Clinical Utility of Lymphocyte Cross-Matching after Renal Transplantation*

Abstract: One of the most important obstacles in renal transplantation is the rejection phenomenon. In spite of the developments in medical technology and immunology, early diagnosis of rejection and specific immunosuppression of the immune attack against the graft have not yet been totally accomplished. Lymphocyte cross-matching (LCM), a test to detect the presence of preformed antibodies in the donor against the recipient, is a practical and cheap method and is performed at almost all renal transplantation centers before transplantation. In this study, we searched for the relation between the post-transplant LCM status and graft survival. LCM was positive in 7 patients and 5 lost their grafts. On the other hand, of 88 patients, whose LCM remained negative, only 8 lost their grafts (p<0.05). In conclusion LCM is a practical and cheap method for detecting the presence of antidonor antibodies which closely relate with graft rejection.

Key Words: Lymphocyte Cross-match (LCM), Renal transplantation Graft survival, DTT, Rejection

Introduction

One of the most important obstacles in renal transplantation is the rejection phenomenon. In spite of the developments in medical technology and immunology, early diagnosis of rejection and specific immunosuppression of the immune attack against the graft have not yet been totally accomplished. Current diagnosis of allograft rejection is still mainly based on clinical and biochemical data such as graft dysfunction and systemic manifestations (1). Lymphocyte cross-matching (LCM), a test for detecting the presence of preformed antibodies in the donor against the recipient, is a practical and cheap method and is performed at almost all renal transplantation centers before transplantation.

In this study, we searched for the relation between the formation of antidonor antibody and grafts loss in renal transplant patients who did not have the antibody prior to transplantation.

Materials and Methods

Ninety-five patients who received renal transplant from living related donors and in whom LCM was performed at least postoperatively were included in the study. Although it was planned to analyse all the patients whose donors were alive retro- and prospectively every six months, this goal was not achieved in most of the patients. LCM was done at four weeks posttransplant period and then repeated at three or six month intervals in the recently performed transplantations.

Criteria such as ABO blood group compatibility and negative LCM were applied for donor selection and the person with the best HLA matching was selected among the potential donors.

The relation between LCM and graft loss was retrospectively evaluated. The clinician was informed about the results of the LCM tests but no comments about medical therapy of patients were made. Patients who received anti-thymocyte globulin for the treatment of rejection were not included in the study because of LCM positivity related to the therapy.

Lymphocyte cross-matching: Lymphocyte cross-matching, including autocrossmatch and dithiothreitol, were performed as described previously (2). The evaluation was made semiquantitatively according to the

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percentage of dead cells (0-10%: negative, 10-20%: 1+, 25-35%: 2+, 35-50%: 3+, 50-80%: 4+, 80-100%: 5+)

Statistical Analysis: Comparisons between patient groups were made using the chi-square test, and the Fisher’s exact test where appropriate. A two-tailed P value of <0.05 was considered statistically significant.

Results

LCM was positive 7 of 95 posttransplant patients. In the patients in whom LCM was carried out 13 graft losses occurred in a follow-up period of three years. The relationship between the LCM results and graft loss is summarized in Table 1. It is found that graft loss is significantly higher in patients with positive LCM (p>0.0005).

Graft loss occurred in five of the seven patients three to twelve months after LCMs were found positive. In one of the two patients in whom graft loss did not occur, LCM continued to be positive for three months, and then became negative (it was tested twice). The other case became negative after it was found to be 2 (+) twice and 1 (+) once.

The relationship between the highest percent of positivity in repeated tests and graft loss was not statistically significant (p=0.5254). This is shown in Table 2.

In only two patients, LCM positivity without DTT changed to negative after DTT treatment. In the first case who had 2+LCM, graft function was well-preserved, but the second case having 3+LCM experienced severe rejection six months after having positive LCM test.

Discussion

It is known that the possibility of hyperacute rejection is very high in the presence of performed antibodies in the recipient against the donor HLA antigens (3-5). Therefore, a positive LCM has been an important contraindication for transplantation except in a few cases (6-8). Although the importance of the presence of donor organ directed antibodies is well-known, there is little knowledge about the frequency and importance of formation of antibodies in the posttransplant period and their roles in allograft rejection. It is known that the allograft rejection is principally based on T-cells except for the hyperacute rejection. In experimental animals where there is no B cell function and the transfer of rejection occurs by T cells only supports this idea (1,9). Antibodies against graft antigens may slough away from the cell surface after they attach to the antigens and these immuno complexes are readily cleared by the reticuloendothelial system. Apart from this, anti-anti-HLA antibodies, ie, antidiotypic antibodies may form in the host and these may block the graft directed antibodies. As the antibody-antigen complexes are rapidly cleared from the circulation, they cannot be detected reliably in order to reflect the real frequency and severity of the event (9-12). Suciu-Foca (13-14) found the frequency of the formation of the anti-HLA antibodies to be 23% and 28% in cadaveric heart and renal transplant patients, respectively, for one year. In our study, this ratio was 7.2% for one year. We think this lower incidence is due to the difference of donor type (Cadaveric versus Living-related).

The formation of cytotoxic antibodies against the donor lymphocytes in the posttransplant period is shown to be closely related to rejection, but it is also reported that the sensitivity of the test is considered low and that its clinical use is limited, as the positivity of the test continues even after the rejection attack subsided (15-18). The clinical application of the test has become less frequent since 1980. As in most of the developed countries, the cadaveric donors are commonly used in many transplantation centres and so, obtaining donor-cells is no longer practical. Besides, more sensitive tests are being widely used to differentiate rejection from infection and cyclosporin toxicity. In our cases, the occurrence of graft loss in 5 of 7 patients in whom LCM was positive and only in 8 of the 88 patients who were negative, shows that this test may be an important predictor of the risk of graft loss. Since fine needle biopsy, serologic virological and flowcytomartic analysis techniques, which are
commonly used for differentiation of rejection from infection or drug nephrotoxicity, are not generally available in our country, we believe that the LCM test still has a value in the early diagnosis of rejection.

We cannot comment whether this finding is associated with the type of relation of the recipient to the donor or number of HLA mismatches, since the number of our LCM positive patients is low. But it is interesting that in all of the 19 patients who had an HLA mismatch of 0-2, LCM remained negative. There is general agreement that the presence of donor-directed IgM antibodies is not a contraindication for transplantation (2,3,8). DTT showed that antibodies were of IgM type. In only two of the patients. One of these patients lost the graft; the other did not. The limited number of patients hinder us from commenting on the importance of IgM type antibodies in renal transplantation but it does not seem to be a frequent event.

Our method only shows the cytotoxic antibodies capable of binding complement at a given concentration. Besides, we cannot deduce that the detected antibodies are directed to HLA antigens. They can be directed to other alloantigens on the surface of lymphocytes. However, in spite of all these limitations the test we applied was found to be useful in predicting the risk of graft loss. In conclusion, LCM is a cheap, practical and rapid (results can be obtained in 3-4 hours) method of determining the risk of rejection in our patients.

References