CELL-MEDIATED IMMUNITY DURING RDT AND THE OUTCOME OF TRANSPLANTATION

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Summary

Previous studies have demonstrated depression of cell-mediated immunity (CMI) in uraemia. We have measured CMI in a group of 248 patients on regular dialysis using a quantitative dinitrochlorobenzene (DNCB) skin test which gives a score of 0 to 15. Ninety-eight of these patients subsequently had first cadaver transplants and the relationship between the DNCB score and graft survival has been examined. Graft survival was found to decline progressively with higher DNCB scores. Blood transfusion also had a major influence on graft survival and the relation between the DNCB score and outcome was observed in all the blood transfusion groups.

Introduction

There is ample evidence that cell-mediated immunity (CMI) is depressed in renal failure using both in vivo [1] and in vitro [2] tests. The test we have used utilises dinitrochlorobenzene (DNCB) applied to the skin and it has been modified to give a quantitative result. We described previously the use of the DNCB skin test to examine the hypothesis that the degree of depression of CMI at the time of transplantation might influence the subsequent incidence of rejection [3]. Such an influence was observed. The purpose of the present study was to extend the previous observations and to examine some of the factors which might determine the degree of depression of CMI as shown by the strength of the DNCB reaction.

Patients and methods

Two hundred and forty eight patients on regular haemodialysis had their skin reaction to DNCB measured. There were 138 males and 110 females with an age range of 14 to 63 years. There was a wide range of underlying renal diseases and all patients were dialysed for four to six hours thrice weekly. Ninety-eight of the patients subsequently received a first cadaver allograft and of this group all but
thirteen received blood transfusion before the transplant. A variety of blood preparations were used namely whole blood, packed cells and/or frozen, thawed red cells. There was a change in blood transfusion policy during the study in that prior to May 1979 blood was given only for clinical reasons and in some cases frozen, thawed cells were used. Subsequent to this date all patients received a minimum of five units of packed cells and the use of frozen, thawed cells was discontinued.

In the DNCB test the initial sensitising dose of 2000μg dissolved in 0.1ml acetone was applied to an area of the forearm 2.5cm in diameter. Fourteen days later, the patient was tested with five doses of DNCB (30, 15, 7.5, 3.7 and 1.8μg), the DNCB being dried onto 1cm diameter felt pads (A1 test patches, Astra Chemicals, Watford, UK) which were applied for 48 hours. The reaction was scored at the end of the 48 hours thus: 0, no reaction or erythema only; 1, erythema and induration confined to the patch; 2, erythema and induration extending beyond the patch; 3, as for 2 plus blistering. The DNCB score recorded was the sum of the scores for each of the five patches, and thus could range from 0 to 15. The test was also done in 15 healthy controls. The DNCB score of the patients undergoing transplantation was not made known to the doctors looking after them. The patients were retrospectively divided into five groups depending on the time of initial sensitisation (as shown in Table II).

Results

The DNCB scores in the 15 healthy subjects ranged from 4 to 14 with a mean of 9.1. By contrast only 72 of the 248 dialysis patients (29 per cent) had scores of four or more and these were arbitrarily classified as strong reactors. Table I shows the DNCB scores in males and females and in five age ranges. A larger percentage

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of patients</th>
<th>Mean DNCB score</th>
<th>Strong reactors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>10–20</td>
<td>8</td>
<td>10</td>
<td>3.75</td>
</tr>
<tr>
<td>21–30</td>
<td>39</td>
<td>27</td>
<td>3.28</td>
</tr>
<tr>
<td>31–40</td>
<td>36</td>
<td>21</td>
<td>3.13</td>
</tr>
<tr>
<td>41–50</td>
<td>36</td>
<td>24</td>
<td>3.16</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>19</td>
<td>28</td>
<td>3.10</td>
</tr>
<tr>
<td>Total</td>
<td>138</td>
<td>110</td>
<td>3.21</td>
</tr>
</tbody>
</table>

of males (33.3 per cent) were strong reactors than females (23.6 per cent) (p < 0.05), while no differences were seen between the age groups. There were, however, few patients over the age of 55 years. Table II shows the relationship between the DNCB score and time of application of the sensitising dose. The
TABLE II. Relation of DNCB score to time of sensitisation after commencement of haemodialysis

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of sensitisation after start of dialysis (months)</th>
<th>Number of patients</th>
<th>Mean age</th>
<th>Sex % of males</th>
<th>Strong reactors Number</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Before</td>
<td>34</td>
<td>39</td>
<td>47</td>
<td>15</td>
<td>42.9</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>124</td>
<td>40</td>
<td>52</td>
<td>41</td>
<td>33.0</td>
</tr>
<tr>
<td>III</td>
<td>7–12</td>
<td>20</td>
<td>35</td>
<td>70</td>
<td>7</td>
<td>35.0</td>
</tr>
<tr>
<td>IV</td>
<td>13–24</td>
<td>33</td>
<td>39</td>
<td>52</td>
<td>7</td>
<td>21.2</td>
</tr>
<tr>
<td>V</td>
<td>&gt; 24</td>
<td>37</td>
<td>36</td>
<td>49</td>
<td>2</td>
<td>5.4</td>
</tr>
</tbody>
</table>

longer the interval from the start of dialysis, the lower was the mean score and this could not be explained by any difference in the sex ratio. However once the sensitising dose had been applied, repeated challenging doses produced very consistent responses in individual patients in almost all cases.

Of the 98 patients who subsequently received first cadaver allografts 27 were strong reactors (28 per cent). Of these, 11 had functioning grafts at six months (41 per cent) compared with 52 of the 71 weak reactors (73 per cent). In addition to this difference in graft survival between strong and weak reactors, there was noted to be a stepwise decline in graft survival with rising DNCB scores. Six month graft survival falls from 71 per cent in the patients with scores of 0 to only 10 per cent for the 10 patients with scores of more than 7.

The effect of pre-transplant blood transfusion is shown in Table III with a rise in six month graft survival from 31 per cent in non-transfused patients to 59 per cent in those given frozen cells only and to 75 per cent in those given whole blood or packed cells. When the patients are also divided into strong and weak reactors, this transfusion effect would still seem to occur although the number of patients in the non-transfused groups are too small for the differences to be significant.

TABLE III. Relation of DNCB score and pre-transplant blood transfusion to renal allograft survival

<table>
<thead>
<tr>
<th>Pre-transplant blood transfusion</th>
<th>Six month graft survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All patients</td>
</tr>
<tr>
<td>No blood (13)</td>
<td>4 of 13</td>
</tr>
<tr>
<td></td>
<td>31%</td>
</tr>
<tr>
<td>Frozen, thawed cells only (32)</td>
<td>19 of 32</td>
</tr>
<tr>
<td></td>
<td>59%</td>
</tr>
<tr>
<td>Whole blood or packed cells (53)</td>
<td>40 of 53</td>
</tr>
<tr>
<td></td>
<td>75%</td>
</tr>
</tbody>
</table>

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Discussion

Further experience has supported our previous findings [3] that our modification of the DNCB skin test is simple and safe and has the advantage over previous methods of giving a quantitative result. Also DNCB has the advantage over other antigens such as tuberculin and candida that it is a new antigen and therefore the first test does not measure the patient's immunological memory.

In 1977, Rolly showed a correlation between the DNCB skin reaction and the outcome of transplantation [4] and our results supported this [3]. What both studies showed was that the patients with more severely depressed CMI had better graft survival. Extended study shows that this is a graded phenomenon with a steady fall in graft survival as the DNCB score rises [5]. One in 10 of our patients had a score of more than 7 and their six month graft survival was only 10 per cent compared with 71 per cent for patients with scores of 0.

There is overwhelming evidence that pre-transplant blood transfusion has a beneficial effect on graft survival. Most of the earlier analyses were retrospective but more recently prospective studies have provided even stronger evidence [6]. Although transplant outcome in our patients was clearly influenced by blood transfusion, the figures in Table III suggest that this effect was independent of the influence of the CMI response. While the numbers in some of the groups were small, graft survival was considerably higher among the weak reactors within all three blood transfusion groups [7].

The goal of successful transplantation for all these patients will depend, among other things, on finding ways of modifying the CMI response in the strong reactors to DNCB, as conventional immunosuppression has failed to do this. Thus it is important that we gain greater understanding of the factors which control the CMI response in uraemic patients, not only for transplantation, but also in explaining infection during regular dialysis treatment. The present study shows less depression in males than females though the difference is not striking. Although the strength of CMI response is known to fall in old age, no differences were demonstrated in the age ranges studied, i.e. those within which transplantation is usually performed. One other observation made in the present study was a fall in the strength of CMI reaction with increasing time on dialysis. When sensitised within the first six months of dialysis, 33 per cent of patients were strong reactors compared with only 5 per cent in patients sensitised more than two years after starting treatment. A similar observation was made by Triolo et al [8]. While dialysis itself could have an immunosuppressive effect, a more likely explanation would be the duration of uraemia. Further investigation of factors controlling the CMI response are clearly required, and DNBC sensitisation should be carried out early after starting the dialysis treatment.

One final point concerns the site at which the CMI response is depressed. We have observed that once the patient is sensitised, the DNBC score usually varies little over periods of several years, but that increasing duration of renal failure before initial sensitisation is associated with a lower response. This would suggest that the depressant effect of uraemia is on the afferent limb of the CMI response.
Open Discussion

JEEKEL (Rotterdam) Did you test with DNCB before and after blood transfusion?

HAMILTON We have found that there was no change in the test score. However, this does not mean that cell-mediated immunity has not changed, as we found in dialysis patients. With a second or a third DNCB test the patient is responding as to a memory or recall antigen. What we need would be a new antigen to use after blood transfusion. So, although the DNCB test score doesn’t change it may be that there has been a change in CMI after transfusion.

GUTTMAN (Montreal) I think you are aware of our paper in press which will be out next month in Kidney International. We have been looking at the late hypersensitivity responses before and after transplantation and certainly find an association with the time on dialysis and the age of the patient as to whether or not they are anergic prior to transplantation. We have some slight difference in our series in that the anergic state is far more predictive of infectious complications, and later on seems to be associated with malignancy more than with rejection per se. Your system seems to be more definitive for predicting a strong allograft response. The comment or question I would like to pose is that some people have said that there are a number of chemicals now in the environment cross-reacting with DNCB and one may actually be sensitising patients for some allergic reactions and I am wondering if you have seen any in your patient group?

HAMILTON No, we have had no trouble yet with sensitised patients. Fortunately, the majority of dialysis patients are such weak responders that even if they met such a cross-reacting antigen later in life they would not get a serious reaction. As far as infection goes, we have seen infection in the non-reactors although it has not been a major clinical problem: their transplants go well. One odd thing we have noticed, however, is that once a kidney is removed from the high reactor patients they have had more morbidity than the non-reactor group. It may be that the reactors have had a lot of steroid pulses, but at the same time we must keep a watch for paradoxes here, since there may be different components of the immune response, only one of which has been tested by DNCB.
JIRKA (Prague) Did you see any differences between different dialysis centres as far as the proportion between strong reacting and weakly reacting individuals was concerned? I am asking because in a similar study started a year ago comprising about 200 patients from 8 dialysis centres we found staggering differences between centres from less than 20% of DNCB reactors to more than 70%. We haven’t found any explanation but maybe it may have something in common with transfusion policy, because in those centres with a high proportion of strong reactors mostly leucocyte-free red cells were given while in centres with a low proportion of these strong reactors either whole or leucocyte-poor blood transfusions were given.

HAMILTON There may be a number of simple explanations of that. The most interesting one is that you have discovered what the controlling mechanism for DNCB reactivity is. But there are technical reasons why you may have found a variation between centres. First, all the testing must be done by one person. It is a rather subjective test to score and hence it must all be done by one person. The little nuances of the test are important, e.g. when the reading is made after removing the patch etc. The second possibility is that your different dialysis centres may have a different mix of dialysis patients who have been transplanted and returned to dialysis. This group would have more high reactors in it. And then the third thing is that the length of time on dialysis clearly plays a role, and some of the confusion in the literature may relate to how long the patients have been on dialysis. And if we could standardise the time of antigen testing on dialysis, perhaps there would be more agreement on the proportion of reactors and non-reactors. I hope this phrase ‘reactor’ and ‘non-reactor’ won’t become misused in the literature as meaning all those with some responses as against those with no responses. The concept should be used for patients above and below your one third to one quarter cut-off point.

JIRKA Well this thing was done by one colleague. No patient had been transplanted beforehand, and we found no differences as far as the length of dialysis was concerned.

HAMILTON You may have inadvertently tumbled on the controlling mechanism for this test: comparison of the dialysis schedules and methods may be interesting.

TRAEGEER (Co-Chairman) When we were looking at skin tests in our patients, we were able to show a big difference between patients treated by haemodialysis and peritoneal dialysis. Did you see this difference?

HAMILTON We have found no difference so far between peritoneal dialysis and haemodialysis patients but we were expecting to find such a difference. First, as this conference has shown, the bone marrow responses of patients on peritoneal dialysis are better, and they run a higher haemoglobin value. Our prediction was that lymphocyte reactivity would be better hence DNCB reactivity would have been higher. That might have the consequence that these patients were more at
risk for transplant rejection. Of course, comparing haemodialysis and peritoneal
dialysis patients involves careful matching of age and the original diseases. Thus,
there are more GN patients on PD, and they are older.

KERR (Newcastle) My colleagues have been slightly reluctant to adopt this test
because of an ill-formulated fear that by sensitising patients to DNBC there is a
risk that you might stimulate non-specifically their cell-mediated immunity. Is
there any experimental work that would reassure them?

HAMILTON The reassurance really comes from cancer immunotherapy. When
BCG, DNBC and other agents are used in cancer immunotherapy each dose gives
a short-lasting, non-specific boost lasting a week or a fortnight. There would be
slight cause for concern if you had sensitised the patient within a week or two
of transplant but I think outside that range the non-specific boost, if any, will
have settled.

WINNEY (Edinburgh) You have shown that as a group there is a relationship
between time on dialysis and responsiveness to DNBC, suggesting that patients
who are strong reactors may be untransplantable. Have you or anybody followed
individual patients for a period of time on dialysis with serial testing and does it
change with time on dialysis in individual patients? That might give hope for
some of the strong reactors who are tested early on.

HAMILTON Strong reactors keep the same test score for years on dialysis. If
you have a score of 8 it will continue within the range 7 to 9 and that misled us
into thinking that CMI was not changing. When we looked at the patients who had
been on dialysis for a while and only then were sensitised for the first time, we
agreed with the Portsmouth group’s report that CMI comes down. There is a
crucial difference between the response to a neo-antigen like the first testing
with DNBC and the repeat tests when you are using it like a recall antigen. Then
the score does not change.

TRAEGGER Well a last comment about your conclusion. Rather than say that
the strong reactors are not transplantable, I would say that in these patients
you may need to add another immunosuppressant; more than usual, that is to
say cyclosporin A or anti-lymphocytic serum.

HAMILTON Yes, I will modify my remarks to agree with you that reactor
patients may need a special regimen of extra immunosuppression after trans-
plantation.