PART II

Chairman: J Traeger
Lyon
France
Influence of Presensitisation on the Fate of Cadaveric Renal Allografts

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Awareness of the importance of lymphocytic antibodies in the fate of renal allografts has increased recently (Patel et al, 1971; Terasaki et al, 1971; Zechack et al, 1972). However, general agreement has not been reached as to the significance of these antibodies. Sheil et al (1972) and Terasaki et al, (1971) have suggested that the initial high rate of failure in kidney transplants reported by the American College of Surgeons/National Institutes of Health Organ Transplant Registry might be due to presensitisation to transplant antigens by blood transfusion or pregnancy. This paper lends support to this suggestion.

In surveying the incidence of allograft rejection at our Medical Center, we noticed that the frequency was less at one hospital (VA Research Hospital) with a recently established haemodialysis programme, than at the other hospital (Passavant Memorial Hospital), whose programme dates back to 1963. We also noticed that, while most patients in the newer programme received fewer blood transfusions with triply-washed erythrocytes only, while the majority of patients in the older programme had been transfused with whole blood.

These findings provided the basis for the current investigation, in the course of which we sought to clarify the following:

(1) the relationship between presence of antibodies and graft rejection;
(2) degree of sensitisation in relation to comparison of transfusions of whole blood or of packed red cells;
(3) status of renal function in sensitised versus non-sensitised patients.

MATERIALS AND METHODS

Serum samples from 50 patients receiving 52 cadaveric transplants were tested monthly in the tissue typing laboratory of the University of Illinois against a panel of 20 to 25 cells for the presence of lymphocytotoxic antibodies
(Amos et al, 1969). The mean percentage of cell donors with whom serum samples reacted positively was determined for each patient.

In 23 patients at least 9 specimens and in 37, at least 3 specimens were examined. Of 42 non-parous patients, 19 had received whole blood (some had been given packed cells in addition), 5 had never been transfused, and 18 patients had received only packed cells. Eight patients had been pregnant (average parity, 3.3), all of whom had been transfused. Patients included in this study were at risk for at least 3 and up to 18 months after receiving a cadaveric graft. All direct cross matches with leucocytes of the specific donor were negative.

RESULTS

Relationship between antibodies and graft rejection

In non-parous patients who had reacted to more than 20 per cent of the donor lymphocyte panels at any one time, 7 of 9 grafts (77%) were rejected within three months (Table I). Two others survive with good renal function (22%).

<table>
<thead>
<tr>
<th>Sensitisation</th>
<th>Rejected</th>
<th>Nephrectomy for other reasons</th>
<th>Functioning grafts average 1 year</th>
<th>Died with good function</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 20% * (N=9)</td>
<td>77%</td>
<td>0%</td>
<td>23%</td>
<td>0%</td>
</tr>
<tr>
<td>0% ** (N=13)</td>
<td>8%</td>
<td>8%</td>
<td>70%</td>
<td>15%</td>
</tr>
<tr>
<td>0% *** (N=25)</td>
<td>24%</td>
<td>0%</td>
<td>64%</td>
<td>12%</td>
</tr>
</tbody>
</table>

* when determined on any single occasion
** at all times
*** for 3 months prior to transplant. This group includes 14 patients who did have lymphocytotoxic antibodies before that time (see text).

Of 13 patients who had not been known to be sensitised, 9 (70%) have good renal function three to fifteen months after surgery (average, eleven months); 2 (15%) died of non-rejection complications with good renal function, and 2 of the group rejected their kidneys within the first two months (15%). Included in this group of 13 are 8 patients whose sera had been examined on only one or two occasions. Possibly sensitisation could have been demonstrated by more complete prior assessment. The statistical comparison between the proportion of patients who rejected grafts in each group was significant (P = < 0.02).

Considering those patients previously sensitised in any one previous month but who were not sensitised at the time of surgery and for at least the
preceding 3 months, 9 of 14 (64%) had good renal function at three months to one year following transplantation (average, eleven months); 2 (14%) have rejected and 3 (21%) have died with good renal function (21%). Of those with more than 5% sensitisation, only two were in this group and both rejected early in their course.

Actuarial analysis of patients who were never sensitised, those with an average sensitisation of less than 5%, and those with an average sensitisation of more than 5% is shown in Figure 1. Total graft failures and those with immunological failures are plotted for each group. At 12 months, total graft survival in the 'zero' sensitisation group was 71% ± 13 SEM; in the < 5% group, 51% ± 8, and 21% ± 9 in the > 5% group respectively. Excluding failures not due to rejection the figures for the three groups were 84% ± 10, 87% ± 7, and 46% ± 12 respectively. These differences are significant (P = < .05).

Renal function in survivors
Mean serum creatinine levels in the survivors in the > 5% and < 5% sensitised group are shown in Table II. The differences are not significant. One
Table II. Serum creatinine levels in transplant recipients *

<table>
<thead>
<tr>
<th>Months</th>
<th>3</th>
<th>6</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 5% sensitisation</td>
<td>$1.7 \pm 0.7$</td>
<td>$1.6 \pm 0.4$</td>
<td>$1.5 \pm 0.2$</td>
<td>$1.6 \pm 0.2$</td>
</tr>
<tr>
<td>&gt; 5% sensitisation</td>
<td>$1.7 \pm 0.8$</td>
<td>$2.5 \pm 1.4$</td>
<td>$1.9 \pm 0.8$</td>
<td>$2.0 \pm 0.7$</td>
</tr>
</tbody>
</table>

* mg/100 ml

patient (average sensitisation = 10.5%) receiving a four antigen typed and matched kidney had a creatinine clearance greater than 100 ml/minute ten months after transplant.

Relationship to blood transfusion

The relationship between blood transfusion and sensitisation is shown in Table III. If groups receiving more than five units are compared, the average degree of sensitisation to donor lymphocyte panels was $20\% \pm 8$ SEM in the whole blood group ($N = 11$), and $0.18\% \pm 0.22$ SEM in those receiving

Table III. Analysis of causes of sensitisation

<table>
<thead>
<tr>
<th>Non-parous patients</th>
<th>Total</th>
<th>0% number of units</th>
<th>&gt; 5% number of units</th>
<th>&gt; 0&lt; 5% number of units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>19</td>
<td>5%</td>
<td>63%</td>
<td>18.6</td>
</tr>
<tr>
<td>Packed cells</td>
<td>18</td>
<td>62%</td>
<td>4.8</td>
<td>11%</td>
</tr>
<tr>
<td>No blood</td>
<td>5</td>
<td>80%</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Correlation Between Blood and Sensitization

![Correlation graph]

Figure 2. Correlation between number of units of whole blood or packed cells and degree of reactivity

460
cells only \((N = 4)\). The whole blood group received \(18 \pm 3\) SEM units and the packed cells group received \(12 \pm 1\) units. Correlation between transfusion and sensitisation, shown in Figure 2 was not significant.

In a group of 5 patients who had never received blood, 3 have good renal function 6 to 18 months later, 1 had rejected and 1 died with good renal function of non-renal complications.

Relationship to parity

Of 8 patients (average number of children, 3.3), 6 were sensitised to less than 5% and 2 were sensitised to more than 5% of donor panels. All had received transfusions. Five of the 6 non-sensitised patients have functioning kidneys 6 to 26 months later (average, 1 year).

Variation in lymphocytotoxicity

Monthly variation in reactivity against donor lymphocytes in 6 patients in whom these variations were well shown is indicated in Figure 3.
or previous transplantation. From a practical view point, patients previously sensitised to a large proportion of random panels have a poor prospect of success (Table I). Although the success rate with lesser degrees of sensitisation may be better, it is clear that the sensitised patient (> 5%) does not do well. It is a stimulant to further research that one highly sensitised patient who did receive a four antigen typed and compatible kidney has had normal renal function for ten months (Table I). A similar case was described by Terasaki et al (1971).

Results of transplantation were much better in patients who were never shown to be sensitised than in those who were highly sensitised. It is possible that some of the non-sensitised patients may have had cytotoxic antibodies in the past. It should be emphasised that the presence of false negatives introduces an error which would weaken the argument that absence of sensitisation is associated with a high graft survival rate. Month to month variations in the degree of lymphocytotoxicity (Figure 3) make it difficult to interpret one or two single measurements of cytotoxic antibodies. Therefore, patients on dialysis awaiting transplantation should be studied for the presence of antibodies at regular intervals.

Once a patient is strongly presensitised to specific antigens, it can be anticipated that an anamnestic response will occur once a kidney containing those antigens is transplanted.

In patients known to have cytotoxic antibodies at some time but who were negative to testing for at least two months prior to transplant surgery, results were good. However, almost all of these patients were in the <5% sensitised group. Two patients who were in the > 5% sensitised group rejected their grafts within one month of transplant.

This paper supports the conclusions of Patel et al (1971) and Terasaki et al (1971) who showed that poor results are frequent in sensitised patients despite a direct negative cross match. Terasaki et al, have suggested that patients be classified as sensitised when their sera react with 5% or more of the donor cells. Cadaveric graft survival was very much better in the non-sensitised group. Zschaeck et al (1972), using 10% as the cut off point, have similar results.

It is important to document the reasons for nonimmunological graft failure in analysing immunologic results of transplantation. It may be that patients dying with good renal function have been exposed to unusually high levels of immunosuppressive drugs in an attempt to overcome incompatibility. In this series, the incidence of nonimmunological causes for failure was the same in the <5% and >5% patient groups.

When the serum creatinine levels are compared at 6, 9 and 12 months in the <5% and the >5% groups, the levels are higher in the latter group although not significantly so. The >5% group have had their number dimin-
ished by graft failure, and only a few remain for late study.

At present there is no clear association between individual HL-A haplotypes and the development of lymphocytotoxicity but much larger numbers are necessary for such an analysis.

In this series, transfusions of whole blood produced a greater degree of lymphocytotoxicity than did packed cells. Only a small number of patients receiving whole blood had no sensitisation (Figure 2). Because the whole blood group did receive more transfusions than the other group, direct comparisons are difficult. Manzler and Nathan (1971) suggested that blood given during haemodialysis is not antigenic. However, in our 9 highly sensitised patients, 6 had received blood during dialysis. Some patients did not have an antibody response to transfusion (Table III) or pregnancy. Since they did not become sensitised, their results of grafting are within the good results seen in the non-sensitised group of patients. Of 5 patients who had never received blood and were not exposed to this mechanism of presensitisation, only one has rejected his kidney.

Opelz et al (1972) have stated that patients who remain without cytotoxicity after a year of dialysis almost never reject kidney transplants. This statement omits consideration of the number of transfusions and the type of blood actually given. Because there is no place for using transfusion as a prospective test of responsiveness, in vitro tests are necessary (Opelz et al, 1972). Until these are available, it would seem preferable to use thawed resuspended (frozen) cells (Chaplin, 1969).

In conclusion, we suggest that not only patients on dialysis but even mildly uraemic patients requiring blood for reasons unrelated to uraemia should receive frozen blood if there is a possibility that they will become transplant candidates. This would require foresight, and would be expensive, but the long term prospects for such patients might be significantly improved.

ACKNOWLEDGMENTS

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OPEN DISCUSSION

J TRAEGGER (Lyon, Chairman): Thank you for this very interesting paper which stresses a fact very important for the actual transplantation team.
This paper is now open for discussion.

W DRUKKER (Amsterdam): I should like to ask why should you transfuse at all? Don't do it, it is not necessary. You can handle patients in the stage just before dialysis is needed without transfusions and when you dialyse them it is the same, transfusion is not necessary. No frozen cells, no packed cells, no blood — this is our feeling!

LEVIN: Thank you for your remarks. I agree, during dialysis it should be possible for most of the time not to give blood and I meant to imply that. But considering someone who has a creatinine clearance of say 30ml/min who has had a large bleed from a duodenal ulcer and is going to need blood, I am suggesting that this patient should get frozen washed blood instead of ordinary whole blood.

DRUKKER: You are right. I don't want to criticise this case, but even in major surgery like gastrectomies in dialysed patients you don't need transfusions.

LEVIN: Yes, I am referring to patients who are not on dialysis but who
may one day require a transplant, say in a year’s time.

DRUKKER: That’s right, and don’t forget the hepatitis risk which we will consider later this afternoon.

B MYERS (Tel Aviv): Have your packed cells been rendered free of leukocytes?

LEVIN: Some were just washed packed cells, some were carefully washed with an attempt made to make them leucocyte-free. As you know, this is very much technician dependent and the degree of removal of leucocytes is very variable. However, even with the best attempts, where perhaps only 5% leucocytes remain, the blood is still antigenic as shown recently by Dr Olga Jonason at the University of Illinois.

TRAEGGER: I would like to ask you whether you have any experience with frozen blood?

LEVIN: We only have begun to use frozen blood when it is really necessary in recent months so we don’t have any direct evidence; but I believe Dr Huggins at the Massachusetts General Hospital has shown over the past few years that frozen blood produces virtually no sensitisation, and also has the benefit of a lower hepatitis risk.

TRAEGGER: May I ask you if before transplantation all your patients were cross-matched with the donors?

LEVIN: Yes, direct cross-matches were done on each patient.

TRAEGGER: Is there another question? Well, I thank you for this paper which is very important, because we are sure now that these patients are high risk patients.