.7	75±57
(n : 17)	(80−1224)[c]	(25.7−88.5)	(58−994)	(2.1−13.7)	(4−37)	(8.9−24.1)	(8−203)
Bacterial infection	504±307[b]	39.7±18.8**	191±59	19.1±1.8*	38±14	21.2±9.1	93±39
(n : 8)	(282−1104)[c]	(20.0−74.3)	(142−284)	(16.7−21.6)	(20−56)	(11.8−41.8)	(33−158)

(a) Cells per mm^3
(b) Mean±S. D.
(c) Range
　* p<0.001
** p<0.01　P value v. s. without complications
Without complicati ons　—Measured value when no complications developed within 6 days after　determination
Acute rejection　　　　—Measured value when acute rejection occurred within 6 days after determination
Bacterial infection　　—Measured value when bacterial infection occurred within 6 days after determination

immunosuppressive procedures are employed, there are patients whose increase in %T. G are slight because the amounts of T. G and non-T. G retained in the peripheral blood are the same, whereas there are cases with marked increase in %T. G, because a comparatively greater amount of T. G than non-T. G remains in the peripheral blood. In other words, no inverse correlation exists between the %T decrease and %T. G increase in the individual cases, and it was considered that the changes in T. G could not be predicted on the basis of
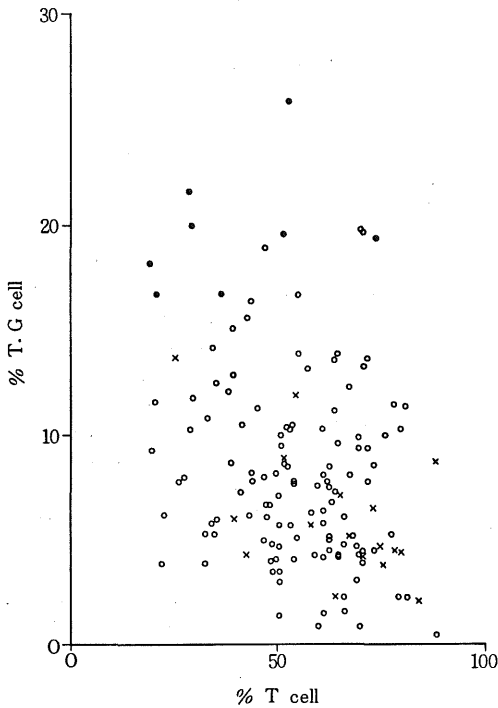
Fig. 3. %T and % T. G cells in renal transplant recipients within 60 days after transplantation

o   Values which indicate the absence of complications within 6 days after determination

●   Values which indicate the development of bacterial infection within 6 days after determination

✕   Values which indicate the development of acute rejection within 6 days after determination

T-cell measurements alone.

Next, review was made of complications and the state of lymphocyte subpopulations within 60 days after transplantation (Table 1). The lymphocyte subpopulations prior to acute rejection showed a significant increase ($p<0.001$) in %T as compared to when complications did not develop, but there was no significant difference in lymphocyte, T-cell, T. G-cell and B-cell counts. Further, the lymphocyte subpopulations prior to bacterial infection demonstrated a significant decrease ($p<0.01$) in %T and increase ($p<0.001$) in %T. G as compared to when complications did not develop, but no significant difference in lymphocyte, T-cell, T. G-cell and B-cell counts was noted. Thus, it is considered that T-cells, particularly %T and

%T. G are more deeply involved in the induction of complications in renal transplant recipients than the B-cell counts.

Therefore, study was focused on %T and %T. G values determined within 60 days after transplantation and the occurrence of complications (Fig. 3). Many instances of acute rejection were found to develop following the increase of %T value, but at times this finding was not observed. On the basis of these findings, it is considered that a close relationship does not exist between occurrence of acute rejection and %T value. Bacteral infection developed in 8 out of 12 determinations when the %T. G value was greater than 15%, but failed to occur when less than 15%. Also in 3 patients who recovered from bacterial infection without developing impairment of renal function, it was observed that their conditions began to improve after their %T. G value decreased. On the other hand, in many instances bacterial infection developed when the %T value was low, but it was found to also occur when the value was high. In other words, the above findings lead to the interpretation that the development of bacterial infection is more closely related to the increase in %T. G value than decrease in %T.

DISCUSSION

Thomas. et al.[6] have reported that the degree of T-cell decrease due to immunosuppressive therapy in renal transplant recipients varies by patient. The authors have ascertained, however, that the difference by patient following immunosuppressive therapy was not due to the T-cells alone, but was also caused by their subsets, the T. G-cells, as well, and that the %T. G increase was due primarily to steroids. As there was hardly any difference in steroid dose administered during the first 10 post-transplant days, it is considered that the variation in %T. G increase was not due to difference in steroid dose, but was caused by difference among patients in the effects on T-cell subsets because %T.G increase was observed even when the same doses were administered to different patients.

Fauci et al.[2] claim there are no differences in binding affinities and dissociation constants to steroids between T. G and IgM–Fc receptor bearing T-cells, and report that the %T. G increase following steroid administration is due

to mechanisms other than T-cell steroid receptors. Therefore, it is presently yet unknown what causes the difference in the effects of steroids on peripheral blood T-cell subsets, but it is felt that it develops as a result of the difference by patient in %T. G increase.

Moretta et al.[5] report that T. G-cells suppress immunoglobulin synthesis of B-cells, while T. M-cells help the synthesis. Further, Lung et al.[4] report that T. G-cells have a weaker response to MLC and CML than T. M-cells. However, Saal et al.[7] state that T. G-cells possess antibody dependent cell mediated cytotoxicity. Therefore, the fact that there is an increase in %T. G in renal transplant recipients cannot be simply attributed to the comparatively large increase in suppressor T-cells alone. When considered only from the standpoint of immunoglobulin synthesis, as the T. M cells which help the synthesis are more greatly decreased than the suppressing T. G-cells, this results in a reduction in the immunoglobulin synthesis capacity. Thus, as many instances of bacterial infection were experienced subsequent to marked increases in %T. G, while such infection was not noted after the degree of increase in %T. G decreased, we presume that renal transplant recipients undergoing steroid therapy are susceptible to bacterial infection when %T. G is markedly increased. Therefore, it is considered that by monitoring the T. G value in renal transplant recipients, it should be possible to prevent excessive administration of immunosuppressive drugs, particularly steroids, and thus avoid excessive immunosuppression, and consequently provide appropriate therapy for the individual patient.

REFERENCES

1. Chatterjee, S. N., Gershwin, M. E. and Eckles, D. 1979. Characterization and alterations of lymphocyte subpopulations in renal affograft recipients. Transplant. Proc. 11 : 374-378.
2. Fauci, A. S., Murakami, T., Brandon, D. D., Loriauh, D. L. and Lipesett, M. B. 1980. Mechanisms of corticosteroid action on lymphocyte subpopulations. VI. Lack of correlation between glucocorticosteroid receptors and the differential effects of glucocorticosteroids on T-cell subpopulations. Cell. Immunol. 49 : 43-50.
3. Leapman, S. B., Strong, D. M., Alpert, S., Fuduska, N. J. and Sell, K. W. 1977. Lymphocyte monitoring as a predictor of renal allograft rejection. Ann. Surg. 186 : 568-572.
4. Lung, P. C. and Singal, D. P. 1981. Functional roles of T-cell subpopulations in T-T cell interactions. Transplant. Proc. 13 : 1160-1163.
5. Moretta, L., Webb, S. R., Grossi, C. E., Lydyard, P. M. and Cooper, M. D. 1977. Functional analysis of two human T-cell subpopulations: Help and suppression of B-cell responses by T cells bearing receptors for IgM or IgG. J. Exp. Med. 146 : 184-200.
6. Thomas, F., Thomas, J., Mendez, G., Lee, H. M. and Lower, R. 1978. Individualization of recipient immunosuppression by use of in vitro monitoring parameters. Transplant. Proc. 10 : 621-625.
7. Sall, J. G., Rieber, E. P., Hadam, M. and Riethmutter, G. 1977. Lymphocytes with T-cell markers cooperate with IgG antibodies in the lysis of human tumor cells. Nature 265 : 1518-60.