Lymphocyte-dependent Antibody and Human Chronic Renal Allograft Rejection

J THOMAS, F THOMAS, H M LEE
Transplant Immunology Laboratory, Medical College of Virginia,
Richmond, Virginia, USA

Summary
Specific anti-donor lymphocyte-dependent antibody (LDA) was found in the sera of 13 of 15 or 86% of patients having clinical chronic renal allograft rejection (CR). In 22 patients without CR, only 1 (4.5%) had demonstrable LDA activity (p < 0.005). In serial studies, LDA activity was found prior to the onset of clinical CR in 7 of 11 (64%) patients studied. These results suggest that LDA plays a causal role in clinical CR and that pre- and post-transplant LDA studies may be useful in identifying patients who are at high risk for development of CR.

Introduction
In the past, chronic renal allograft rejection (CR) has constituted a significant risk for graft loss, although early acute rejection was clearly responsible for the large share of immunologically induced graft loss. Recently, with the introduction of more effective early post-transplant immunosuppression using anti-thymocyte globulin (ATG), irreversible acute rejection early post-transplant occurs in less than 10% of recipients, and CR has, therefore, become the leading cause of allograft loss at all time periods post-transplant in our unit.

The immunobiology of chronic rejection is still poorly understood. Two groups (Jeannet et al, 1970, Pierce et al, 1975) have reported low levels of circulating IgG antibodies to kidney cells by sensitive mixed agglutination assays in patients with CR, and others have described deposits of immunoglobulins in the glomeruli of chronically rejected kidney allografts (Hume et al, 1970, Porter et al, 1968). These studies have implicated anti-donor humoral antibody...
in the aetiology of CR, but the precise relationship of circulating antibodies to this disease process remains elusive.

Recently, several investigators have studied the relationship of lymphocyte-dependent-antibody (LDA) to human disease processes and transplantation immunity (Schneider et al, 1975, D’Apice and Morris, 1974, Descamps et al, 1975a, Jeannet et al, 1975, McConnachie and Dossetor, 1973, Ting and Terasaki, 1974). Anti-donor LDA is detectable in a significant portion of the transplant population either before or after transplantation. Two reports have associated LDA with early acute rejection (Jeannet et al, 1975, Ting and Terasaki, 1974) although others have failed to demonstrate such an association. None of these reports have commented on the relationship of LDA to chronic rejection.

In this study, 37 transplant patients, 15 of whom had clinical CR, were studied for LDA activity. A close association of LDA activity to clinical CR was found and the results suggest a causal role of antibody dependent cell-mediated cytotoxicity in CR.

METHODS

A total of 37 recipients were tested for lymphocyte-dependent-antibody (LDA). Of these, 31 had HLA haplo-identical related donors, four had HLA identical related donors, and two had HLA non-identical cadaver donors. At the time of LDA testing, 35/37 had functioning renal allografts, and 2/37 had been returned to dialysis.

All of the recipients were transplanted and followed at the Medical College of Virginia, and were maintained on routine immunosuppressive (IS) therapy as previously described. Imuran and Prednisone were withdrawn for 24 hours prior to obtaining recipients’ blood samples for testing. Recipient serum was heat inactivated and dialysed against phosphate buffered saline before assay.

Lymphocyte-dependent-antibody (LDA) assays were performed according to the technique of Trinchieri et al (1973) with minor modifications as previously described (Thomas et al, 1976). Target cells were PHA-induced lymphoblasts tagged with $^{51}$Cr. In each experiment cells from the recipient, donor, and unrelated third and fourth parties were used as target cells. Effector cells were from healthy normal volunteers who did not possess the incompatible HLA donor antigens. Sera with known LDA activity were used as positive controls. In addition, recipient serum diluted in positive control serum was included as a control to detect possible false negative reactions due to inhibition of cytotoxic effector cell activity by recipient serum.

Isotope release was measured after four hours of incubation at 37°C and expressed as per cent lysis according to the formula (ER/SR)/(MR-SR) x 100

where ER, SR, and MR are experimental, spontaneous, and maximum release, respectively. The per cent specific lysis was calculated by subtracting the per
cent lysis obtained in serial dilutions of recipient serum in foetal calf serum. Specific lysis greater than 4% was considered positive.

RESULTS

Of 37 recipients tested, 14 or 38% had significant positive LDA at periods ranging from three months to 11 years post-transplant. The mean post-transplant follow-up at the time of testing was 5.8 ± 4.2 (SD) years in the LDA positive group and 5.5 ± 3.5 (SD) in the LDA negative group. The highest per cent specific lysis at any dilution was taken for analysis of the data. In the LDA negative group the mean per cent specific lysis of donor cells was 1.03% ± 1.3 (SD). In the LDA positive group the mean per cent specific lysis was significantly higher (p < 0.01) at 24.1 ± 14.8 (SD).

The LDA positive recipients were not distinguished from the LDA negative group in the number of mismatched HLA antigens or in the degree of MLC responsiveness (SI) to the specific donor. The mean SI in the LDA positive and negative groups was 9.4 ± 9.8 (SD) and 11.4 ± 7.4 (SD), respectively. The mean number of mismatched donor HLA (A + B) antigens per recipient was 1.3 ± .93 SD and 1.3 ± .77 (SD). In the LDA positive group 7% had no defined HLA A or B locus mismatch; 14% had one defined A locus mismatch; 50% had one defined B locus mismatch, and 29% had both an A and B locus mismatch. In the LDA negative group 17% had no defined A or B locus mismatch; 9% had a single defined A locus mismatch; 27% had a single defined B locus mismatch, and 47% had both an A and B locus mismatch.

The majority of positive sera demonstrated LDA against unrelated target cells sharing the mismatched donor HLA antigens, but cross reactions to non-HLA A and B locus antigens were evident in 28%. Positive LDA was present in the serum of one recipient with a four antigen matched sibling donor.

The presence of anti-donor LDA in the post-transplant period was found to be associated with clinical symptoms of chronic renal allograft rejection (Table 1). Of 14 LDA positive recipients, 13 had persistent proteinuria (>1 gram per 24 hours) at the time of testing. In contrast only two of 23 LDA negative recipients had proteinuria. Mean serum creatinine and creatinine clearance values were also significantly different in the LDA positive and negative groups. Of the 13 LDA positive patients with clinical CR symptoms, seven have had transplant biopsies or nephrectomy (two cases), and all seven have pathologically documented CR.

TABLE 1 Post-Transplant Anti-donor LDA and Chronic Renal Allograft Rejection

<table>
<thead>
<tr>
<th>Urinary Protein (grams/24 hrs)</th>
<th>LDA Positive Group</th>
<th>LDA Negative Group</th>
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<tbody>
<tr>
<td></td>
<td>2.4 ± 1.6</td>
<td>p &lt; 0.005</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min)</td>
<td>34.5 ± 21</td>
<td>p &lt; 0.005</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>2.9 ± 1.2</td>
<td>p &lt; 0.005</td>
</tr>
</tbody>
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Data is expressed as mean ± standard deviations. P values were calculated by Wilcoxon Rank Sum Test.
Of interest was the finding that of 11 patients whose early post-transplant frozen sera were available for retrospective LDA testing, 7 (64%) had positive anti-donor LDA three months to five years prior to symptoms of deteriorating renal allograft function. In the four other recipients tested retrospectively, the earliest available LDA positive samples were coincident with proteinuria in the clinical course. In eight LDA positive recipients whose pre-transplant sera were available, four were positive.

DISCUSSION

In this study, LDA activity was found in 86% of renal transplant recipients with clinical CR whereas only 5% of recipients without CR had LDA (p<0.005). Furthermore, serial retrospective studies of LDA activity in 11 patients showed that 64% had LDA activity demonstrable in their serum three months to six years prior to the onset clinical of CR. All four patients determined to have LDA activity pre-transplant developed CR. Histological confirmation of the clinical diagnosis of CR was obtained in all seven patients in whom a renal biopsy or transplant nephrectomy was performed. The results obtained in this study suggest that LDA activity may be a causal factor in clinical CR.

The LDA reaction or antibody-dependent cell-mediated cytotoxicity first described by Moller (1965), and studied more recently by Perlmann and Perlmann (1970), MacLennan (1972), Kovithavongs and Dossetor (1975) and numerous other investigators, is an interesting immunological mechanism from a variety of viewpoints. It represents an interface of humoral and cell-mediated immunity, and the resulting complement-independent cytolysis of specific target cells can be considered a product of synergistic activity of these two components of the immune system. LDA can sensitize target cells to become lysed by Fc receptor bearing effector cells at very low antiserum concentrations. This might explain why chronic kidney allograft damage occurs with low levels of anti-donor antibody in the serum of patients with CR (Jeannet et al, 1970, Pierce et al, 1975). If LDA were in fact a principal mechanism of CR, one could also explain operative cytolysis despite failure to detect antibody by less sensitive, conventional, complement-dependent cytolysis techniques.

The specificity of the LDA antibody would also appear to be of some significance for future studies. Kovithavongs and Dossetor (1975) have offered evidence that the LDA antibody can have non-HLA A and B locus specificity. In this study, LDA activity was demonstrable in one patient who had CR and was HLA identical with the donor. Moreover, in 28% of sera tested, there were cross reactions to target cells not sharing the mismatched donor antigens. Absorption studies are necessary to define the specificity of LDA antibodies in patients with CR, and such studies are currently in progress.

Questions concerning the clinical significance of LDA crossmatching pre-transplant have been raised in recent studies. Some investigators have associated
pre-transplant LDA and early post-transplant LDA with severe, early acute rejections (Jeannet et al, 1975, Ting and Terasaki, 1974). Others, however, have not observed such an association (D’Apice and Morris, 1974, Descamps et al, 1975a). Chronic rejection was not discussed in these reports. In the present study all four patients with pre-transplant LDA activity ultimately developed CR, although the clinical onset varied from months to years post-transplant. This data suggests that a positive LDA assay pre-transplant may constitute a relative contra-indication to transplantation. This area of investigation would appear to be of importance in current clinical transplant management, and a larger data base is needed.

It would also be important better to understand the capability of the clinical immunosuppressive agents to modulate the cellular effectors of antibody-dependent cellular cytotoxicity. MacLennan’s data (1972) suggests that chronic Azathioprine therapy can depress K cell effectors, and Descamps et al, (1975b) have offered evidence that Prednisone is an effective suppressor of K cell activity in man. Few studies of other agents are available. One of our patients with LDA and CR was changed from Azathioprine to Cytoxan with a resultant fall in levels of K cell cytotoxicity (measured by the chicken red cell assay) to about 50% of previous levels, although her relentless CR course was not modified. This suggests to us, however, that immunosuppressive agents other than Prednisone or Azathioprine may be useful for pharmacological suppression of the cellular effectors of LDA activity.

A better concept of the validity of the putative association of LDA activity and clinical CR will hopefully develop from multi-centre studies. The clinical importance of CR suggests the appropriateness of such studies, and the results presented here provide a rationale for further study of this hypothesis.

Acknowledgment

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Open Discussion

AHLMEN (Göthenburg) From this impressive work, I would very much like to ask you whether you have used the theoretically possible suppressor cell activity in any prospective clinical way?

THOMAS, F I think that is a very critical question to which we must address ourselves. Before we do, however, we have to accept that we must make a clear distinction between early post transplant rejection activity and late post transplant rejection activity in relation to the T cell. Evidence from our laboratory which we first presented about two years ago and which is now being reproduced by a number of other groups in our Country indicates very clearly that the primary actor in the acute rejection scenario is the T cell, early post-transplant. Therefore we feel that activating the T cell would lead in most cases to a very disastrous result in the early post transplant period, and we feel that suppression of the T cell at this time is the most appropriate therapy. In a later post transplant period, I think it might be appropriate to try to selectively activate the suppressor T cell. But I think we have to be very careful that we are not talking about apples and oranges, which often happens in these discussions. There is a clear distinction between early and late post transplant rejection activity.

CASTRO (London) I would like to comment on your studies on serum blocking of MLC. We recently carried out a survey of blocking in 20 patients who had kidney allografts surviving for two years or more. In fact we found neither specific nor non-specific blocking in any of these patients, findings which agreed with the published ones of DeBray-Sachs. Furthermore, prospective studies in our patients on haemodialysis showed that those patients exhibiting non-specific blocking had a poor survival of kidney transplants.

I would like to ask what your criteria of blocking in MLC were and how you explain the difference between our results and yours.
THOMAS, F  Yes, I think this is also a very important question. Our criteria for blocking in the MLC was a statistically significant fall in MLC reactivity. Therefore, the biological significance of this in vitro finding is questionable. Furthermore, as I mentioned in our paper, (and I think this is perhaps the most important message of the paper today) we do not feel at the present time that these blocking factors are an adequate explanation for long-term graft survival. We think that perhaps there are other factors which are at least as important as blocking factors and perhaps even more important. In summary, we do not know the role of serum blocking factors, and we feel that there is certainly a possibility that they have been highly overrated as factors in long-term allograft survival in non-identical patients.

CHAIRMAN One more short question specifically researched by a Turkish colleague.

HABERAL (Ankara) Are you monitoring LDA during the period of rejection and after the period of rejection? Do you think LDA is useful for monitoring the treatment of rejection?

THOMAS, J We found no correlation between LDA activity and acute rejection although a number of patients with acute rejection did have LDA activity. I think the second part of your question is of critical importance to us as clinicians, because we need to know more about the pharmacological modulation of this LDA activity. We need to know how we can decrease or prevent it. We have done some preliminary studies and there is some suggestion, for example, that Cytoxan is a better agent for regulating K cell activity, if indeed this is the effector cell. There is some evidence that the effector cell may be the B cell or macrophage. But the K cell activity appears to be regulated better by Cytoxan than Imuran. We have not found a correlation with high doses of steroids, and decreased LDA activity has been reported by Dr Hamburger's group, but this also needs to be followed up further.