ENDOTHELIAL ANTIBODIES IN RENAL TRANSPLANTATION

L C Paul, L A van Es, F H J Claas, M W Kalff

University Hospital, Leiden, The Netherlands

Summary

Sera of 97 consecutive transplant recipients were studied for the presence of antibodies directed against their renal allografts. A highly significant correlation (p < 0.001) was found between irreversible rejection and donor-specific endothelial antibodies (EAb). EAb reacted not only with the endothelium of peritubular capillaries and venules, but also with glomeruli. Absorption of EAb to platelets, B- or T-lymphocytes did not remove its EAb activity or its cytotoxicity for monocytes. Furthermore, it was shown with a panel of randomly chosen donor kidneys, that EAb are directed against a polymorphic system of endothelial-monocyte antigens, which is unrelated to HLA–A, –B, –C or –D.

Introduction

In previous studies we established the correlation between the presence of antibodies directed against the endothelium of the peritubular capillaries and venules of the donor kidney, and irreversible vascular rejection within 50 days after transplantation [1–3]. We hold EAb responsible for graft rejection because EAb can be demonstrated along peritubular capillaries of irreversibly rejected grafts, together with histological lesions in these capillaries [1]. Furthermore, the presence of EAb before transplantation results in accelerated graft rejection [4]. It was shown by cytotoxicity and immunofluorescence experiment that the EAb are directed against antigens which are also present on monocytes but not on lymphocytes [4,5] and thus are not HLA–A, –B, –C or –DR antigens. Furthermore, panel analysis of the eluted EAb demonstrated the independence of the endothelial-monocyte antigens from the known antigens of the HLA–complex [5].

This study is an extension of our previous observations and concerns the presence of the endothelial-monocyte antigens in glomeruli, and the polymorphism of these antigens.
Materials and Methods

Sera were collected and studied using an indirect immunofluorescence technique on unfixed sections of pretransplant biopsies of donor kidneys as described previously [1]. As controls, normal AB serum, pretransplant sera that were EAb negative, and human antisera directed against ABO blood group antigens [2] were used.

To study the presence of endothelial antigens in glomeruli, sections were chosen from five kidneys which did not show IgG in the glomeruli [6]. EAb-positive sera from ABO-compatible renal allograft recipients were tested against those sections. In order to study the polymorphism of the EAb, eleven EAb-positive sera were tested against a panel of eleven randomly chosen pretransplant renal biopsies.

Results

Table I summarises the incidence of EAb in 97 consecutive renal allograft recipients; as shown, EAb could be demonstrated in 7 out of 12 recipients who rejected their grafts irreversibly within 7 weeks after transplantation. Five recipients rejected their grafts without EAb in their sera. These antibodies were not found in recipients who lost their grafts due to non-immunological causes. These data

<table>
<thead>
<tr>
<th>Clinical results</th>
<th>EAb present</th>
<th>EAb absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irreversible vascular rejection</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>&lt; 50 days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Graft survival &gt; 50 days</td>
<td>2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>74</td>
</tr>
<tr>
<td>Non-Immunological failure</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>88</td>
</tr>
</tbody>
</table>

Table I. Incidence of Circulating Endothelial Antibodies (EAb) in 97 Consecutive Renal Allograft recipients<sup>a</sup>

a. Two patients are excluded, one because of ABO-incompatibility [7] and another because pretransplant donor kidney tissue was not available.

The correlation between the presence of EAb and vascular rejection is significant (p < 0.001 on Fisher 2x2 table)

b. EAb present during severe rejection episodes

(Reproduced with permission of the Publisher of Transpl. Proc.)

show a significant correlation between the presence of EAb and irreversible vascular rejection; it should be noted, however, that factors other than EAb can also induce irreversible vascular rejection.

When EAb-positive sera were tested on sections of ABO-compatible pretransplant graft biopsies, it was found that IgG antibodies bound to the glomeruli, the peritubular capillaries (Figure 1), and the venules, indicating that the endo-
Figure 1. Photomicrograph of a pretransplant graft biopsy incubated with an EAb-positive ABO-compatible recipient serum and stained with fluorescein-conjugated swine antihuman IgG. Binding of IgG is present in the glomeruli and to the endothelium of peritubular capillaries (arrows). Magn. x 700
Magn. x 700

Endothelial antigens are present on the renal endothelium starting in and beyond the glomeruli (Table II). No binding of immunoglobulins was observed when sections were incubated with normal AB serum. However, incubation with blood group antisera resulted in binding of immunoglobulins not only to glomeruli, peri-

<table>
<thead>
<tr>
<th>TABLE II. Intrarenal Distribution of Endothelial- and ABO-antigens Determined on Sections of Pretransplant Biopsies of Renal Allografts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endothelial antigen</td>
</tr>
<tr>
<td>--------------------</td>
</tr>
<tr>
<td>Arteries</td>
</tr>
<tr>
<td>Arterioles&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glomeruli</td>
</tr>
<tr>
<td>Peritubular capillaries</td>
</tr>
<tr>
<td>Venules</td>
</tr>
<tr>
<td>Veins</td>
</tr>
</tbody>
</table>

<sup>a</sup>: Arterioles with an internal elastic lamina  
<sup>+</sup>: Antigen not present according to the immunofluorescence test  
<sup>+</sup>: Antigen present according to the immunofluorescence test
TABLE III. Reaction Pattern of EAb-positive Sera Against a Panel of Randomly Chosen Kidneys

<table>
<thead>
<tr>
<th>Panel Kidney</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
<th>XI</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ba</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
<td>(+)</td>
<td>-</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mo</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Z</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>W</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Br</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>(+)</td>
<td>-</td>
<td>(+)</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Li</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S</td>
<td>-</td>
<td>+</td>
<td>NT</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>La</td>
<td>-</td>
<td>NT</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>K</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ma</td>
<td>-</td>
<td>-</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

NT: not tested
(+): positive reaction may be due to ABO-incompatibility

tubular capillaries and venules, but also to the endothelium of arteries and afferent arterioles. When EAb-positive sera were tested against a panel of randomly chosen kidneys (Table III), positive reactions were obtained with a certain number of kidneys, as can be expected of alloantibodies. Although the results show a certain degree of polymorphism, no conclusions can as yet be drawn since no (cross-) absorption experiments were carried out and the number of sera and kidneys tested is small.

Discussion

In 1977 Moraes and Stastny [8] described an antigen system expressed in umbilical cord endothelial cells and monocytes. The alloantibodies against the endothelium of the graft which we found in 7 out of 12 recipients who lost their grafts due to irreversible vascular rejection (Table I), are probably directed against the same antigen system. These endothelial antigens are also present on monocytes but not on B— or T—lymphocytes [4,5]. In the kidney, the endothelial antigens seem to have a limited distribution; using the indirect immunofluorescence test, we found that EAb only bound to the glomeruli (Figure 1), the peritubular capillaries [1—4] and the venules [2,3] but not to the arteries and arterioles i.e. vessels with an internal elastic lamina.

Although the degree of polymorphism of the endothelial-monocyte system has not yet been determined, it can be expected on the basis of our preliminary data (Table III) that mismatches for these antigens should be quite common between donors and recipients. The question can be raised how the endothelial-monocyte system relates to the HLA system. Graft survival is usually excellent when kidneys are transplanted between HLA—identical siblings [9,10] although
irreversible rejections may occur [11,12]. In this situation the endothelial-mono
cyte system does not seem to play a major role. One explanation could be that
these antigens are coded for by a locus on the same (sixth) chromosome which
carries the HLA complex. The data obtained by Stastny [13] support this view.
However, we were unable to find a correlation between EAb and DR typing sera,
when tested against a panel of unrelated donors [5]. If the locus for endothelial-
monocyte antigens is not on the sixth chromosome, another explanation is needed
for the excellent survival of kidneys transplanted between HLA identical siblings.
One possibility could be that antigenic differences for HLA (especially for the
D-locus) are required for the induction of an immune response against endothelial-
monocyte antigens. Family studies should determine if the genes coding for
endothelial-monocyte antigens segregate with the HLA antigens.

Acknowledgments

We thank Miss Mary Mentink for her assistance in the preparation of the manu-
script.

References

1 Paul, LC, van Es, LA, van Rood, JJ, van Leeuwen, A, Brutel de la Rivière, G and
de Graeff, J (1979) Transplantation. 27, 175
2 Paul, LC, van Es, LA, Kalff, MW and de Graeff, J (1979) Transpl. Proc., 11, 427
3 Paul, LC, Fleuren, GJ and van Es, LA (1979) Transplantation (In press)
Med., 300, 1258
5 Claas, FHJ, Paul, LC, van Es, LA and van Rood, JJ (1979) Tissue Antigens (In press)
7 Paul, LC, van Es, LA, Brutel de la Rivière, G, Eernisse, G and de Graeff, J (1978)
Transplantation. 26, 268
504
10 Singal, DP, Mickey, MR and Terasaki, PI (1969) Transplantation. 7, 246
11 Lundgren, G, Magnusson, G, Moller, E, Nordenstrom, H, Werner, B and Westberg, G
(1972) Tissue Antigens. 2, 32
Antigens. 8, 233

Open Discussion

KOENE (Nijmegen) If these antibodies are important in rejection could you
comment on the fact why rejection of this type is not more frequently seen in
transplantation between identical siblings?

VAN ES There are various possibilities. One is that the locus which codes for
this antigen is on the 6th chromosome but at quite a distance from the locus that
codes for HLA. That would explain the excellent transplantation results in sib-
lings. There are preliminary data that this does not have to be the case. Even in
family transplantation the HLA and the endothelial-monocyte antigens may dissociate. This is preliminary and we are not very sure. There is a second possibility and that is that the immune response against endothelial-monocyte antigens depends on the presence of a difference for the HLA antigen and in particular the antigen of the HLA D locus. At this moment we cannot differentiate between those two possibilities.

DOSSETOR (Edmonton, Canada) I think this is an important antigen system. In some work that we have done with similar sera which are again only marking monocytes, we did find in informative segregant studies in families, that sera did segregate with the HLA haplotype even though they were clearly not HLA—A, —B or DR antigens. I wonder if you have done any informative segregant family studies?

VAN ES No we have not.

VRIESMAN (Maastricht) Is it possible that you have two sets of antigens, one on peritubular capillaries and one on the glomeruli which looked a little bit like a GBM type of pattern?

VAN ES We haven’t done any cross absorption studies. By the way, from the panel studies that we did with eleven sera that we have now against endothelial-monocyte antigens it becomes very clear that the system is polymorphic, but to determine the degree of polymorphism we have to do cross-absorptions. Cross absorptions would also answer your question but since we haven’t done it yet, I cannot answer your question.