HISTOCOMPATIBILITY IN ORGAN TRANSPLANTATION

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Summary

The major causes of failure in cadaveric renal transplantation are irreversible rejection of the renal allograft or complications of the immunosuppressive drug therapy. These could be overcome by the better definition of factors affecting histocompatibility or the induction of specific suppression of the rejection reaction.

A review of 183 primary cadaver renal allografts fails to show a marked influence of HL-A matching on the outcome of the graft. Nevertheless, it does suggest that the very well-matched transplant is more likely to be successful, so that regional, national, and international kidney sharing schemes are justified.

Recipients can be divided into high and low responders based on the reactivity of their serum with lymphoblastoid cell lines. Low responders show an 83% one-year survival of a cadaver renal allograft. The definition of these two groups of patients may influence the management of end-stage renal failure.

Specific suppression of rejection of renal allografts in rats has been produced both by antigen pretreatment of the recipient in the form of whole blood and by donor-specific antibody treatment (passive enhancement) of the recipient. The latter is likely to be most applicable to cadaver transplantation, as it does not require pretreatment of the recipient with the risk of sensitization, its effect is augmented by the concurrent use of anti-thymocyte serum, it can be achieved with small doses of antisera, and it does not have to be directed at all donor–recipient incompatibilities.

Thus many of the problems of transplantation should be overcome in the next decade.

Introduction

John Hunter's ingenuity and imagination were no better illustrated than by his experimental attempts at transplantation. He is best known for the transplantation of teeth not only in man both as autografts and allografts, but even as heterografts into the comb of a cock, although his observations about the success of these experiments seem to have been rather optimistic. He also performed both autografts and allografts of testes in poultry and may have attempted bone transplantation.

It must be admitted that Hunter did not recognize the uniqueness of the individual with respect to histocompatibility antigens, considering that allografts and autografts would behave in a similar manner, a successful transplant depending on "the disposition in all living substances to unite when brought into contact with one another, although they are of different structure, and even although the circulation is Hunterian Lecture delivered on 24th May 1973 (Ann. Roy. Coll. Surg. Engl. 1973, vol. 53)
only carried on in one of them. Nevertheless, he was certainly the first person to perform experiments in transplantation, and in all fairness to Hunter it must be pointed out that the first attempts at renal transplantation in the human were done without much knowledge of histocompatibility differences between unrelated individuals.

Today renal transplantation has attained a standard of success not imagined possible a decade ago. This is well illustrated by the Australian and New Zealand experience in cadaveric renal transplantation, where approximately 60% of kidneys are surviving one year (Fig. 1). The majority of the losses occur within the first few months after transplantation, although there is steady attrition thereafter. Three-quarters of these failures are due to uncontrollable rejection of the renal allograft or death of the patient from sepsis due to the immunosuppressive drug therapy.
How, then, are we likely to overcome these problems in renal transplantation, which primarily represent either failure to suppress the rejection reaction or complications of the drug therapy? Firstly, one solution would be close matching of donors and recipients for their histocompatibility antigens which might result in a diminished rejection, so allowing a lower dosage of immunosuppressive drugs. Certainly when the donor is a living relative this can be achieved, but the role of tissue typing in cadaveric renal transplantation is uncertain. Thus I intend to discuss some aspects of our experience with tissue typing in cadaveric renal transplantation over the past 6 years, as well as other related aspects of the recipient’s response to a renal allograft. Secondly, specific suppression of the rejection reaction of the recipient against donor histocompatibility antigens would provide the ultimate answer to both problems, and in this context I wish to discuss some of the work of Dr. John Fabre and myself in the induction of specific suppression of rejection of renal allografts in the rat with relevance to cadaveric renal transplantation.

The HL-A system

The major system of histocompatibility antigens in man is known as the HL-A system. It is defined serologically in most laboratories.

### TABLE I

**THE HL-A SYSTEM OF LEUCOCYTE ANTIGENS WITH CAUCASIAN GENE FREQUENCIES**

(G = 1 \( - \sqrt{1-F} \), where F = Antigen Frequency).

<table>
<thead>
<tr>
<th>First or LA Locus Antigens</th>
<th>Gene Frequency</th>
<th>Second or Four Locus Antigens</th>
<th>Gene Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>HL-A1</td>
<td>0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HL-A2</td>
<td>0.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HL-A3</td>
<td>0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HL-A9</td>
<td>0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W23 (9.1)</td>
<td>0.02</td>
<td>HL-A13</td>
<td>0.03</td>
</tr>
<tr>
<td>W24 (9.2)</td>
<td>0.09</td>
<td>W5</td>
<td>0.09</td>
</tr>
<tr>
<td>HL-A10</td>
<td>0.06</td>
<td>W10</td>
<td>0.06</td>
</tr>
<tr>
<td>W26 (10.2)</td>
<td>0.03</td>
<td>W14</td>
<td>0.04</td>
</tr>
<tr>
<td>W25 (10.1)</td>
<td>0.02</td>
<td>W15</td>
<td>0.06</td>
</tr>
<tr>
<td>HL-A11</td>
<td>0.07</td>
<td>W16</td>
<td>0.04</td>
</tr>
<tr>
<td>W19</td>
<td>0.13</td>
<td>W17</td>
<td>0.04</td>
</tr>
<tr>
<td>W29 (19.1)</td>
<td>0.05</td>
<td>W18</td>
<td>0.05</td>
</tr>
<tr>
<td>W30 (19.3)</td>
<td>0.02</td>
<td>W21</td>
<td>0.03</td>
</tr>
<tr>
<td>W31 (19.4)</td>
<td>0.07</td>
<td>W22</td>
<td>0.03</td>
</tr>
<tr>
<td>W32 (19.5)</td>
<td>0.04</td>
<td>W27</td>
<td>0.04</td>
</tr>
<tr>
<td>W28</td>
<td>0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>0.93</td>
<td>TOTAL</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Some of the previously defined HL-A antigens have now been shown to comprise more than one component, e.g. HL-A9, and these components have been given a provisional W number.
today by a microlymphocytotoxic technique, using peripheral blood lymphocytes. The HL-A antigens can be divided into two segregant series (Table I). The antigens of each series behave as if they were determined by mutually exclusive alleles at each of two loci, known as the first and the second locus (or the LA and the Four locus). These two loci are generally inherited en bloc. Bearing in mind that the same two loci exist on a paired homologous autosome in the cell nucleus, it is apparent that any individual can express at the most 4 HL-A antigens, assuming heterozygosity at each locus. It must also be apparent that there can be an enormous number of different HL-A phenotypes found in the population, with a relatively small chance of two unrelated individuals being HL-A identical. In the case of a common phenotype, it is of the order of 5 chances in 100, but for most phenotypes it is far lower than this.

In addition to the serologically determined products of the two loci mentioned above, it has more recently become evident that the mixed lymphocyte reaction (MLR) is determined by products of a third locus closely linked to the second (Four) locus (see Morris3 for references). The role of the MLR products in determining histocompatibility between donor and recipient is not clearly defined but is likely to be an important component of HL-A in this respect. Thus our assessment of histocompatibility between donor and recipient of a cadaver renal transplant must be far from complete at the present time.

**Matching for HL-A in cadaver renal transplantation**

The analyses to be presented are based on 225 consecutive cadaver renal transplants, performed between 6 months and 5 years before the analyses. There were 208 first grafts, 16 second grafts, and one third graft. Only the first transplants will be considered further. Twenty-five first transplants have been excluded from the matching analyses owing to failure of the graft or death of the patient within 2 weeks of transplantation for non-immunological reasons (with the exception of hyperacute rejection, which is not directly related to matching itself). The reasons for exclusion from analysis were renal ischaemia (6 patients), hyperacute rejection (4), infection (5), haemorrhage (5), myocardial infarction (3), ureteric necrosis (1), and cerebrovascular accident (1).

HL-A typing is carried out on both peripheral blood lymphocytes and spleen lymphocytes, the latter allowing better lymphocyte preparations and giving more consistent reactions with weak antisera4. Over the past 18 months all cadaver kidneys used for transplantation in Melbourne have been maintained on a Belzer preservation machine for approximately 24 hours by Dr. David Scott. This allows careful typing, mostly in daylight hours.

The following HL-A antigens have been identified either prospectively or retrospectively throughout this series: HL-A1, 2, 3, 9, 5,
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7, 8, 12, 13, W5, W28, W14. Over the last 30 months (134 transplants), additional antigens have been determined: HL-A10, 11, W19, W10, W15, W17, M2 (W18), W22, W27, and M3. Again throughout the series the two complex specificities, 4a and 4b, have also been determined. As the definition of HL-A specificities has not been uniform throughout the series, the matching analysis presented here will be based on the number of shared antigens, rather than on the number of incompatibilities, between donor and recipient.

The assessment of graft outcome has been based on renal function and survival one year after transplantation, as well as by the construction of actuarial survival curves.

The results of these comparisons are shown in Figure 2 and Table II. The actuarial survival curves do not suggest a major influence of tissue matching in cadaver renal transplantation, other than perhaps where 3 antigens are shared between the donor and the recipient (although this is
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TABLE II

COMPARISON BETWEEN GRAFT OUTCOME ONE YEAR AFTER TRANSPLANTATION AND MATCHING FOR HL-A BASED ON THE OUTCOME OF SHARED ANTIGENS BETWEEN DONOR AND RECIPIENT.

<table>
<thead>
<tr>
<th>No. of identical antigens</th>
<th>Graft function at one year</th>
<th>Nephrectomy, patient death</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( S \text{Cr} = \text{or} &lt; 2.0 \text{mg/100 ml} )</td>
<td>( S \text{Cr} &gt; 2.0 \text{mg/100 ml} )</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>34</td>
<td>18</td>
</tr>
<tr>
<td>1</td>
<td>31</td>
<td>26</td>
</tr>
<tr>
<td>0</td>
<td>19</td>
<td>24</td>
</tr>
</tbody>
</table>

\( S \text{Cr} = \text{serum creatinine.} \)

2, 3 \( v \) 0, 1—0.05 \(< P < 0.1; 2, 3 \( v \) 0—\( P < 0.05 \)

not statistically different from the other curves at present). Certainly there is no evidence based on survival to suggest that one can distinguish between the lesser degrees of identity between donor and recipient. However, our assessment of function at one year, which takes into account serum creatinine as well as survival, does suggest a better course for the better-matched donor and recipient, and this may be reflected by survival figures with the passage of time. The assessment of matching based on incompatibilities rather than identities produces rather similar results. Nor are the results any different if only transplants performed in the last 2 years are considered, during which time an increased number of HL-A antigens have been determined in our laboratory.

Is there a strong argument for regional, national, and international sharing of kidneys based on HL-A matching? I think there is, as it would seem from our data and those of others that the well-matched kidney does have a better graft outcome, although there are data which fail to find a correlation between matching and graft outcome. Obviously the chances of finding a reasonable number of donors and recipients sharing 3 or 4 antigens will be significantly increased as the size of the donor and recipient pool increases. Nevertheless it must be stressed that the evidence showing an influence of HL-A matching in cadaver renal transplantation is not compelling, and other factors are equally important. This is well illustrated by the difference in survival of cadaver kidneys in Australasia and here in London, which can be attributed in the main to the use of better donor kidneys in Australia and New Zealand. For it is well to remember that all the tissue typing in the world will not help a kidney that is dead at the time of transplantation.

Matching for first- and second-locus HL-A antigens

It has been suggested recently that incompatibilities at the second locus exert more influence on graft outcome than those at the first locus. This has been of particular interest to me, in that for some years I have claimed that incompatibility for 4a and 4b of all the HL-A
specificities seemed to exert the most influence on graft outcome⁶. These two are complex specificities and all the second-locus antigens appear to cross-react with either 4a or 4b, so that these recent observations would not be incompatible with our own. However, although incompatibility for 4a and 4b still appears to be important in determining the early course of the graft as judged by the number and severity of rejection episodes, we have not been able to show a significant influence of matching for the second-locus antigens over matching for first-locus antigens. However, the numbers suitable for analysis in this regard are rather small.

**Lymphocytotoxins before transplantation**

After the initial observation that lymphocytotoxic antibodies, usually of donor specificity, developed after rejection of renal allografts in man¹⁴, ₁₅, their importance in the pregraft recipient soon became evident. For it became apparent that transplants performed in the presence of antibodies directed against donor histocompatibility antigens (a positive cross-match) generally resulted in hyperacute rejection of the kidney¹⁶-²₀. Thus a positive cross-match between donor lymphocytes and recipient serum became an absolute contraindication to renal transplantation. This led to a significant reduction in the occurrence of non-functioning cadaver kidneys in most units, probably of the order of 50%. More recently it has been shown that recipients with pregraft lymphocytotoxins have a worse graft survival despite a negative cross-match between donor and recipient than patients who remain negative for cytotoxins up to transplantation²¹. Our own experience, although much smaller, is similar (Fig. 3).

Initially I felt that the worse prognosis of these kidneys was due to a false negative cross-match between donor and recipient at the time of transplantation, resulting in a second-set type of rejection of a subsequent renal allograft. This does explain some of our cases²². I now have 6 patients who have rejected their renal transplants within 3 months of transplantation in the presence of a negative cross-match with peripheral blood lymphocytes, but in whom I have been able to demonstrate a positive cross-match with donor kidney cells in tissue culture. Unfortunately our technique requires the establishment of kidney epithelial cells in tissue culture before they can be tested with the recipient's serum, so that the information is not available for a minimum of 7 days after transplantation, and so provides only retrospective information.

However, I now feel that the worse prognosis of these patients with preformed lymphocytotoxins is more likely a manifestation of their overall immune reactivity. These patients, then, are not only more likely to respond to challenge with histocompatibility antigens in the form of
Fig. 3. Actuarial survival curves of primary cadaveric renal allografts in patient with (antibody + ve) and without (antibody - ve) demonstrable lymphocytotoxic antibodies against HL-A before transplantation.

blood transfusions by the demonstrable production of lymphocytotoxins, but they will respond more vigorously to a subsequent renal allograft despite immunosuppressive drugs. These two groups of patients might be termed high responders and low responders, if we borrow the terminology of our colleagues working with immune response genes.

At the beginning of 1970 we began a policy of restricting transfusions to an absolute minimum in patients awaiting transplantation. This policy was aimed at reducing the number of patients developing lymphocytotoxins before transplantation. But as seen in Table III, this

| TABLE III |
| NUMBER OF TRANSFUSIONS RECEIVED BY PATIENTS BEFORE TRANSPLANTATION COMPARED WITH FREQUENCY OF LYMPHOCYTOTOXINS |
| Mean no. of Antibody |
| Period | transfusions ± SE | frequency |
| 1967-69 | 26.8 ± 3.3 | 8/42 (19%) |
| 1970-72 | 6.8 ± 1.1 | 24/135 (18%) |

SE = standard error.
policy has been unsuccessful. Although there was a significant \( P < 0.05 \) reduction in the number of transfusions given to the patients in the second period, this did not result in a decrease in the proportion of patients developing antibodies. This again lends support to the suggestion just made.

**High responders and low responders**

Can the high responders and low responders be distinguished more precisely before transplantation other than by the presence or absence of lymphocytotoxins? Obviously the response to transfusions after a year on dialysis would be one method, as shown by Opelz, Mickey, and Terasaki\(^ {23} \), but no one is likely to be enthusiastic about extensively transfusing potential recipients so that the group who will not develop antibodies (low responders) becomes more clearly defined in a shorter time. We have tackled this problem in another way—namely, by assessing the reactivity of the recipients’ serum against established lymphoblastoid cell lines. These cells not only carry an increased density of HL-A antigens on the cell membrane and so are more sensitive target cells for HL-A antibodies, enabling weak HL-A antibody to be detected in a patient’s serum, but also carry antigens peculiar to these cells which are either the Epstein–Barr virus membrane antigen (MA) group or a new antigen group that we have called ‘D’. Most members of the normal adult population appear to have antibodies to this antigen—for example, 95\% of adult untransfused males will react with these cell lines. The antibody to the D antigen is heat labile (56°C for 30 minutes), as opposed to anti-HL-A and anti-MA antibodies, which are heat stable. However, only 55\% of potential renal allograft recipients react against these cultured cell lines. These patients are the high responders. This 55\% includes about 30\% or so of patients who have heat-stable anti-HL-A antibodies. The 45\% of patients who are non-reactive to these cultured cell lines (the low responders) have a remarkably good graft outcome, with a one-year kidney survival of 83\% (Fig. 4), this being strikingly different from the high-responder group of patients \((P<0.001)\). Furthermore, two-thirds of these patients have either no or only one rejection episode in the first 3 months after transplantation.

Both Festenstein’s and van Rood’s groups\(^ {10, 11} \) have suggested that tissue matching correlates better with graft outcome in the group of patients with pregraft antibodies. In our data there is a trend suggesting that this is so in our high-responder group, but it is not statistically significant.

Thus the lymphoblastoid cell lines enable us to define two distinct groups of patients—the high responders who have anti-HL-A antibody or just anti-D antibody, and the low responders who have neither—using a relatively simple technique. The low responders have a very good
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graft outcome, while the high responders have a stormier early course and a much poorer graft survival. The recognition of these two groups of patients represents a very important step forward in tissue matching in its broadest concept, for it embraces another facet important in graft rejection—namely, the patient’s immune reactivity.

**Specific immunosuppression of rejection**

As I have pointed out earlier the likelihood of obtaining truly well-matched cadaver donors and recipients even among relatively large pools of recipients will be possible only in a minority of cases. This then means that attempts to improve cadaver renal transplantation must be directed at the induction of immunosuppression against donor histocompatibility antigens. That this is likely to be possible in the not too distant future is borne out by the demonstration in recent years that survival of renal allografts in the rat can be very significantly prolonged by various methods of immunological manipulation of the recipient animal. And in the human the first steps have been taken.

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Fig. 4. Actuarial survival curves of renal allografts in patients with (LCL +ve) and without (LCL -ve) antibodies reactive with lymphoblastoid cell lines before transplantation.
in this direction by Batchelor and his colleagues in living related donors and recipients. I intend to review briefly some of the work in this area done by Dr. John Fabre and myself over the past few years, in which we have used both antigen pretreatment and antibody treatment of the recipient animal to suppress rejection of a renal allograft in the rat.

Experimental model. Two strains of rats have been used in most of these experiments. DA and Lewis, and their F1 hybrids. These two strains differ at the major histocompatibility locus in the rat, AgB, and skin grafts exchanged between these strains will be rejected in less than 10 days. The transplanted kidney is inserted orthotopically with end-to-end anastomosis of renal artery and vein and end-to-end anastomosis of the ureter. Ischaemia times range from 17 to 25 minutes, and the procedure is carried out by two operators with an operating microscope. The recipient animal's own kidneys are removed at the end of the procedure so that survival can be used as an end-point of rejection. Blood urea levels are also monitored regularly.

Antigen-induced suppression of rejection. In these experiments we were interested in the ability of donor antigen in the form of whole blood to suppress rejection of a tissue allograft in the rat. These experiments began some 4 years ago following our observation that there was no correlation in man between the number of transfusions given to a patient before transplantation and the outcome of the graft. Our first experiments were performed with skin grafts, but we could produce only a very modest survival of DA skin allografts on Lewis

\begin{table}
\centering
\begin{tabular}{|l|l|l|l|}
\hline
Strain & Treatment & No. of rats & Survival (days) \\
\hline
F1 (DA × Lewis) to Lewis & Nil & 10 & 10.9 ± 1.3 (MST ± SD) \\
4 weeks & & 5 & 13, 40, 63, 110, > 231 \\
10 weeks & & 7 & 7, 17, 31, 36, > 181 \\
& & & > 259, > 264 \\
& 20 weeks & 3 & 19, > 197, > 200 \\
F1 (DA × Lewis) to DA & Nil & 9 & 18.0 ± 8.5 (MST ± SD) \\
4 weeks & & 6 & All > 100 \\
\hline
\end{tabular}
\caption{Results of F1 (DA × Lewis) to Lewis or DA renal allografts in untreated recipients and in recipients treated with 0.5 ml of DA or Lewis blood intravenously twice weekly for the times noted.}
\end{table}

recipients by pretreatment with donor-strain (DA) blood by either single or multiple intravenous injections. However, the same protocols which produced a slight prolongation of skin allografts produced a very striking prolongation of survival of F1 (DA × Lewis) renal allografts (Table IV). Even more striking was the effect of a single injection of donor-strain
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TABLE V
RESULTS OF F1 (DA × LEWIS) TO LEWIS OR DA RENAL ALLOGRAFTS IN
UNTREATED RECIPIENTS AND IN RECIPIENTS TREATED WITH A SINGLE INJECTION
OF 0.5 ML OF DA OR LEWIS BLOOD INTRAVENOUSLY BEFORE TRANSPLANTATION AT
THE TIMES NOTED

<table>
<thead>
<tr>
<th>Treatment at Day</th>
<th>F1 (DA × Lewis) to DA</th>
<th>F1 (DA × Lewis) to Lewis</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rats</td>
<td>MST ± SD (days)</td>
<td>No. of rats</td>
</tr>
<tr>
<td>Nil</td>
<td>9</td>
<td>18.0 ± 8.5</td>
</tr>
<tr>
<td>-1</td>
<td>6</td>
<td>9.1 ± 5.0</td>
</tr>
<tr>
<td>-7</td>
<td>6</td>
<td>&gt; 90*</td>
</tr>
<tr>
<td>-28</td>
<td>5</td>
<td>&gt; 50*</td>
</tr>
</tbody>
</table>

MST = median survival time; SD = standard deviation.

*P < 0.01 †P < 0.02

blood in the F1 (DA × Lewis) to DA direction (Table V), where complete
suppression of rejection with indefinite survival of the recipient
animal resulted38. In the stronger strain combination, F1 (DA × Lewis)
to Lewis, a single injection produced accelerated rejection when given
one day before transplantation, and only a very modest increase in sur-
vival when given 7 days before transplantation.

Thus it seemed that these two models—namely, the F1 (DA × Lewis)
to Lewis and the F1 (DA × Lewis) to DA—differ markedly in their
response to a renal allograft as well as in the ease of induction of
antigen-induced suppression of renal allograft rejection. Thus our pre-
vious suggestion that exposure to allogeneic blood transfusions in man
before transplantation might induce specific immunosuppression in cer-
tain patients receives some support from these experiments.

The mechanism by which this antigen-induced suppression has been
produced remains uncertain. Our investigations, which have been de-
scribed fully elsewhere37, would suggest that active enhancement is more
likely than classic tolerance, but are by no means conclusive. The main
arguments against a mechanism of enhancement were, firstly, our failure
to demonstrate donor-specific antibodies in rats with long-surviving
renal allografts, and secondly, failure to transfer the immunosuppression
passively with serum from long survivors. That some form of graft
adaptation occurs is shown by the delayed rejection of long-surviving
renal allografts retransplanted into untreated syngeneic recipients. How-
ever, as a fresh renal allograft of donor specificity is not rejected when
transplanted into the long-surviving immunosuppressed recipient, adap-
tation cannot be an important mechanism in this model.

There are two main arguments against a mechanism of tolerance.
Firstly, rats with long-surviving renal allografts reject skin allografts
of donor specificity, although this might be explained by the presence
of skin-specific histocompatibility antigens. Secondly, and most com-
pelling, lymphocytes from rats with long-surviving renal allografts can
react in a normal manner with donor histocompatibility antigens in
a popliteal node graft-versus-host reaction. Thus it is not possible to
draw firm conclusions about the nature of the mechanism of this type
of antigen-induced suppression, but I feel that our findings are more
compatible with active enhancement. Jenkins and Woodruff\textsuperscript{38} have pro-
duced almost identical results in a rat cardiac allograft model.

These experiments raise the question of pretreating potential human
transplant recipients with donor antigen—in the form of regular blood
transfusions, for example. At present the risk of sensitization rather
than immunosuppression occurring would be too high for any of us to
think of embarking on this unless perhaps in the low-responder group
of patients that I have already defined. However, I feel that this potential
type of therapy must wait until we have defined the mechanism more
precisely in the experimental animal.

\textbf{Antibody-induced suppression of rejection (passive enhance-
ment).} This form of immunosuppressive therapy is likely to be the most
applicable to renal transplantation in man, as there is less risk of harm-
ing the transplanted kidney. Since the first successful induction of
passive enhancement of renal allografts in the rat by Stuart and his
co-workers\textsuperscript{26} and French and Batchelor\textsuperscript{29} there have been a number of
other successful experiments in rats in different strain combinations. I
intend to discuss briefly some of our own work in this field which has
clinical relevance.

We were fortunate that in one of our strain combinations (F1 (DA $\times$
Lewis to Lewis) it proved impossible to produce indefinite survival
of renal allografts by passive enhancement, although a very significant
delay in the onset of rejection did occur. In passing it should be
noted that this finding suggests that passive enhancement alone will
not completely suppress rejection across a strong histocompatibility
barrier.

In collaboration with Batchelor\textsuperscript{39} we decided to test the hypothesis
that suppression of rejection was a competition between protection of
the kidney allograft by the passively administered enhancing serum
and the cellular immune response of the recipient mediated by thymus-
derived killer cells (T cells) until the animal produced its own enhancing
antibody. If this were the case, then we felt that it should be possible
to suppress rejection completely in the strong F1 (DA $\times$ Lewis) model by
treating the recipient with both a rabbit anti-rat-thymocyte serum and an
enhancing serum. This we did, using a weak rabbit anti-rat-thymocyte
serum, which could by itself prolong skin allograft survival by only 2
days and renal allograft survival a little longer, together with a weak
enhancing serum which produced a modest prolongation of renal allo-
graft survival when used alone. This resulted in complete suppression
of rejection and indefinite survival of 5 of 6 animals (Table VI). These
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TABLE VI
RESULTS OF F1 (DA × LEWIS) TO LEWIS RENAL ALLOGRAFTS IN RECIPIENTS TREATED WITH A RABBIT ANTI-RAT-THYMOCYTE SERUM (ATS) INTRAPERITONEALLY, A LEWIS ANTI-DA ENHANCING SERUM (ES) INTRAVENOUSLY, OR A COMBINATION OF BOTH.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of rats</th>
<th>MST ± SD (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>6</td>
<td>10.6 ± 1.8</td>
</tr>
<tr>
<td>ATS</td>
<td>5</td>
<td>14.2 ± 3.2</td>
</tr>
<tr>
<td>(2 ml days 2, 5)</td>
<td>5</td>
<td>18.2 ± 5.5</td>
</tr>
<tr>
<td>ES</td>
<td>5</td>
<td>&gt; 110</td>
</tr>
<tr>
<td>ATS + ES</td>
<td>6</td>
<td>&gt; 110</td>
</tr>
</tbody>
</table>

MST = median survival time; SD = standard deviation.

results not only support our hypothesis, but suggest that the combination of more conventional immunosuppressive therapy with enhancing serum could be a profitable approach in the human.

We have also attempted to define the minimum dosage required to produce passive enhancement of a renal allograft in the rat. For if we extrapolated to the human the conventional dosage used by ourselves and others to produce enhancement in the rat on a weight-for-weight basis, it amounted to well over a litre of enhancing serum that would be necessary to treat a single human patient. This would present considerable logistic difficulties in the provision of such hyperimmune anti-HL-A antiserum. We therefore carried out extensive dose–response studies in both our strong and weak models, and found that as little as a single injection of 50 μl of enhancing serum given at the time of transplantation was just as effective as the larger doses spread over several days (Table VII). In fact, in the weaker model even 10 μl was

TABLE VII
RESULTS OF F1 (DA × LEWIS) TO LEWIS OR DA RENAL ALLOGRAFTS IN DA OR LEWIS RECIPIENTS TREATED WITH SUCCESSIVELY LOWER DOSES OF DA ANTI-LEWIS OR LEWIS ANTI-DA ENHANCING SERA.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>F1 (DA × Lewis) to DA</th>
<th>F1 (DA × Lewis) to Lewis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of rats</td>
<td>MST ± SD (days)</td>
</tr>
<tr>
<td>Nil</td>
<td>8</td>
<td>19.0 ± 12.6</td>
</tr>
<tr>
<td>2 ml Day 0</td>
<td>5</td>
<td>&gt; 149</td>
</tr>
<tr>
<td>1 ml Day 1</td>
<td>5</td>
<td>&gt; 149</td>
</tr>
<tr>
<td>0.5 ml Days 3, 5</td>
<td>5</td>
<td>&gt; 149</td>
</tr>
<tr>
<td>1 ml Day 0</td>
<td>6</td>
<td>&gt; 107</td>
</tr>
<tr>
<td>250 μl Day 0</td>
<td>5</td>
<td>&gt; 169</td>
</tr>
<tr>
<td>50 μl Day 0</td>
<td>5</td>
<td>&gt; 144</td>
</tr>
<tr>
<td>10 μl Day 0</td>
<td>6</td>
<td>&gt; 115</td>
</tr>
</tbody>
</table>

MST = median survival time; SD = standard deviation.

*P < 0.01 †not significant.
effective in some animals. This suggests that perhaps 15–25 ml of enhancing serum might be sufficient in human practice.

Again, if passive enhancement is to be of value in cadaver transplantation, then it is necessary to demonstrate that the specificity of the enhancing serum does not have to be directed at all the incompatibilities carried by a renal allograft in order to achieve some degree of specific immunosuppression. For it seems unlikely that it will be possible to provide antisera that will cover both known and unknown donor–recipient incompatibilities. Our experiments in this area, which are still incomplete and are shown in Table VIII, suggest fortunately that an enhancing serum directed against only some of the donor–recipient incompatibilities can produce an immunosuppressive effect.

**TABLE VIII**

**Results of F1 (Lewis × AS2) to DA Renal Allografts in Untreated Recipients and Recipients Treated with Either DA Anti-AS2 or DA Anti-Lewis Enhancing Serum.**

<table>
<thead>
<tr>
<th>Enhancing serum</th>
<th>No. of rats</th>
<th>Survival (days)</th>
<th>Blood urea at Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nil</td>
<td>5</td>
<td>All &gt; 50</td>
<td>400 mg/100 ml</td>
</tr>
<tr>
<td>DA anti-AS2</td>
<td>5</td>
<td>18, 39, &gt; 90</td>
<td>Normal</td>
</tr>
<tr>
<td>(1 ml Day 0)</td>
<td></td>
<td>&gt;120, &gt;120</td>
<td></td>
</tr>
<tr>
<td>DA anti-Lewis</td>
<td>5</td>
<td>All &gt; 100</td>
<td>Normal</td>
</tr>
<tr>
<td>(1 ml Day 0)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thus passive enhancement seems likely to have a definite role in renal transplantation, not only in the living related donor–recipient situation, where Batchelor has taken the first steps, but also in cadaver transplantation. For any degree of specific immunosuppression which will allow a diminution in dosage of the current immunosuppressive drugs will represent a bonus to transplanters.

**Conclusion**

In conclusion, I do not feel that there is much justification for the present air of pessimism that pervades the clinical transplantation field because of the lack of any real improvement in the results of renal transplantation over the past few years. The work discussed in this lecture, as well as that of others, makes me optimistic that many of the major problems of organ transplantation will be solved over the next decade.
HISTOCOMPATIBILITY IN ORGAN TRANSPLANTATION

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