TRANSFUSION-INDUCED ANERGY: SKIN TEST AS AN INDEX FOR PRETRANSPLANT TRANSFUSIONS

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Summary

Cell-mediated immunity in vivo was studied by delayed cutaneous hypersensitivity (DCH) to seven antigens in 156 chronic haemodialysis (HD) patients, using a disposable multipuncture device. Anergy was found in 46.8 per cent of patients, and a positive correlation was seen between anergy and female sex, time on HD, glomerulonephritis as primary renal disease, younger age and previous blood transfusions (BT).

The effect of BT on DCH was studied prospectively in 29 responsive patients. A significant decrease in DCH response was seen. The transfusion-induced anergy remained for a variable time. The pretransplant BT policy suggested by our data would be to periodically undertake skin tests and to transfuse only responsive patients, thereby avoiding the adverse effects of multiple BT.

Introduction

Depression of cell-mediated immunity in chronic renal failure (CRF) is well known. By studying delayed cutaneous hypersensitivity (DCH) to different antigens previous studies have demonstrated that reactions are weaker and less frequent in uraemic patients [1].

Several authors have shown a relationship between the outcome of renal transplantation and the degree of depression of cell-mediated immunity measured by DCH to dinitrochlorobenzene (DNCB) [2–5].

In addition, the beneficial effect of pretransplant blood transfusions (BT) on kidney graft survival is well documented [6], but the possible influence of BT on DCH has not been reported.

The purpose of the present study is to evaluate the response to DCH recall antigens in a population of CRF patients using an easy and reproducible test, attempting to define those factors which might determine the depression of cell-mediated immunity and also its spontaneous evolution. Finally, the effect of BT on DCH was studied prospectively.
Patients and methods

A group of 156 stable chronic haemodialysis (HD) patients were studied. There were 86 males and 70 females with an age range from 12–70 years. All patients were dialysed for four to five hours three times weekly.

In those patients and in 51 healthy controls we tested DCH to seven recall antigens using a disposable plastic device containing a 70 per cent glycerinated solution of the antigens ready to use by multipuncture application (Multitest®).

The antigens were tetanus (550,000 Mérieux units/ml), diphtheria (1,100,000 Mérieux units/ml), streptococcus (2,000 Mérieux units/ml), tuberculin (300,000 IU/ml), candida (2,000 Mérieux units/ml), tricophyton (150 Mérieux units/ml) and proteus (150 Mérieux units/ml), while a 70 per cent glycérin dilution was provided as a negative control.

Multipuncture was performed in an area of the forearm and the reaction (diameter of induration to each antigen) was measured at 48 hours, by the same person, considering positive 2mm or more of induration, and total score the sum of positive reactions in millimetres.

According to the score patients were classified: anergic (less than 5mm); responsive (more than 10mm); and intermediate (between 5 and 10mm).

The results of the skin tests were correlated with several variables including age, sex, time on HD, primary renal disease, previous BT, ABO blood group, HLA tissue typing, haematocrit, total serum proteins, serum albumin and transferrin, HBs antigen, HBs and HBC antibodies, lymphocytotoxic antibodies and previous maximum lymphocytotoxic antibody titre.

To study the effect of BT on DCH, 29 responsive patients received one unit of packed red cells and skin tests were carried out again after one month. Seventeen of these patients were also tested six and 12 months after BT. Five patients received several BT (2–5 units) and skin tests were repeated after each BT.

A control group of 19 responsive patients did not receive BT and were followed up for a six month period.

The spontaneous evolution of DCH during six and 12 months was studied in a group of 40 patients with different scores (anergic, responsive and intermediate) that did not receive any BT.

During the study, 16 tested patients were grafted from cadaver donors, and the outcome was evaluated in relation with the score. Seventeen patients previously grafted were also tested at different times after transplantation.

Statistical analysis was by the $\chi^2$ test, paired and unpaired t test and Dunnet test.

Results

Table I shows the distribution of healthy controls and patients according to the DCH reaction, and its relationship to patient’s sex, age, primary renal disease, time on HD and previous BT.
<table>
<thead>
<tr>
<th></th>
<th>Healthy controls</th>
<th></th>
<th>Chronic renal failure patients</th>
<th></th>
<th>Time on HD</th>
<th>Previous BT</th>
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<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Sex M F</td>
<td>Age (years) M F</td>
<td>Primary renal disease M F</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;40  &gt;40</td>
<td>GN PN CRD RVD AU</td>
<td>&lt;1 year</td>
<td>&gt;1 year</td>
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<tr>
<td><strong>Anergic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Yes No</td>
</tr>
<tr>
<td>Number (%)</td>
<td>3 (5.8)</td>
<td>73 (46.8)</td>
<td>30 (34.8) 43 (61.4)</td>
<td>38 (60.3) 37 (39.7)</td>
<td>33 (67.3)</td>
<td>23 (43.3)</td>
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<td></td>
<td></td>
<td>23 (46.8)</td>
<td>30 (34.8) 43 (61.4)</td>
<td>38 (60.3) 37 (39.7)</td>
<td>33 (67.3)</td>
<td>23 (43.3)</td>
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<tr>
<td>Intermediate</td>
<td>9 (17.8)</td>
<td>32 (20.5)</td>
<td>18 (21) 14 (20)</td>
<td>11 (17.4) 20 (21.5)</td>
<td>10 (20.5)</td>
<td>4 (11.4)</td>
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<td>Number (%)</td>
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<td></td>
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<td>19 (16.5)</td>
<td>13 (31.7)</td>
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</tr>
<tr>
<td>Responsive</td>
<td>39 (76.4)</td>
<td>51 (32.7)</td>
<td>38 (44.2) 13 (18.6)</td>
<td>14 (22.3) 36 (38.8)</td>
<td>6 (12.2)</td>
<td>22 (45.3)</td>
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<tr>
<td>Number (%)</td>
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<tr>
<td></td>
<td></td>
<td>24 (25.3)</td>
<td>18 (44)</td>
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<tr>
<td><strong>TOTAL</strong></td>
<td>51 (100)</td>
<td>156 (30.6)</td>
<td>86 (16.9) 70 (13.9)</td>
<td>63 (12.6) 93 (18.7)</td>
<td>49 (9.8)</td>
<td>53 (10.6)</td>
</tr>
</tbody>
</table>

p < 0.0001  p < 0.01  p < 0.05  p < 0.01  p < 0.05  p < 0.01
GN Glomerulonephritis  
PN Pyelonephritis  
CRD Congenital renal disease  
RVD Renal vascular disease  
AU Aetiology uncertain  
HD Haemodialysis  
BT Blood transfusions
Normal controls

Only three (5.8%) controls were anergic, whereas 39 (76.4%) were responsive and nine (17.8%) intermediate. All the male controls were responsive, but only 63.6 per cent of females.

CRF patients

Seventy-three patients were anergic (46.8%), 51 responsive (32.7%) and 32 intermediate (20.5%) (p<0.0001 compared to the normal controls).

The mean number of positive antigens was 3.2 ± 0.06 in the responsive patients, and 0.3 ± 0.06 (X ± SEM) in the anergic patients (p<0.0005).

Anergy was more frequent in female (61.4%) than in male patients (34.8%) (p<0.01), and in patients younger than 40 years (p<0.05). A higher incidence of anergy was also found in relation with glomerulonephritis as primary renal disease (p<0.01) and time on HD. The mean score decreases as time on HD increases (Figure 1), and there was statistical difference in the score of patients on HD during more than one year (p<0.05).

![Graph showing relationship between time on haemodialysis and score.](image)

**Figure 1.** Score (X ± SEM) according to time on haemodialysis

Previous BT

Anergy was significantly more frequent in previously transfused than in non-transfused patients (p<0.01). Otherwise, the mean number of blood units
received by all the anergic patients was greater ($\bar{X}: 7.5 \pm 1.1$) than that received by responsive ones ($\bar{X}: 3.4 \pm 0.6, \bar{X} \pm \text{SEM}$) ($p<0.005$).

Figure 2 demonstrates the relationship between score and time since previous transfusion. Mean score was lower in patients who received their last transfusion within four months before test. When skin tests were done after five or more months from the last transfusion the scores were higher and with greater variation.

![Graph showing the relation between score and time since last transfusion](image)

**TIME SINCE LAST TRANSFUSION (months)**

Figure 2. Relation between score ($\bar{X} \pm \text{SEM}$) and time since last transfusion

**Other parameters**

There was no significant correlation between anergy and ABO blood group, HLA tissue typing (A, B or DR), haematocrit, total serum proteins, serum albumin and transferrin, HBs and HBe antibodies, and HBs antigen, although nine out of 15 (60 per cent) Hbs positive antigen patients (carriers) were anergic.

There was no difference in the lymphocytotoxic antibody value, but the maximum previous value was higher in anergic than in responsive patients ($17.3 \pm 3.9\%$ versus $4.4 \pm 1.9\%$ ($\bar{X} \pm \text{SEM}$) $p<0.01$).
Effect of BT on DCH

Figure 3 shows the decrease of the score in all responsive patients that prospectively received one unit of packed red cells. Mean score fell from 17.3 ± 1.3mm before BT to 5.2 ± 0.6mm (X ± SEM) one month later (p<0.0001). Sixteen became anergic, 10 intermediate and three remained responsive, but showing a marked decrease in the score.

![Graph A](image)

![Graph B](image)

![Graph C](image)

Figure 3. Effect of one unit of packed red cells on the score. A: decrease at one month in 29 responsive patients (p<0.0001). B and C: follow-up at one, six and 12 months after transfusion in 17 responsive patients (B: X ± SEM. Open circles: control group of 19 non-transfused responsive patients)

Follow-up at six and 12 months showed an increase in the mean score, although some patients remained anergic at one year, while others recovered a responsive state at six months.
No change in the score was observed in the control group of 19 non-transfused responsive patients at six months (Figure 3B). Patients who received repeated BT showed a different decrease in the score after each BT.

**Spontaneous evolution of DCH**

The follow-up at six and 12 months of DCH tests in 40 non-transfused patients did not show any significant change in the score. The initial mean score was 5.4 ± 1mm, at six months 5.5 ± 0.7mm and at 12 months 4.4 ± 1.1mm (X ± SEM).

**Kidney graft survival**

Sixteen patients were grafted from cadaver donors during the time of the study. Ten were anergic and none of them lost the kidney by rejection at six months. However, two out of six responsive patients lost the graft because of irreversible acute rejection at one and two months (p not significant).

It was impossible to analyse the relation of BT and DCH on kidney graft survival because of the small number of patients.

**DCH in transplanted patients**

In 17 previously transplanted patients (one month to four years before) skin tests were performed. Thirteen were anergic (76.4%), three intermediate with scores lower than 8mm, and only one was responsive (12mm). There was no relationship between the score and the time since transplantation or prednisone and azathioprine doses.

**Adverse reactions**

No local or general adverse reactions were seen in any of the cases.

**Discussion**

Skin tests are a good method of measuring in vivo cell-mediated immunity. The results of our work demonstrate a high incidence of anergy in a wide population of uraemic patients, as in several previous reports[2–5], but in contrast to an incidence of 14 per cent described by Guttman [7].

The main factors related to the anergic state were in our experience female sex, time on HD, glomerulonephritis as primary renal disease, younger age and previous BT. Higher incidence of anergy in female sex has also been reported in normal controls [8] and CRF patients [1, 5, 7]. Time on HD increases the incidence of anergy as has been shown by Watson et al [5] and Guttman et al [7]. The latter author pointed out the association between anergy and age in contrast with our data.

The relationship of anergy and previous BT has been stressed by Watson et al [4] in a retrospective analysis. We have confirmed this observation in retrospect.
and this fact prompted us to make a prospective study of the effect of BT on DCH.

All our patients were in a stable clinical situation, and no correlation was seen between anergy and haematocrit or serum proteins. We have not studied DCH in malnourished patients, a situation which probably may affect DCH. No relation was seen with ABO groups, HLA tissue typing and HB markers, although anergy is predominant in HBs antigen carriers.

A higher maximum lymphocytotoxic antibody activity observed in anergic patients suggests a relationship with the greater number of BT received by these patients.

The multipuncture test used in this work has been shown to be a simple, safe and reproducible method. Non-transfused patients keep the same score up to one year and no adverse reactions were seen.

Some authors emphasise the advantage of DNCB over other antigens because it is a new antigen and the test does not measure the patient's immunological memory [4, 5]. However, some severe adverse reactions have been reported [9].

The multipuncture test used in this study has a wide number of antigens, is easier to use and more reliable than intradermal tests.

The main conclusion of our work is that DCH can be modified by transfusions, and responsive patients converted to anergic. This means that in a good nutritional state DCH is not a stable parameter. Immunosuppression depresses DCH also, as may be suggested from our data.

The change in the immune response induced by BT could be in relation to the beneficial effect of pretransplant BT on renal allograft survival. Weak response to skin tests is correlated with the outcome of kidney transplantation as shown by different authors [2–5], and our results are suggestive in this sense.

Our data demonstrate that transfusions induce anergy, but a high number of cases are necessary to differentiate the effect of spontaneous and transfusion-induced anergy on graft survival. However, the results reported by Watson et al [5] suggest that the beneficial effect of BT is independent of the influence of the anergic state on the outcome of renal transplantation.

In this way we conclude that DCH is a good index for transfusion requirements in patients awaiting kidney transplantation. The controversy concerning the optimum number of BT and the optimum interval between BT and transplantation may be explained by our results. Transfusion-induced anergy persists during a variable time, and some patients convert to a responsive state early while others remain anergic one year after BT. According to that, the pretransplant BT policy would be to perform DCH tests periodically and to transfuse only to responsive patients. This may avoid the adverse effects of BT in CRF patients [10].

References

2 Rolley RT, Sterioff S, Parks LC et al. Transplant Proc 1977; 9: 81
3 Diamondopoulos AA, Hamilton DNH, Briggs JD. Proc EDTA 1978; 15: 283
4 Watson MA, Briggs JD, Diamondopoulos AA et al. Lancet 1979; i: 1323
Open Discussion

HAMILTON (Glasgow) We have extensive experience of the dinitrochlorobenzene skin test in the same situation as you have described. In our data from the year before deliberate blood transfusion studying the effect of blood given on clinical grounds, we also noticed the transfused patients had a lower DNCB skin reactivity which we, in fact, interpreted very cautiously as being some common effect on the marrow. I congratulate you on your bold alternative explanation of your findings. Since Professor Terasaki is the Chairman my question is, did your reactive patients show any increased incidence of antibody formation during the deliberate blood transfusion?

VALDERRÁBANO No, we have seen no correlation between cytotoxic antibody titre and the skin test, but analysing the highest titre of cytotoxic antibody we have seen that in anergic patients it is higher than responsive patients, but we feel that this is related to the greater number of transfusions received by anergic patients.

TERASAKI (Chairman) Following up on that question, you mention that you had six patients that were responsive, were those six patients transfused?

VALDERRÁBANO Well some of these patients were previously transfused and others not. The number of transplanted patients was small, only 16 and really it is impossible to differentiate the effect of spontaneous and transfusion-induced anergy. I feel that we need a larger number of patients to differentiate this effect.

TERASAKI Yes but I was wondering if these patients were responsive and despite transfusion did not lower their immune response. You mention that patients who are transfused have a lower response, they are anergic, but it is possible that these six patients after transfusion did not respond.

VALDERRÁBANO One of the patients who rejected the kidney was transfused and the other was not.

TERASAKI The others were not transfused and were responsive from the beginning.
VALDERRÁBANO Yes, anergy induced by transfusion is variable from one patient to another. In some patients we have made repeat transfusions and we have seen a different decrease in the score after each transfusion.

TERASAKI So does that then mean that possibly your failure is due to the fact that those patients were not transfused, not because they were responsive. If you say that your responsive patients were not transfused then it’s possible that you divided your patients only according to whether they had been transfused, those are the non-responsive, and the patients who were not transfused, those were your responsive patients.

VALDERRÁBANO In both groups (anergic and responsive) we have patients transfused and non-transfused and at different times before transplantation.

BARNES (Birmingham) I was interested you were using seven different antigens. Was there any difference in the discriminating value one against the other or was it necessary to use quite as many as that? Could you have got the same result by just using two antigens?

VALDERRÁBANO I feel that in our area we have a high incidence of tuberculin positive reactions. One of the advantages of this method is the fact that using seven different antigens we obtain good information about the immune response of the patient, and not only the immunological memory.

BARNES Have you analysed any difference between any of the antigens? Are they all necessary or could you dispense with one or two of them?

VALDERRÁBANO No, we have only analysed the score of the patient.

PERDUE (Los Angeles) You showed a quite dramatic effect with the single packed cell transfusion in 29 responsive patients. Had those patients received this as an initial transfusion or had some or all of them had transfusions prior to this responsive state?

VALDERRÁBANO No, none of these patients were transfused in the one year before, but some patients had received transfusion more than one year before.

WILLIAMS (Chairman) You did not mention pregnancy as a possible factor influencing your findings in your tests. Did you look at this in the female patients?

VALDERRÁBANO Well we analysed the high incidence in the female sex but there was no relation with previous pregnancies.
PERAINO (Houston) I believe you showed that blood transfusion and time on haemodialysis was associated with an increasing percentage of anergy. Are they additive or can you account for one in terms of the other?

VALDERRÁBANO I think that transfusions are an additive factor to time on haemodialysis.