HUMAN GAMMA GLOBULIN ENHANCES THE SURVIVAL OF RENAL ALLOGRAFTS

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Summary

Ninety seven transplant recipients participated in a controlled, clinical trial measuring the graft-enhancing property of a human gamma globulin preparation. The latter was prepared from the blood of pregnant women (RPGG) and contained antibodies with specificities for HLA locus products. Data analysis indicated that RPGG treated recipients had enhanced survival rates of their grafts if they had received one or more blood transfusions prior to transplantation.

Introduction

Human source gamma globulin is under clinical investigation as an immunosuppressive agent for use in renal transplantation. This report details our experience with a preparation recovered from the blood of pregnant women and used in adult recipients of cadaveric grafts. The rationale for its use in this situation is based on in-vitro findings, summarised and published elsewhere [1], that suggest the product may interfere with immunological pathways that ultimately lead to the rejection of allografts. The data reported here, the results of a clinical trial now 31 months in progress, indicate that the product, retroplacental source gamma globulin (RPGG), can significantly improve the survival rate of cadaveric grafts beyond that presently obtainable with standard immunosuppressive therapy.

Materials and Methods

RPGG is prepared from blood harvested from the retroplacental space following delivery of the placenta: approximately 50ml is collected from each woman. A pool is formed from that amount of blood recovered from 5,000 deliveries. The fractionation process, essentially a salting out method, yields a collection of gamma globulin proteins that are stabilised with glycine (pH7) and concentrated as a 16% solution. Broad reactivity against products of the A, B, C, D and DR
loci are routinely observed with each lot using selective immunological assays [2].
The biopotency standard used for selection of lots states that each lot of RPGG
must inhibit allogeneic MLC proliferative responses by a factor of 50% or more at
a microwell concentration of 1mg/100ml RPGG.

Recipients of either sex, 16 years of age and older, were asked to participate
in the study. Patients were randomly assigned to one of two study groups and all
received standard immunosuppressive therapy consisting of azathioprine and
prednisolone. Those assigned to the RPGG Group were treated as follows: 1.6g
just prior to and immediately after transplantation; then 1.6g on the first post-
operative day and daily thereafter for 27 days; then 1.6g weekly thereafter for
12 weeks. The cumulative dose of RPGG given therefore is 65.5g in a four month
period.

The pairing procedure of a cadaveric graft recipient with the appropriate donor
kidney has remained unchanged at our Centre since 1973. The donor organ and
recipient must be ABO compatible and produce a negative cross match indicating
absence of specific sensitisation to donor tissue. While we attempt to pair individ-
uals with the highest degree of HLA antigens shared, we have not excluded those
pairings that are completely mismatched provided that they meet the earlier
requirements. Analysis of the study groups revealed the absence of significant
weighting of either group for the following: age, sex, proportion of responders
(immunised recipients) to non-responders, HLA—A, B numerical matching grades,
and ABO blood groups.

Life tables and survival functions were statistically analysed using the general-
ised Wilcoxon (Breslow) test method [3]. The Breslow Test was selected because
it gives greater weight to early observations, and is less sensitive to late events
where fewer graft performances exist and where the graft attrition rate is notably
less.

No attempt was made to correlate outcome of a transplant with the type of
blood preparation used. This was due in large part to the variety of preparations
used at the 21 dialysis centres that refer patients to us for transplantation. Blood
products known to have been infused were: 1. whole blood; 2. packed cells;
3. buffy coat-poor packed cells; 4. washed and packed red cells and 5. frozen and
thawed washed packed cells. Any one of these preparations was therefore con-
sidered a "blood transfusion".

Blood transfusions given prior to and/or during the transplant procedure per se
were counted as pretransplant transfusions. Data were insufficient for analysis as
to the exact time prior to transplantation a transfusion was given; most were
reportedly given several months prior to surgery but within a period of one year.

Results (See Table I)

The cumulative survival rate (CSR) of first cadaver transplants at two years in
recipients receiving RPGG is 55.2 ± 9.1% compared with 32.0 ± 8.1% for the
Control Group (p > 0.05). RPGG treated recipients of second grafts had a CSR
of 51.6 ± 12.4% compared with the Control CSR of 25.0 ± 12.5% (p < 0.05).
The effect of blood transfusions on the CSR of grafts varied with the study group.
TABLE I. Allograft Survival Rates of Cadaveric Transplant (In Adults)

<table>
<thead>
<tr>
<th>Transplant Number</th>
<th>Group</th>
<th>Cumulative survival rate ± SE% at two years</th>
<th></th>
<th>Control Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RPGG Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First Cadaver</td>
<td>All patients</td>
<td>55.2 ± 9.1 (33)</td>
<td></td>
<td>32.0 ± 8.1 (34)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Transfused</td>
<td>71.5 ± 11.2 (20)</td>
<td></td>
<td>31.3 ± 9.4 (25)</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Not transfused</td>
<td>29.4 ± 12.9 (13)</td>
<td></td>
<td>33.3 ± 15.7 (9)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Responders</td>
<td>74.4 ± 9.9 (20)</td>
<td></td>
<td>41.2 ± 11.5 (19)</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Non-responders</td>
<td>28.0 ± 13.0 (13)</td>
<td></td>
<td>25.6 ± 12.5 (15)</td>
<td>NS</td>
</tr>
<tr>
<td>Second Cadaver</td>
<td>All patients</td>
<td>51.6 ± 12.4 (17)</td>
<td></td>
<td>25.0 ± 12.5 (13)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Among first graft recipients in the Control Group, preoperative blood transfusions did not affect the outcome: CSR of untransfused controls was 33.3 ± 15.7% while that of the transfused group was 31.3 ± 9.4% (p > 0.05). On the other hand, transfusions had a pronounced effect on the CSR of RPGG treated recipients: the CSR of untransfused patients was 29.4 ± 12.9% compared with 71.5 ± 11.2% (p = 0.007) for the transfused group.

The immunisation status of a recipient, determined by pretransplant screening of sera for complement-dependent leucocytotoxins, influenced the outcome of grafts in this study. Responder status did not significantly alter the outcome of grafts in the Control Group (see Table I): its effect on RPGG treated patients was notable. Here, the CSR first grafts was 74.4 ± 9.9% for responders and 28.0 ± 13.0% for non-responders (p < 0.01).

Discussion

Human gamma globulin of retroplacental origin enhances the survival rate of renal allografts beyond that presently obtainable with standard immunosuppressives alone. Not all graft recipients benefited from its use; those who had not received a pretransplant blood transfusion had survival rates comparable with those found for the untransfused controls.

Of note in this study was the failure of blood transfusions alone to enhance graft survival rates. This finding is in contrast with the many favourable reports of blood transfusions cited by van Es and Balner in a recent review article [4]. It should be pointed out that most of the patients in this study were poorly matched (i.e. shared less than two antigens with the donor) and most were highly immunised, two factors cited in the review article that could minimise or negate the favourable effect of blood.

At the present time, we cannot explain the synergistic effect of blood and RPGG on graft survival rates. In a previous report wherein we noted the beneficial effect of RPGG on preimmunised graft recipients [5], we had thought that gamma globulin might be suppressing immunological responses associated with humoral-type rejection. This explanation becomes less tenable or beclouded by the introduction of another factor, namely the added transfusion requirement.
A unifying hypothesis that might shed light on this observed combined effectiveness of blood and gamma globulin, must be postponed until additional data are acquired and examined.

Acknowledgments

This study was supported in part by a grant from The John A Hartford Foundation, Inc., and NIH Grant HL 11822.

References

4 van Es, AA and Balner, H (1979) Transplant. Proc., 11, 127
5 Riggio, RR, Kim, SJ, Saal, SD, Stubenbord, WT, Cheigh, JS, Stenzel, KH and Rubin, AL (1978) Lancet, i, 233

Open Discussion

JEEKEL (Co-Chairman) What is the mechanism behind this? Did you do any in vitro studies like blocking effects?

RIGGIO Our initial interest in this material was found when we were screening pregnancy sera in mixed lymphocyte culture, where we would have random responder and stimulating cells and by the addition of pregnancy sera, in contrast to non-pregnancy sera, we found that the mixed lymphocyte response was inhibited significantly, approximately by a factor of 70–80% of the untreated control response. It was our impression at the time that the mixed lymphocyte response was the in vitro model of the in vivo immune response, possibly the recognition phase of the immune arc, and that possibly by giving it we could block in some fashion the whole recognizable phase of the immune arc. That was the rationale for its use. We subsequently screened material from pregnancy sera, this material was made in Vienna for us by the Immunopharmaceutical Company and prepared from essentially women of Austrian origin. We had not attempted to determine the gene frequencies of the antibodies directed against the HLA alleles representatives of this ethnic group. It almost always blocks mixed lymphocyte responses in vitro.

MICHEIlsen (Leuven) How do you explain the extremely low kidney survival rate in your control group?

RIGGIO I have no explanation for it. I tried to possibly allude to this finding. Incidentally this is first cadaver transplant in adults. First cadaver transplants in our hospital, including children, bring our overall survival rate at 2 years close to 39%, and survival rate of children with first cadaver grafts is significantly greater than it is in adults. So if I might add that our overall patient survival rate is 39%
and in adults 32%.

I don’t know that that is a highly significant difference from the international transplant registry report at 2 years, I believe the value there is 42%, which is based on 25,000 grafts. I believe the vagaries of the ‘Centre Effect’ are relevant. Possibly interracial transplantation between a sizeable number of blacks and whites may contribute. I don’t have a final answer.

THOMPSON (Melbourne) Do you know if your immunoglobulin preparation contains a significant quantity of aggregated immunoglobulin as such aggregates may inhibit the role of the macrophage in either the primary or secondary immune response.

RIGGIO As far as we have been able to detect, using ammonium sulphate fractionation and an additional focusing procedure there are no aggregates in this material, in contrast to cold ethanol produced products. The largest material we have here by sedimentation analysis is 7S material, but the majority of it is 4S material. I think this allows us to give this drug with relative impunity intravenously.

VINCENTI (San Francisco) Do you think the human gamma globulin has any steroid sparing effect?

RIGGIO We have not considered that. If I may take the other end, did it have a patient survival effect since it had immunoglobulins of other types, our cumulative patient survival rate was almost identical to yours, 81% at five years. Most fortunately, recently we have had one death in the past one hundred first cadaver transplants performed at our hospital, but it is identical to our control population. I would think that in a selected patient we might use less steroids but we don’t use much to begin with. Maintenance is about 15 or 20mg a day within a month of transplantation.

VRIESMAN (Maastricht) It seems unlikely that immunisation with gamma-globulin at the time of transplantation will elicit anti-idiotypic antibodies in time to prevent recipient sensitisation. So (1) why not immunise prior to transplantation? (2) cannot an alpha globulin (Mowbray) within the 50% ammonium sulphate cut be responsible for the observed effect?

RIGGIO There is less than 1% alphaglobulins in this, in terms of the mitogen blocking effect. You raised the question and mentioned the word idiootype and as you did mention we give it simultaneously with transplantation. I think what you have said is what we plan to do now. It appears that anti-idiotypes are the key to this, namely that if the recipient can raise an anti-idiotypic antibody, he will come out ahead. If the patient is not able to muster this type of response before his rejection apparatus takes place, he will lose the graft. It is conceivable that, since the material seems to work well in our responders, they may have an unusual capacity to raise anti-idiotypic antibodies. But, as you have said we plan to immunise patients with this, prior to getting grafts, which we are unable to do now owing to the nature of our cadaveric programme.