T CELL SUBSETS IN RENAL TRANSPLANTED PATIENTS DEFINED BY THEOPHYLLINE SENSITIVITY AND MONOCLONAL ANTIBODIES

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Summary

Peripheral T cell subsets were determined in 26 patients after renal transplantation. Thirteen patients were undergoing acute rejection episodes at the time of blood sampling. Two methods were used for the determinations: the theophylline sensitivity test (THST) and the monoclonal antibodies method OKT8 and OKT4. In both rejecting and non-rejecting groups, the percentages of T suppressor lymphocytes (TS) was found to be higher by the OKT8 method than by the THST. Furthermore, no significant difference in TS percentages could be revealed by the OKT8 method between the two groups. However, with the THST the differences were significant with a mean value of 13.5±7.9 per cent in the rejecting group and 21±5.9 per cent in the non-rejecting group (p<0.01). The different results between the two methods could be attributed to the fact that by THST only TS cells are defined while the OKT8 conjugates also with cytotoxic T lymphocytes. The measuring of T helper cells (TH) revealed much higher percentages of TH in the rejecting group than in the non-rejecting group, 51±9.5 per cent mean value and 29±13 per cent mean value, respectively (p<0.05). The ratio OKT4+/OKT8+ was below one in the non-rejecting group and above 1.5 in the rejecting group. We concluded that the THST as well as OKT4+/OKT8+ ratio may be a helpful laboratory test to confirm a clinically suspected acute rejection episode.

Introduction

Observations by many investigators showed that the determination of the percentages of certain functional T cell subpopulations, helper T lymphocytes (TH), and suppressor T lymphocytes (TS) in the peripheral blood of recently transplanted kidney patients might be indicative of their immunological response state [1–5].

A decrease in the amount of TS lymphocytes or elevated ratios of TH/TS
have often been shown to correlate with clinical symptoms of acute rejection episode or even predict acute rejection if this pattern were found during clinical quiescence [1,3–5].

The method most utilised for quantifying T cells and their subsets is the use of monoclonal antibodies which recognise different antigens on the surface of the different T cell subsets [6,7]. At our centre the theophylline sensitivity test (THST) is used regularly for enumerating TS lymphocytes [5]. Therefore, we undertook this study to compare the accuracy of both methods in correlating clinically established acute rejection episodes.

Materials and methods

Twenty-six consecutive HLA non-identical first cadaver renal graft recipients entered this study. From each patient one sample of heparinised peripheral blood was drawn during the fourth post-transplant week. Thirteen patients had clinical and chemistry laboratory evidence of acute rejection when the blood sample was drawn. Percentages of TS lymphocytes were determined in each sample by the monoclonal OKT8 antibody indirect immunofluorescence method and by the THST. The suppressor activity of the separated theophylline sensitive T cells was confirmed by the local xenogeneic graft versus host reaction (GVHR) [5].

In addition, the percentages of TH lymphocytes was defined by monoclonal antibodies (OKT4) in five patients during acute rejection episode and in seven non-rejecting patients.

Results

The mean percentage of OKT8-positive (+) (Ts lymphocytes) in the rejecting patients was 35±11.6 per cent, while in the non-rejecting patients it was 53±20 per cent. These differences are not statistically significant (p>0.05). At the same time in the same samples lower values of TS lymphocytes were found by the THST with mean values of 13.5±7.9 per cent in the rejecting group and 21±5.9 per cent in the non-rejecting group. The differences between the groups are statistically significant (p<0.01) (Figure 1).

The five patients with acute rejection who were also tested for TH lymphocytes using OKT4 showed much higher mean percentages than the patients who were in quiescence, 51±5 per cent as compared to 29±13 per cent TH lymphocytes respectively. These results were statistically significant (p<0.05). The mean value of the OKT4/OKT8 ratio in the non-rejecting patients was below one and in the rejecting group above 1.5 (Figure 2).

Discussion

Three different patterns could be revealed in recent publications in respect to the number of circulating OKT8+ cells during acute rejection episodes:

1. Decrease in circulating OKT8+ cells as demonstrated by Cosimi and his colleagues [4].

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Figure 1. Suppressor T lymphocyte percentages as measured by the theophylline sensitivity test and by the OKT8 monoclonal antibody technique

2. Increase in circulating OKT8+ cells as demonstrated by Smith and his colleagues [2].

3. No difference in the circulating OKT8+ cells between patients in rejection and those in quiescence as demonstrated by Stelzner and his colleagues [1].

This last observation corresponds with ours when OKT8 antibodies were used. The above discrepancies could be attributed to the fact that OKT8 antibody
conjugates with two T lymphocyte subsets; suppressor as well as cytotoxic cells [4]. As yet we have very little information on the changes occurring in cytotoxic cells. If their proportion correlates with the number of TS during rejection episodes or quiescence it might well be that there is no consistent relationship between the percentage of the cytotoxic cells and that of the TS during different immunological response states. This could explain the variations in the total number of the OKT8+ cells which were observed by the different groups.

However, by using the THST and the xenogeneic GVHR the suppressor lymphocyte subset alone is determined. As shown in this study and in a previous report [5], this method gives a significant correlation between the percentages
of TS and the immunological response of the kidney transplant patient. This essential difference between the two methods may also explain the variance in our results since both of them were used for determining TS.

On the other hand, the use of monoclonal antibodies for defining OKT4+ is a more reliable indicator since it was observed by some authors to correlate sufficiently with the immunological response state [2,4]. This may also be concluded from the present study. Furthermore, the ratio of OKT4+/OKT8+ was found to correlate with the clinical status of the patients. Elevated ratios above 1.3–1.7 were found to be significant related with the clinical state of rejection [1,3,4]. In our study a ratio above 1.5 was found in the rejecting group while a ratio below one was typical of the patients in quiescence.

Morris et al [8] as well as Toledo-Pereyra et al [9] could not, however, confirm such correlations. They suggested that the different results in the OKT4+/OKT8+ ratio in the different studies deriving from the actual immuno-suppressive protocol employed by each group and the actual type of the rejection process.

The THST is a far less costly method and could be considered as a satisfactory alternative to the determination of OKT4+/OKT8+ ratios. However, it remains to be seen whether the development of monoclonal antibodies such as Leu-15 which will conjugate solely with TS would make this method the most accurate one for confirming or, in case of serial monitoring, for predicting acute rejection.

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References

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