Does Histocompatibility Affect Homograft Valve Function After the Ross Procedure?

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Background—Homograft valves have been shown to be immunogenic, but it is unknown whether this affects valve function. Therefore, we prospectively studied the degree of histoincompatibility (defined as the number of human leukocyte antigen [HLA] mismatches between valve donor and recipient) and the response of the recipient (measured by antibodies against HLA) in relation to echocardiographic parameters of homograft valve function after the Ross procedure.

Methods and Results—Twenty-six patients (mean age 41±14 years; 20 males, 6 females) and the cryopreserved pulmonary homograft valves that were implanted during a Ross procedure were typed for HLA-A, HLA-B, and HLA-DR. After a mean follow-up of 15±6 months, 14 (54%) of the patients were anti–HLA class I antibody positive. In all but 1 patient, these antibodies were shown to be donor specific. During follow-up, there was a significant increase of the maximal (+6.2±7.1 mm Hg) and mean (+3.2±4.3 mm Hg) transhomograft pressure gradients but not of homograft regurgitation. Neither the number of HLA mismatches nor antibody status was found to have significant impact on homograft valve function. In a multivariate analysis, smaller homograft size (P=0.001) and younger recipient age (P=0.044) were shown to be significantly associated with increased transhomograft pressure gradients.

Conclusions—Implantation of a cryopreserved pulmonary homograft during the Ross procedure can induce a specific humoral response. We observed a significant increase of the transhomograft pressure gradients within 15±6 months after surgery. For this period, we were unable to demonstrate a relationship between this increase and the degree of histoincompatibility. (Circulation. 2001;104[suppl I]:I-25-I-28.)

Key Words: valves ■ surgery ■ transplantation ■ immunology

The pulmonary autograft, or Ross procedure, for the treatment of aortic valve disease is associated with excellent long-term results.1-3 However, a 1-valve disease is turned into 2-valve-surgery, and although it is apparently rare, reoperation of the homograft used for reconstruction of the right ventricular outflow tract (RVOT) occurs.1 A more sensitive marker for homograft failure than the rate of reoperation is the pressure gradient across the homograft, which can be measured noninvasively by echocardiography. Some.4-6 but not all,3 echocardiographic studies following the Ross procedure have recently shown an increase of the transhomograft pressure gradient with time. The cause of this increase is unknown. Homograft valves are known to be immunogenic,7,8 but are transplanted without donor-recipient matching for blood group or human leukocyte antigens (HLAs). Therefore, homograft degeneration may be immunologically mediated, but this has not been explicitly examined for the Ross procedure so far. Therefore, in the present prospective study, we investigated whether homograft valve function is adversely affected (1) by the degree of histoincompatibility (defined as the number of HLA-A, HLA-B, or HLA-DR mismatches between valve donor and recipient) or (2) by the provoked immune response (measured by the presence of specific alloantibodies against HLA class I determinants of the donor during follow-up).

Methods
Of all the patients undergoing a Ross procedure between January 1998 and July 1999 (n=41), a random sample of 26 patients (63%; mean age 41±14 years; 20 males, 6 females) was studied. The present study was approved by the local ethics committee, and informed consent was obtained from the patients.

Operative Technique
All operations were performed through a median sternotomy with hypothermic (26°C) cardiopulmonary bypass and cold crystalloid cardioplegia (4°C, St Thomas’ Hospital solution) for myocardial protection. For the Ross procedure, in brief, the diseased aortic valve was excised first. Then the pulmonary autograft was harvested from the RVOT, if it was found to be morphologically normal, it was implanted as an aortic valve substitute either as a total root replacement (n=2) or by placement inside the aortic root in the

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subcoronary position (n=24). Cryopreserved pulmonary homografts (mean diameter 25.5±1.6 mm) were then thawed and implanted into the RVOT during rewarming. During or after surgery, only 8 patients (31%) had any kind of allogenic blood transfused.

HLA Genotyping of Valve Donor and Recipient
All recipients were serologically typed for HLA-A and HLA-B (Lymphotype HLA-ABC, Biotest). Serological HLA typing of the valve donor was available from the homograft bank in 17 patients. In the remaining 9 patients, HLA typing of the valve was performed from a small specimen of the homograft myocardium that had been harvested during surgery and stored at −80°C. In brief, the DNA was first isolated enzymatically. HLA class I typing was performed by using a polymerase chain reaction (PCR) protocol with sequence-specific primers (Micro SSP Generic HLA Class I DNA Typing Tray, One Lambda Inc). HLA-DR typing was performed by using a PCR protocol with sequence-specific oligonucleotides (INNO-LiPA DRB/DQB Key Amplification, Innogenetics NV) and standard interpretation software (INNO-LiPA Expert Genotyping Program, Innogenetics NV).

Screening for Anti-HLA Antibodies
The patients underwent anti–HLA class I antibody determination before surgery, ~1 week after surgery, and at least once during follow-up. Whole blood was centrifuged immediately, and serum samples were stored at −80°C until analysis. All reagent antibodies in serum specimens were detected by using complement-dependent cytotoxicity testing (National Institutes of Health standard).9 Lymphocytes from 50 HLA-typed blood donors were used to determine panel reactive antibodies (PRAs). Results are expressed as percentage PRA, ie, the number of positive donors divided by the number of donors tested times 100. A PRA >6% was considered a positive antibody determination.

Echocardiographic Data Acquisition and Measurements
Echocardiography was performed before discharge (6.9±2.6 days after surgery) and at the most recent follow-up appointment (14.8±6.0 months after surgery). Resting transthoracic echocagrams were created with 2.5-MHz ultrasound transducers (Hewlett-Packard Sonos 2500 System) in standard longitudinal and cross-sectional views and were recorded on videotape. A modified echocardiogram lead I was continuously recorded. Maximum velocities across the pulmonary homograft valve were calculated by a continuous-wave Doppler imaging transducer. For determination of the pressure gradient, a modified Bernoulli equation (Δp=4v², where Δp is the pressure gradient, and v is the velocity across the valve) was used. To assess pulmonary homograft regurgitation, pulsed wave, continuous wave, and color flow Doppler were performed. Semiquantitative assessment from grade 0 to 3 of pulmonary homograft regurgitation was based on the length and width of the regurgitant jet and the distance that it reaches into the RVOT on the parasternal short-axis view.

Statistical Analysis
Data are presented as absolute numbers and percentages or as mean±SD. Relative frequencies were compared by use of the Fisher exact test, and means were compared by use of the t test or Mann-Whitney U test. The Wilcoxon test was used to analyze paired data. Linear regression analysis was used to test for an association between the number of HLA mismatches and the PRA value. For analysis of the determinants of the pressure gradients across the homograft at follow-up, multiple regression analysis using the natural logarithm of the (not normally distributed) gradients was performed. All analyses were performed with the use of Minitab, release 12, or the SAS package.

Results
The implanted pulmonary homograft had no HLA-A, HLA-B, or HLA-DR mismatch in 3 patients, 1 or 2 HLA-A mismatches in 7 and 3 patients, 1 or 2 HLA-B mismatches in 5 and 4 patients, and 1 or 2 HLA-DR mismatches in 10 and 3 patients. No patient had detectable levels of anti–HLA class I antibodies before surgery.

Echocardiographic Results
Before discharge, the maximal and mean transhomograft pressure gradients measured 5.3±2.5 and 3.3±1.4 mm Hg, respectively. One patient had mild homograft regurgitation; homograft regurgitation could not be determined in 8 patients (no suitable echocardiographic window in 5 patients and missing study in 3 patients), and there was no regurgitation in the other patients. At the most recent follow-up, the maximal and mean transhomograft pressure gradients measured 13.0±7.9 and 7.5±4.7 mm Hg, respectively. Six (24%) patients had mild homograft regurgitation; there was no homograft regurgitation in the other patients. Therefore, there was a significant increase of the maximal (P=0.002) and mean (P=0.005) pressure gradients during follow-up but no significant evidence of an increase in the degree of homograft regurgitation (P=0.06). The pressure gradients and the degree of homograft regurgitation at follow-up did not differ significantly whether or not the patients had received blood transfusions.

Anti–HLA Class I Antibodies
In a single patient, anti–HLA class I antibodies were detected as early as 1 week after surgery, but all other blood samples taken early after surgery were negative for alloantibodies. At the most recent follow-up, 14 (54%) patients were positive for anti–HLA class I antibodies. Table 1 shows the distribution of various clinical characteristics according to antibody status. In all but 1 patient, these antibodies could be shown to be donor specific. The 1 patient in whom antibody specificity could not be determined had broad-panel reactivity, precluding the determination of specificity. Alloantibody-positive patients had a significantly higher number of HLA-A (P=0.008) and HLA-B (P=0.025) mismatches than did antibody-negative patients. The median PRA measured 54%. There was a significant association between the number of HLA-A (P=0.016) or HLA-B (P=0.016) mismatches (either
According to Anti–HLA Class I Antibody Status

### TABLE 2. Transpulmonary Homograft Pressure Gradients and Regurgitation Across Pulmonary Homograft at Latest Follow-Up According to Anti–HLA Class I Antibody Status

<table>
<thead>
<tr>
<th>Anti–HLA Antibody Status</th>
<th>Negative</th>
<th>Positive</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \Delta p ) maximal, mm Hg</td>
<td>11.3±4.1</td>
<td>14.6±10.1</td>
<td>0.85</td>
</tr>
<tr>
<td>( \Delta p ) mean, mm Hg</td>
<td>6.2±2.3</td>
<td>8.7±6.1</td>
<td>0.46</td>
</tr>
<tr>
<td>Transhomograft regurgitation, n (%)</td>
<td></td>
<td></td>
<td>0.38</td>
</tr>
<tr>
<td>None</td>
<td>8 (67)</td>
<td>11 (85)</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>4 (33)</td>
<td>2 (15)</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SD or as indicated.

 alone or combined) and the PRA value (Figure). There was no significant relation between HLA-DR mismatches and the presence of anti–HLA class I antibodies (\( P=0.77 \)).

**Histoincompatibility and Homograft Function**

Baseline transhomograft pressure gradients and regurgitation did not differ according to anti–HLA class I antibody status at the most recent follow-up. Table 2 summarizes the echocardiographic results at most recent follow-up according to anti–HLA class I antibody status. There was no evidence of a significant influence of antibody status on homograft valve function. There was no association between the number of HLA-A or HLA-B mismatches and the maximal transhomograft pressure gradient (\( P=0.86 \) and \( P=0.70 \)), the mean pressure gradient (\( P=0.73 \) and \( P=0.58 \)), or the degree of homograft regurgitation (\( P=0.20 \) and \( P=0.75 \)). There was no association between the number of HLA-DR mismatches and homograft regurgitation (\( P=0.89 \)), but a higher number of HLA-DR mismatches was associated with significantly lower maximal (\( P=0.020 \)) and mean (\( P=0.023 \)) transhomograft pressure gradients. To further analyze these significant negative associations, a multivariate analysis, including the number of HLA-DR mismatches as well as clinical variables that might affect transhomograft pressure gradients (sex, age, weight, height, and homograft diameter), was performed, and only homograft diameter and age at operation remained significant; ie, a larger homograft and older age were associated with lower maximal (\( P=0.001 \) and \( P=0.044 \)) and mean (\( P=0.001 \) and \( P=0.040 \)) transhomograft pressure gradients at follow-up.

**Discussion**

The present study provides evidence that the implantation of a cryopreserved homograft during the Ross procedure can induce a specific humoral immune response. However, no relationship between the degree of histoincompatibility and homograft valve function could be demonstrated during a mean follow-up of 15±6 months.

The Ross procedure offers excellent hemodynamic and clinical results in the vast majority of patients. It compares at least favorably with aortic homograft implantation and even offers growth potential in children. However, it puts 2 valves at risk for failure. The risk of pulmonary homograft failure leading to reoperation appears to be low (15% at 20 years). Regarding valve function, we (as well as others) report a significant increase of the transhomograft pressure gradients with time. Therefore, knowledge of the factors that might exert a negative effect on homograft valve function is of great importance for further improving the results of the Ross procedure.

Implantation of a cryopreserved homograft is reported to be associated with early cellular autolysis and severe degeneration with disruption of normal architecture and loss of endothelial and interstitial cells. In explanted homografts, calcifications are often observed, and if stenotic, the obstruction is often located above the valve level. Inflammatory cell infiltrates are usually reported to be absent or trivial, but this finding is controversially discussed. Rajani et al found that explanted homografts of infants showed thickened leaflets and that the leaflets and aortic sleeves contained a hyperplastic intimal layer with numerous spindle cells (similar to those seen in coronary arteries in transplant vasculopathy) and multiple foci of inflammation. In addition, after heart transplantation with immunosuppression, the aortic valve shows remarkable structural preservation and a low tendency for calcification. These findings may indicate that there is immune-mediated dysfunction of homograft valves.

With the present preservation protocols, homograft valves can be expected to contain at least some viable cells at the time of implantation, resulting in a specific cellular and humoral immune response directed against HLA determinants of the donor. For aortic homograft valves, but not for pulmonary homografts, clinical or echocardiographic and immunologic parameters have been studied simultaneously. Dignan et al recently reported that 2 mismatches at the HLA-DR locus resulted in a significantly higher rate of structural deterioration of aortic homograft valves. There was no such association for HLA class I mismatches. Multivariate analysis revealed age of operation <25 years and a time from procurement until cryopreservation of <4 hours (but not HLA-DR mismatch) as independent predictors for structural deterioration. In the present study, we also found a significant, but inverse, relationship between the number of HLA-DR mismatches and homograft valve function. In the multivariate analysis, however, only homograft size, not studied by Dignan et al, and age at operation were found to affect the transhomograft pressure gradient. Although
HLA-DR matching has a significant impact on graft survival in cardiac transplantation\textsuperscript{18} and although the immunogenicity of homograft valves can be reduced in vitro by HLA-DR matching,\textsuperscript{19} it should be noted that the immunogenicity of homograft valves seems to be mainly mediated by endothelial cells, which rapidly lose HLA class II expression during storage and antibiotic treatment of valve tissue.\textsuperscript{20}

Anti-HLA antibodies are associated with decreased survival after cardiac transplantation.\textsuperscript{21} However, after a study of aortic valve homograft implantation, no significant differences were found regarding long-term valve function according to anti-HLA class I or II antibody status (or the degree of HLA class I or II mismatch),\textsuperscript{22} which is in accordance with our results in pulmonary homograft recipients. Our finding that smaller homografts were associated with higher pressure gradients at follow-up is consistent with a “shrinking” process of the homograft. The fact that younger age at operation was also associated with higher pressure gradients at follow-up may raise the hypothesis that this shrinking process is immune-mediated, but our immunologic studies provide no evidence for such a hypothesis.

Limitations of the Study
We have not studied blood group incompatibilities, but a recent study indicates that ABO antigens are not expressed on cardiac valves.\textsuperscript{23} Because of the noninvasive nature of the present study, we were not able to investigate whether or not infiltration of the homograft with inflammatory cells occurred. Therefore, we cannot disprove rejection, but we were unable to demonstrate any relationship between the supposed shrinking process and either the degree of histoincompatibility or the humoral response of the recipient. However, we have demonstrated a specific response in the recipient that is graded according to the degree of histoincompatibility, and the missing association between immunologic markers and homograft valve function may be explained by the small number of HLA-matched patients. Therefore, it will be crucial to extend follow-up length and sample size, the main limitations of the present study, to validate our findings. The significant difference in the length of follow-up between antibody-negative and -positive patients seems of minor concern. First, the longer follow-up in antibody-positive patients should be expected to bias the results toward a group difference. Second, we have found an almost identical prevalence (57%) of anti-HLA antibodies in a cross-sectional study (47 patients, examination 25±14 months after surgery),\textsuperscript{24} and others have reported that by 1 year the maximal prevalence of antibody positivity has already been reached.\textsuperscript{25}

In conclusion, we observed a significant increase of the transhomoft graft pressure gradients during a mean follow-up of 15±6 months, but we were unable to provide evidence that this increase is dependent on the degree of histoincompatibility between valve donor and recipient.

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References
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