

Tolerance to aortic valve homografts in man

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This study was designed to look for evidence of host reaction to aortic tissue by testing the response of host lymphocytes in tissue culture. Aortic tissue of each donor was ground in a tissue homogenizer, and an aqueous extract was prepared. Extracts in Freund's adjuvant injected into rabbits produced up to four precipitating antibodies. Lymphocytes of each recipient were cultured alone, in the presence of phytohemagglutinin (PHA), and with the tissue extract. Lymphocytes obtained the day before surgery gave a mean transformation response of 3.6 per cent blastlike forms in the presence of extract compared to 1.9 per cent in controls and 42.2 per cent with PHA. The lymphocytes of 7 patients studied 2 months after surgery again did not demonstrate significant response to tissue extract. In 2 more patients whose valves failed, extracts of the recovered homografts gave similarly low responses. Histologically, their valves showed no evidence of immune tissue injury. In a comparative study, lymphocytes responded with a mean of 21.5 per cent transformation in the presence of killed allogenic leukocytes. These observations suggest that the absence of clinical and histologic signs of immune rejection may be due to the paucity of histocompatibility antigen in aortic tissue.

Twelve years ago Murray¹ first successfully inserted an aortic valve homograft in a recipient's descending thoracic aorta. The surgical technique has been further refined so that the diseased aortic valve may be replaced directly with a homograft.² Cumulative experience has exceeded 400 aortic valve transplants. Although most transplanted valves remain functional indefinitely,^{1-7, 10, 11, 13, 15, 16} about 30 per cent fail. One important consideration for failure is immune rejection. This study was designed to look for such a reaction by testing the response of lymphocytes from homograft recipients to aortic valve antigens in cell culture.

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Table I. *Summary of patients entered in study*

<i>Patients, 29 (ages 27-66)</i>	<i>Pathology</i>	<i>Results</i>
Male, 20	Rheumatic heart disease, 26	Deaths, 10 (autopsied, 7)
Female, 9	Endocarditis, 3	Homograft removed, 6 (2nd homograft, 2)
		Initially improved, 18
		Improved > 1 year, 12/15
		Improved with 2nd homograft, 2/2

MATERIALS AND METHODS

Twenty-nine patients scheduled for aortic valve homotransplantation were included in this study. There were 20 men and 9 women who ranged from ages 27 to 66. Twenty-six had rheumatic heart disease with aortic stenosis and/or aortic insufficiency. Most of these also had associated mitral valvular disease. Three other patients had bacterial endocarditis with destruction of the aortic valve. Four patients died on the day of surgery. Six others died 10 days to 10 months after surgery, and 5 were autopsied. The grafted valves failed in 6 other patients and were removed 3 to 30 months after transplant. Two of these 6 had a second homograft procedure—the others received a prosthetic valve. One year following surgery, 12 patients remained clinically improved (Table I).^{6, 10, 11} Both of the patients who underwent a second homograft procedure demonstrated improvement. Immunosuppressive drugs were not used following surgery.

TECHNIQUE

Cadaver aortic valves were sterilized in beta propiolactone, attached to a plastic frame, and stored in sterile Ringer's lactate solution containing bacitracin and neomycin. The sterilized valves were kept refrigerated at 4° C. up to a maximum of 4 weeks.⁹

A 2 cm. square of the donor tissue trimmed from donor valve was obtained at the time of surgery. The donor tissue was frozen at -72° C., pulverized, ground in a tissue homogenizer with sterile saline under sterile conditions, and centrifuged. The supernatant aqueous extract contained between 2 and 4 mg. per milliliter protein. The immunogenicity and heterogeneity of this protein was then studied by injecting portions of the extracts of 5 valves in Freund's adjuvant into each of 5 rabbits once every 2 weeks for a total of three injections. Rabbit serum was obtained prior to the first injection and then once every 2 weeks for the following 6 weeks. Precipitating antibody was identified by the Ouchterlony double diffusion technique. At 4 to 6 weeks all rabbit sera showed 4 to 6 precipitin bands to valve material in dilutions up to 1:8. Each rabbit antiserum reacted similarly to all valve extracts tested, but none reacted to human serum in any dilution. No human sera, including those from several valve recipients, contained or developed precipitins to valve extracts.

Blood for cell culture studies was collected in heparin from each patient the day before surgery and 2 months after surgery when possible. The blood was allowed to stand at room temperature until the red cells had settled out. One milliliter aliquots of the lymphocyte-rich plasma were added to 4 ml. of TC199 culture medium in sterile plastic culture flasks and incubated at 37° C.¹² The lymphocytes of each recipient were cultured alone, in the presence of phytohemagglutinin, and in the presence of 0.1 ml. of the donor aortic tissue extract. The PHA-stimulated cultures were harvested after 3 days, and the rest were harvested at the end of 10 days,* fixed and stained, and the number of transformed cells per 300 cells counted and validated by "blind" observation as described earlier.¹² Most of the work was completed before tritiated thymidine uptake studies were begun in our laboratory. We have found a good correlation between the uptake of triti-

*Other histocompatibility studies conducted in our laboratory indicated that lymphocyte transformation as stimulated by tissue antigens is optimal at 10 days of culture.

Table II. Comparison of response before and after transplantation

	Patients	Per cent transformation		
		Zero	PHA	Valve
-1 Day	29	1.9 $\pm$ 1.5	42.2 $\pm$ 17.1	3.6 $\pm$ 2.5
+2 Months	7	1.7 $\pm$ 1.1	41.4 $\pm$ 11.8	0.8 $\pm$ 1.1
Excised valve	2	0.7	33	1
		0	40.3	4

PHA-stimulated response is highly significant ($p < 0.001$). Valve extract does not stimulate any transformation significantly greater than control culture.

Table III. Transformation capabilities in several clinical situations

	Patients	Per cent transformation		
		Zero	PHA	Allogeneic WBC
Immune defect	5	1.0 $\pm$ 0.9	27.5 $\pm$ 3.6	5.8 $\pm$ 2.8
Normal recipients (kidney, skin, valve)	19	2.1 $\pm$ 2.08	31.3 $\pm$ 9.3	21.3 $\pm$ 9.2
Identical twins	2	2.7 3.0	42.0 48.0	0 0

Stimulation by PHA in all patients and by other foreign tissue to normal recipients is highly significant ($p < 0.001$). Lymphocytes of patients with immune defects demonstrate barely significant capabilities ($p < 0.1$).

ated thymidine and morphologic transformation induced by PHA; this gives further support for the validity of the cell counts.

For comparison, similar studies were done with the use of allogeneic leukocytes instead of aortic tissue extract. These cells were obtained from volunteers, washed free of plasma, lysed in sterile water for 1 hour, and then added to viable lymphocytes in culture. These lymphocytes were obtained from normal volunteers, 2 of the transplant recipients, and patients with immune deficiencies.*

RESULTS

Cultured lymphocytes obtained the day before surgery contained a mean of 3.6 per cent blastlike forms in the presence of the aortic tissue extract compared to 42.2 per cent with phytohemagglutinin and 1.9 per cent with nothing added. The lymphocytes of 7 patients studied again 2 months or more after surgery reacted no differently to the tissue extract, and, in 2 more patients whose valves failed, the extracts of the recovered homografts gave similarly low responses (Table II). Lymphocytes responded with a mean of 21.3 per cent transformation in one-way mixed lymphocyte cultures. Individuals with immune deficiencies and delayed skin graft rejection had a mean transformation of 5.8 per cent in the one-way mixed lymphocyte cultures. One set of identical twins also had a negative response against the donor twin's lysed lymphocytes (Table III).

DISCUSSION

Aqueous extracts of human aortic valve tissue caused production of precipitating antibodies in rabbits which did not cross-react with human serum; other

*The 5 patients with immune deficiencies all had very low levels of IgG, IgM, and IgA. They varied in cell-mediated immune competence; for example, skin graft rejection varied in time from 21 days to 1 year.

organ extracts were not studied. Though they were antigenic, the valve extracts did not induce the transformation of lymphocytes from the transplant recipients that one observes when other allogeneic tissues, such as skin, kidney, and leukocytes, are used.

In our laboratory aqueous skin extracts, which contain relatively more nuclear material than aorta, caused brisk transformation (26 per cent in 4 studies) in lymphocytes of immune-competent recipients. Patients with immune defects demonstrated delayed rejection of the skin grafts and had a comparable picture in cell culture. The immune defect group is not homogenous, as two of the patients did not reject skin grafts at the end of one year and had little or no response in cell culture, whereas one patient rejected a skin graft in 21 days and did show a moderate response in cell culture. This comparison of positive and negative controls attests to the degree of sensitivity of the method.

Berah, Hors, and Dausset¹⁸ reported a negligible concentration of transplant antigens in aortic material compared to other tissues. In general, the concentration of transplant antigens correlates with the amount of nuclear material, but the significance of this to histocompatibility is uncertain.

Experience with human aortic valve homografts at the University of Washington has been similar to that in other institutions. There was an over-all 30 per cent cumulative mortality; but most of the surviving patients demonstrated improvement, as they were able to return to work and tolerate exercise with fewer symptoms.^{10, 11} We have been particularly interested in relating our negative findings to the histological changes noted in those instances in which the valves failed. Histologic sections of the transplanted valve and adjacent tissue were reviewed in the 5 autopsied patients and in the 6 patients requiring removal of a transplanted valve. Although some of these valves did have adherent proteinacious material, there were no infiltrating plasma cells or lymphocytes. Valve failure was adequately explained by perforation of the cusp, tear of a leaflet, or infection. Similar observations have been made elsewhere. Hudson¹⁴ described good preservation of the aortic elastic tissue but also observed thickening of the cusp base. There was little or no evidence of an immunologic response to foreign tissue. The major complications and causes of failure included sepsis, graft detachment, cusp perforation, and aortic dissection.

Mohri and associates¹⁷ compared viable and nonviable aortic valve homografts in dogs. Although leaf mobility is well maintained in nonviable grafts, transplanted valves with viable donor cells demonstrated limitation of mobility. A small number of lymphocytes and plasma cells were present briefly at the graft-host junction of viable grafts. In dogs, at least, viable donor cells appeared to induce some immune response by the host. This may relate to a continued production of foreign (antigenic) material from the living donor cells and, thus, continued stimulation of host immunoresponsive tissue. The nonviable donor valve seems to serve as a template for host cells (as demonstrated by Morhi and associates), much like a bone graft and vascular allografts.

In this study, we have shown that the lack of lymphocyte response to allogenic tissue in cell culture can serve to predict host tolerance. We must emphasize that this is not the same as specific immune tolerance. The aortic valve

appears to have a paucity of histocompatibility antigens and, within species at least, will induce no significant immune reaction. The favorable experiences with homografts are likely to stimulate interest in the use of valve xenografts.^{15, 16} It would be worthwhile to determine the host tolerance of xenografts by testing the lymphocyte response of potential recipients before surgery rather than to rely only upon information obtained retrospectively from histological examination.

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