**The Effect of Allogeneic or Xenogeneic Immune Responses and Preservation Techniques on Transplanted Aortic Valve Grafts**

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**Summary:** We examined the effect of allogeneic and xenogeneic immune responses on the histopathological changes in aortic valve grafts and the influence of preservation techniques on these changes. Brown Norway rats and Syrian hamsters were used as allogeneic and concordant xenogeneic donors of aortic valve grafts, respectively. The allografts and xenografts were implanted heterotopically in the abdominal aorta of Lewis rat recipients immediately after harvest (homovital), after cryopreservation, or after preservation with antibiotics at 4 °C (fresh preservation). Allografts and xenografts were explanted at days 7, 28 or 56 and at days 3, 7 or 14, respectively, for the histopathological examination. The allografts underwent histological changes characteristic of graft arteriosclerosis. No significant effect of cryopreservation on these changes was observed. The fresh-preserved graft was, however, predisposed to focal destruction of the elastic fibers and to early disappearance of the leaflet. The lesions in xenografts were characterized by severe destruction of the elastic fibers. Compared to homovital xenografts, both cryopreserved and fresh-preserved xenografts showed more prominent disruption of the elastic fibers, well-developed valvular and vascular thrombi and earlier disappearance of the leaflet. In conclusion, it could be assumed that failure in retention of cellular and extracellular components during fresh preservation accelerates structural deterioration of allografts. As for xenografts, even the extracellular matrix may have potential xenogeneic immunogenicity. There is a possibility of these preservation techniques reducing xenogeneic immunogenicity of the endothelial cells, probably because of loss of these cells. However, it appears that, even in this setting, other cellular and extracellular components could trigger immune responses causing structural deterioration of xenografts.

**Key words** aortic valve, allotransplantation, xenotransplantation, cryopreservation, rejection

**INTRODUCTION**

The allograft heart valve is acknowledged to be a preferred substitute for aortic valve replacement, because of its excellent hemodynamics, low thrombogenicity without anticoagulation and resistance to infections [1-4]. Cryopreserved allografts in combination with sterilization by low-dose antibiotics or homovital (nontreated) allografts are now widely recognized as the ideal allografts for producing long-term performance [5,6]. However, there are variable factors, such as warm ischemic time, the age of donors and of recipients, the valve position in donors or in recipients and insertion techniques, which can affect the grafts’ durability [5]. Even if we can obtain truly homogeneous allografts, it is still not clear to what degree immunological reactions affect the structure and function of the allograft heart valve.

In a review of previous reports describing explanted allografts from infants, we noted lymphocytic infiltration in the leaflets suggesting that rapid valve failure in infants is caused by transplant rejection [7,8]. However, in spite of previous investigations indicating a donor-specific immune response...
evoked by allograft insertion in human and in animal models [9-12], explanted allografts from adults show no evidence of rejection in the leaflet tissue itself [8,13]. Moreover, Shoen et al. emphasize the importance of a residual connective tissue matrix of the allograft for long-term performance, because the allograft is morphologically nonviable [13].

From this viewpoint, because of a shortage of allografts from donors, especially in Japan, there is currently an interest in the use of the viable xenogeneic matrix, which has mechanical properties superior to glutaraldehyde-fixed xenografts [14]. One approach to overcoming xenogeneic immune responses is removal of the original cells while maintaining the viable extracellular matrix [15,16]. However, the effect of actual immune responses on xenograft valves, containing viable cellular and acellular components, and the magnitude of these immune responses remains unknown. To compare allogeneic and xenogeneic immune responses may facilitate the clinical application of viable xenografts as a new device for heart valve replacement. The aim of this study is to observe the behavior of allogeneic or xenogeneic immune responses on graft tissue and to determine the impact of preservation techniques on the degree of tissue damage.

MATERIALS AND METHODS

Animal models

Strongly allogeneic models were experimentally established with a combination of Brown Norway (RT-1\(n\)) and Lewis (RT-1\(1\)) rat strains, and a combination of Syrian hamsters and Lewis rats was used as concordant xenogeneic models. Inbred male Brown Norway rats weighing 200 to 250 g and inbred male Syrian hamsters weighing 150 to 200 g were used as aortic valve donors. Inbred male Lewis rats weighing 350 to 400 g were used as recipients. Donor aortic valve grafts were implanted in recipient Lewis rats immediately after harvesting as the homovital model. Others were cryopreserved or fresh-preserved until implantation.

Cryopreservation

For 24 hrs after harvesting, aortic valve grafts were sterilized in a culture medium containing TC-199 with 10% calf serum, 5% HEPES buffer and the following antibiotics: cefoxitin (240 mg/L), vancomycin (50 mg/L), lincomycin (120 mg/L), and polymixin B (100 mg/L). After sterilization, grafts were placed in individual containers with a medium containing TC-199 with 10% calf serum, 5% HEPES buffer and 10% dimethylsulfoxide (DMSO) for cryopreservation. The grafts were then frozen at a rate of 1 °C/min to -80 °C with the use of a computer-programmed freezer (Planer KRYO 10, Diamed Laboratory Supplies, Mississauga, Ontario, Canada) and stored at -196 °C in the vapor phase of liquid nitrogen for 2 to 3 months. At implantation, the graft was thawed quickly in a hot water bath at 40 °C and then placed in Ringer’s solution for 15 min to wash out DMSO, which could injure the graft cells at higher temperatures.

Fresh preservation

The culture medium for fresh preservation was composed of TC-199 with 10% calf serum, 5% HEPES buffer and the antibiotics previously described. Aortic valve grafts were stored with the

Principles of Laboratory Animal Care formulated by the Institute of Animal Resources and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Harvest of aortic valve grafts

Donor Brown Norway rats and donor Syrian hamsters underwent general anesthesia with 60 mg/kg of sodium pentobarbital administered by intraperitoneal injection. Under clean conditions, the heart with a segment of the ascending aorta was explanted and rinsed in a container with chilled Ringer’s solution. The aortic valve with a cuff of ventricular muscle and a segment of the ascending aorta 1.0 cm in length was dissected. The coronary arteries were ligated with an 8-0 polypropylene suture (Prolene, Ethicon Inc., Somerville, NJ). Blood in the valved aortic conduit was gently washed out, and excess myocardial muscle was trimmed. Some donor grafts were implanted in recipient Lewis rats immediately after harvesting as the homovital model. Others were cryopreserved or fresh-preserved until implantation.
medium at 4 °C for 2 weeks until implantation.

**Heterotopic implantation of aortic valve grafts**

Each recipient underwent heterotopic implantation of an aortic valve graft using a modification of the surgical technique described by Yankah et al. [17]. Under general anesthesia with 50 mg/kg pentobarbital sodium administered by intraperitoneal injection, a median laparotomy was performed. The infrarenal abdominal aorta was dissected and divided for proximal and distal clamping. Aortic valve grafts were anastomosed in an end-to-end fashion with 8-0 polypropylene sutures using 2.5× magnifying glasses. While the proximal anastomosis between the proximal abdominal aorta and the proximal end of the graft was being carried out, the anterior leaflet of the aortic valve graft was incorporated into the suture line to make valvular incompetence. Then the distal anastomosis between the distal abdominal aorta and the distal end of the graft was performed. The entire procedure was completed within 45 min. After the patency of the anastomosis was confirmed by palpation of the distal aorta, the laparotomy wound was closed and recipients were allowed to recover. No anticoagulants and no antibiotics were administered during or after the surgery. The recipient rats were fed a standard diet postoperatively.

**Explantation of aortic valve grafts**

The recipient rats were sacrificed and the grafts were explanted, including the recipient’s native abdominal aorta. Allografts were explanted at 7, 28 or 56 days after implantation, and xenografts were explanted at 3, 7 or 14 days after implantation, as shown in Tables 1 and 2. Any recipients that developed claudication postoperatively or died before the designed time were excluded from this study. Those recipients in which graft occlusions were observed at explantation were also excluded.

**Morphological and histopathological study**

Explanted aortic valve grafts were fixed in 10% buffered formalin solution. For removal of any air in the graft and for proper fixation, 10% formalin was injected into both ends of the explanted graft. After the dehydration process, grafts were embedded in paraffin and then were sectioned into 4-μm sections. These sections were stained with hematoxylin-eosin and elastic van Gieson stain. Intimal thickening, media necrosis and perivascular infiltration with inflammatory cells were examined and graded on a scale of + to +++. Overall valve damage was also examined. The scale used is as follows:

- : nil
+ : mild
++ : moderate
+++ : severe

The six models of aortic valve grafts before implantation were also examined as controls. Morphological and histopathological analyses were performed, blinded from the original protocol and the study design.

**RESULTS**

**Surgical results**

Of the 72 rats undergoing implantation, 66 rats survived without developing claudication during the designed time period before they were sacrificed for explantation. The claudication that did occur, in 6 rats, was observed immediately following surgery. Seven grafts were totally occluded with thrombi at the time of explantation, which left 59 grafts that were acceptable for histopathological examination (Tables 1 and 2).

| TABLE 1. Number of explanted allografts in each group at each time point (successful grafts without occlusion / total explanted grafts) |
|---|---|---|---|
| Group | 7 days | 28 days | 56 days |
| H-A | 3/3 | 3/3 | 2/3 |
| C-A | 2/3 | 3/3 | 3/3 |
| F-A | 3/3 | 3/3 | 3/3 |

H-A: homovital-allogeneic; C-A: cryopreserved-allogeneic; F-A: fresh-allogeneic

| TABLE 2. Number of explanted xenografts in each group at each time point (successful grafts without occlusion / total explanted grafts) |
|---|---|---|---|
| Group | 3 days | 7 days | 14 days |
| H-X | 3/3 | 3/3 | 3/3 |
| C-X | 5/5 | 4/5 | 4/5 |
| F-X | 5/5 | 4/5 | 3/5 |

H-X: homovital-xenogeneic; C-X: cryopreserved-xenogeneic; F-X: fresh-xenogeneic
Preimplant grafts

Before implantation, neither fresh-preserved grafts nor cryopreserved grafts showed evidence of histological changes (that is, the grafts maintained cellularity and the fibers maintained elasticity) compared with nontreated homovital grafts.

Morphological and histopathological findings of explanted grafts

Tables 3 and 4 summarize the morphological and histopathological findings of explanted aortic valve grafts at the designed time points.

No histological change was observed in the recipient’s native aorta just proximal or distal to the graft, indicating that the surgery did not provoke pathologic lesions outside the transplanted portion. Homovital-allogeneic (H-A) grafts: At 7 days, severe inflammatory infiltration consisting mainly of polymorphonuclear neutrophils was seen in the adventitia, and the media showed few or no infiltration of these inflammatory cells. The intima was lost focally without infiltration by inflammatory cells. The leaflet was preserved during this time period (Fig. 1). At 28 days, the intima showed mild to moderate thickening consisting of noninflammatory cells. Focal areas of media necrosis were observed; however, the elastic fibers appeared normal in the unaffected areas of the

| TABLE 3. |
| Histological assessment of the aortic valve allograft at each time point |

<table>
<thead>
<tr>
<th>Graft</th>
<th>IT</th>
<th>MN</th>
<th>PI</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-A</td>
<td>7 days</td>
<td>0+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>28 days</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>56 days</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>C-A</td>
<td>7 days</td>
<td>0+</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>28 days</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>56 days</td>
<td>+++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>F-A</td>
<td>7 days</td>
<td>0+</td>
<td>+</td>
<td>++</td>
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<td></td>
<td>28 days</td>
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<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>56 days</td>
<td>+++</td>
<td>++</td>
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</tr>
</tbody>
</table>

H-A: homovital-allogeneic; C-A: cryopreserved-allogeneic; F-A: fresh-allogeneic; IT: intimal thickening; MN: media necrosis; PI: perivascular inflammation; PL: preserved leaflet

| TABLE 4. |
| Histological assessment of the aortic valve xenograft at each time point |

<table>
<thead>
<tr>
<th>Graft</th>
<th>IT</th>
<th>MN</th>
<th>PI</th>
<th>D</th>
<th>PL</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-X</td>
<td>3 days</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>0+</td>
<td>0+</td>
<td>++</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>focal</td>
</tr>
<tr>
<td>C-X</td>
<td>3 days</td>
<td>0</td>
<td>0+</td>
<td>+</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>focal</td>
</tr>
<tr>
<td></td>
<td>14 days</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>diffuse</td>
</tr>
<tr>
<td>F-X</td>
<td>3 days</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>no</td>
</tr>
<tr>
<td></td>
<td>7 days</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
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<tr>
<td></td>
<td>14 days</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>diffuse</td>
</tr>
</tbody>
</table>

H-X: homovital-xenogeneic; C-X: cryopreserved-xenogeneic; F-X: fresh-xenogeneic; IT: intimal thickening; MN: media necrosis; PI: perivascular inflammation; D: destruction of the elastic fibers; PL: preserved leaflet

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media and there were a limited number of inflammatory cells. On the other hand, the adventitia showed prominent cellular infiltration, mainly by lymphocytes and macrophages. The leaflet was also preserved at this time; however, it was dysplastic, with an occasional valvular thrombus. At 56 days (Fig. 2), the intima was thickened resulting from marked proliferation, consisting of spindle cells and few inflammatory cells, with luminal narrowing. The elastic fibers in the media appeared normal, although the media showed diffuse and full-thickness necrosis. In the adventitia, dense lymphocytic infiltration remained but had regressed. Free leaflets no longer persisted at this time because they had been covered with neointimal proliferation.

Cryopreserved-allogeneic (C-A) grafts: The characteristics of histopathological changes in the C-A graft at 7 days did not differ significantly from those in the H-A graft.

Fresh-allogeneic (F-A) grafts: The histopathological characteristics in the F-A graft did not differ significantly from those in the H-A graft. However, media necrosis was complete and diffuse with focal destruction of the elastic fibers at 28 days (Fig. 3). In addition, the aortic valve showed dysplastic leaflets with well-developed valvular thrombi at 7 days (Fig. 4), and free leaflets had disappeared at 28 days.

Homovital-xenogeneic (H-X) grafts: At 3 days, focal monocellular infiltration involved the adventitia, and medial cellularity was preserved without damage to the internal elastic lamina. Regarding the aortic valve, preserved leaflets focally showed attachment and infiltration of inflammatory cells, and no thrombi were associated with these pathological leaflets (Fig. 5). At 7 days, the leaflet showed more prominent infiltration, although the leaflet was preserved with no valvular thrombi (Fig. 6). At 14 days, there was loss of medial cellularity with focal disruption of the internal elastic lamina and the increase of adventitial thickness due to more prominent infiltration. The sections at the valvular levels no longer showed preserved leaflets, but showed focal thrombi, which were rarely seen in the distal aortic conduit. There was intimal thickening only focally in the H-X graft at 7 and 14 days.

Cryopreserved-xenogeneic (C-X) grafts: At 3 days (Fig. 7), the endothelial cells were sparsely preserved and intimal thickening was not observed. Contrary to the H-X graft, the leaflet appeared normal without cellular infiltration; however, valvular thrombi had already developed. The media showed loss of cellularity without destruction of the internal elastic lamina. Perivascular monocellular infiltration in the C-X graft was as prominent as that in the H-X graft at each time point. The sections of the aortic root no longer showed preserved leaflets at 7 days (Fig. 8). The aortic conduit at this time point showed neointimal proliferation and vascular thrombi, which

Fig. 1. H-A graft at 7 days showing preserved leaflet, inflammatory cell infiltration in the adventitia. Medial cellularity is preserved and the elastic fibers are intact. (Original magnification ×50. Hematoxylin-eosin stain)

Fig. 2. H-A graft at 56 days. Intimal proliferation develops towards the lumen (triangle), and monocellular infiltration in the adventitia is regressed. The media shows almost normal elastic fibers, although loss of medial cellularity is seen. (Original magnification ×25. Hematoxylin-eosin stain)
Fig. 3. F-A graft at 28 days showing complete and diffuse media necrosis and intimal proliferation. Focal destruction of the elastic fibers is seen (arrow). (Original magnification ×25. Elastic van Gieson stain)

Fig. 4. F-A graft at 7 days. The leaflets (small arrow) are dysplastic associated with well-developed valvular thrombi (large arrow). (Original magnification ×25. Hematoxylin-eosin stain)

Fig. 5. H-X graft at 3 days. The leaflets are preserved, although they are focally infiltrated by inflammatory cells (arrow). (Original magnification ×50. Hematoxylin-eosin stain)

Fig. 6. H-X graft at 7 days showing preserved but diffusely infiltrated leaflets. Medial cellularity is preserved with the intact elastic fibers. (Original magnification ×50. Hematoxylin-eosin stain)
resulted in luminal narrowing, and it became concentric at 14 days. Diffuse media necrosis was established at 7 days with focal destruction of the internal elastic lamina. Disruption and disappearance of the elastic fibers were diffusely observed at 14 days (Fig. 9).

*Fresh-xenogeneic (F-X) grafts*: Histological changes in the F-X graft, including early development of valvular thrombi, complete and diffuse media necrosis associated with marked destruction of the elastic fibers, and intimal thickening causing luminal narrowing, did not differ significantly from those in the C-X graft (Figs 10, 11 and 12).
DISCUSSION

It has been demonstrated that human homovital or cryopreserved aortic valve allografts elicit a donor-specific immune response, including donor-specific immunoglobulin G antibodies and T cell-mediated reactions to human leukocyte antigens [10-12]. Nevertheless, it remains unclear whether this allogeneic response induces histopathological and morphological changes resulting in late valve failure. Because of a shortage of allografts, there is also a new interest in the xenogeneic extracellular matrix; however, actual immune responses to the xenograft valve containing viable cellular and acellular components are unknown. This study has demonstrated the effect of allogeneic and xenogeneic immune responses on the histological changes of aortic valve grafts in rodent combinations. To our knowledge, so far a study using aortic valved conduits with a note on morphological changes in the leaflet and on the impact of preservation techniques has not been reported.

Aortic valve allografts

In agreement with previous investigations of the aortic allograft in rats [18,19], this study suggests that acute and chronic rejection play an important role in the aortic allograft, because the aortic wall underwent histopathological changes, including an increase in intimal cellularity and a decrease in medial cellularity associated with perivascular mononuclear infiltration, which are characteristic of graft arteriosclerosis in vascularized allografts. Indeed, vascular allografts, such as rodent aortic grafts [20-22] or porcine coronary artery grafts [23], have been used as an experimental model to investigate graft arteriosclerosis.

Of course, nonspecific inflammation mainly by polymorphonuclear neutrophils soon after implantation and endothelial injury during procurement and preservation, could be additional causes of these histological changes. Moustapha et al. described a loss of medial cellularity and intimal proliferation even in the syngeneic graft, and they suggested that nonspecific inflammation, which is another potential source of the release of cytokines, growth factors and other factors, is also responsible for these histologic changes [18]. However, intimal thickening and media necrosis due to these causes are less prominent and not progressive [18,19]. Therefore, the prominent and progressive changes of the allografts in this study could be considered as evidence of transplant rejection.

As for the leaflet itself, the results of this study did not allow us to determine the degree of leaflet tissue damage by alloreactivity because the leaflets were covered with vascular neointimal proliferation. However, the development of valvular thrombi could be a consequence of an allogeneic immune response to the leaflet tissue. A potential mechanism of such findings may be the expression of cell adhesion molecules on the endothelial cells upregulated by immune effectors, such as cytokines, growth factor and other factors [24,25].
The cryopreservation technique has been thought to attenuate allograft immunogenicity because of the reduced viability or cellularity of the endothelium [24,26] or the diminished expression of leukocyte adhesion molecules [25]. This study, however, showed no significant effect of cryopreservation on these histopathological changes in the aortic wall or in the leaflet, indicating that cryopreservation does not alter allograft immunogenicity as noted in previous studies in the rat model [18,27]. On the other hand, the fresh-preserved graft was predisposed to prominent media necrosis causing focal destruction of the elastic fibers, despite less prominent adventitial lymphocytic infiltration. Furthermore, the leaflets in this group showed earlier dysplasia and disappearance. These findings in the fresh-preserved graft may have been due to the reduced cellular viability resulting from the cytotoxic effect of this preservation technique and failure in retention of the extracellular matrix [26,28,29]. Preservation with antibiotics has also been thought to reduce allograft viability resulting from the cytotoxic effect of this preservation technique and failure in retention of the extracellular matrix [26,28,29]. However, in this study using the rat model, retention of cellular viability and of the extracellular matrix also have potential xenogeneic immunogenicity resulting in medial elastic injury. In our study, all 3 types of xenograft examined had developed considerable degeneration of the elastic fibers in the media within 14 days of implantation, whereas the allograft showed elastin injury only focally. In addition, elastin injury in the xenograft was characterized by disruption and disappearance of the elastic fibers. These changes were never observed in the allograft, suggesting that the extracellular matrix also have potential xenogeneic immunogenicity [36].

Regarding the impact of preservation techniques, there were two notable differences between the histological and morphological changes of the homovital graft and those of the cryopreserved or the fresh-preserved graft. The first was the timing of loss of medial cellularity associated with destruction of the elastic fibers. Such findings in the homovital graft and in the latter 2 types of preserved grafts became evident at 14 days and at 7 days, respectively. The second was the histological changes of the leaflet tissue. The 2 types of preserved grafts showed well-developed valvular thrombi and earlier disappearance of the leaflet, whereas the leaflet in the homovital graft was preserved until 7 days after implantation. In addition, thrombi in the aortic conduits were well developed in these preserved grafts.

From these results, it may be hypothesized that these preservation techniques reduce the xenogeneic immunogenicity of the endothelial cells, probably because of loss of these cells during preservation, and that other xenogeneic immune responses to the smooth muscle cells and even to the extracellular matrix are activated earlier than in the homovital graft. Histological changes of the leaflet in these xenografts is similar in strength and specificity to an allogeneic response [30,31].

As the aortic allograft has been used as an experimental model for graft arteriosclerosis, the aortic xenograft of rodents has been used as a model to investigate the mechanism of rejection in vascularized xenografts. In a previous study reported by Allaire et al. [35], they described how the endothelial cells of the hamster-to-rat aortic graft are rejected by the immune effectors, including monocytes and immunoglobulin G, between 5 and 8 days after implantation, and how the smooth muscle cells in the media are rejected by the same effectors at 15 days. Therefore, unlike the case with allografts, the xenogeneic extracellular matrix also has immunogenicity resulting in medial elastic injury. In our study, all 3 types of xenograft examined had developed considerable degeneration of the elastic fibers in the media within 14 days of implantation, whereas the allograft showed elastin injury only focally. In addition, elastin injury in the xenograft was characterized by disruption and disappearance of the elastic fibers. These changes were never observed in the allograft, suggesting that the extracellular matrix also have potential xenogeneic immunogenicity [36].

Aortic valve xenografts

The detailed mechanism underlying xenograft rejection is still unknown, although in the past decade rapid progress has been made in understanding and controlling the rejection of vascularized xenografts in concordant rodent combinations. Platt has described the phases of xenograft rejection, which have been termed hyperacute rejection, acute vascular rejection and cellular rejection [30,31]. Hyperacute rejection is mediated by the reaction of natural antibodies of the recipient or the recipient complement system activated by the alternative pathway [30-34]. In concordant combinations such as hamster-to-rat, however, the vascularized graft does not undergo hyperacute rejection [30-34]. In this setting, the vascularized xenograft is subject to acute vascular rejection, which is probably also antibody-mediated, perhaps by the same naturally occurring antibodies that mediate hyperacute rejection and which leads to graft destruction in several days [30,31]. Acute vascular rejection is also characterized by changes in endothelial cells and local activation of cytokines [30,31]. The cellular immune response to xenografts is the least well studied of the rejection responses; however, some researchers believe that the cellular rejection response to xenografts is similar in strength and specificity to an allogeneic response [30,31].

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preserved grafts could be explained by the similar hypothesis that rejection of fibroblasts, smooth muscle cells and extracellular collagen matrix in the leaflet tissue may result in early deterioration of the leaflet elementary structure. Further investigation of the mechanisms underlying xenogeneic rejection of the leaflet tissue, which must maintain its elementary mechanical properties responsible for the long-term fate of valve function, is necessary to confirm this hypothesis.

CONCLUSION

This study has shown that the aortic allograft in the rat undergoes histological changes characteristic of graft arteriosclerosis. No significant effect of cryopreservation on these changes indicates that cryopreservation does not alter allograft immunogenicity. However, it could be assumed that failure in retention of cellular viability and of the extracellular matrix during antibiotics-treated preservation would accelerate structural deterioration of the aortic valve allograft. As for xenografts, even the extracellular matrix may have potential xenogeneic immunogenicity. There is a possibility of antibiotics-treated preservation and cryopreservation reducing xenogeneic immunogenicity of the endothelial cells, probably because of the loss of these cells. However, even in this setting, fibroblasts, smooth muscle cells and the extracellular collagen matrix may trigger the xenogeneic immune responses resulting in early structural deterioration of the aortic valve xenograft. Therefore, removal of viable cellular components alone may not be effective for the clinical application of xenograft valves. Another approach such as seeding of human endothelial cells on the leaflets using tissue engineering [16], which is expected to reduce immunological reaction and to allow self-repair and ingrowth of host cells, may also be required.

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