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Antigenic Pattern of the Pig Aortic Valve and its Simplification by Culture and Transplantation *

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The antigenicity of the pig aortic valve was studied by immunoelectrophoresis on the valves before and after storage in a growth culture medium, and before and after grafting into dogs.

Nine precipitation bands were detected in the fresh pig aortic valve. When the valve was cultured in a growth medium for five days, six bands were found. When the cultured valve was transplanted into the dog only three bands were detected. It seems therefore that when pig aortic valve is cultured and xenotransplanted an antigenic simplification occurs.

The use of fresh aortic valve allografts has probably given the best clinical results among the many techniques employed as heart valve substitutes (12). These grafts, however, have some drawbacks such as the difficulty of obtaining a sufficient number of healthy valves soon after death (1). Aortic xenografts would obviate these problems (5, 6).

The initial clinical results achieved with modified, stored, pig aortic valves were similar to those obtained with allografts (2, 9, 15), but they later developed valve distortions and ruptures. These poor results could be attributed to the methods of storage (14) as suggested by the experimental work of BUCH (4). Fresh xenografts on the other hand, have not been used clinically. However in the experimental animal, some authors do not find any evidence of rejection (7) while the majority, using the criteria of microscopic changes in the transplanted valve (3, 8, 13) or a positive response to the administration of immunosuppresive drugs (10, 16, 18), argue for the existence of rejection.

The aim of the present work is to investigate the antigenicity of fresh pig aortic valves and, if so, to introduce modifications in this xenogenic material to-

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make it as similar as possible to an allograft without altering its viability.

Three different approaches were used: 1) The antigenicity of pig aortic valves was studied by immunoelectrophoresis (11, 17) on the valves either before and after storage in a growth culture medium or, before and after grafting into dogs. 2) The titre of circulating antibodies against pig valves grafted into dogs was studied using Boyden's passive haemaglutination technique (19) as an indication of the immunological response of the recipient animal to the antigenic insult. 3) In addition, all specimens were examined histologically to determine the viability of the graft after culture and signs of rejection after transplantation.

In this work we report only the first approach to the problem.

Materials and Methods

Fresh pig hearts. — Nine pig hearts were obtained under clean but not sterile conditions immediately after the death of the animal in the slaughter house.

Fresh dog hearts. — Nine dog hearts were collected under similar conditions from laboratory animals.

Fresh pig aortic valves. — The aortic valve was dissected out under strict sterile conditions including a small cuff of myocardium below and a cylinder of aorta above.

Fresh dog aortic valves were collected in the laboratory under similar conditions as the pig valves.

Cultured pig aortic valves. — The valves were kept at 37° C in a tissue growth medium * for five days with one change of medium on the third day. Implanted cultured pig aortic valves. In 44 adult mongrel dogs a cultured pig valve was implanted between the external and internal oblique muscles of the abdomen. At the time of surgery, a swab taken from each graft was kept at 37° C in thioglycolate for 6 days. If bacterial growth was observed the grafted dog was discarded (16 animals). Similarly discarded were those animals where signs of local infection were detected (8 dogs). The implanted valves in the remaining 20 dogs were removed surgically at regular intervals between 5 and 70 days after implantation.

Transplanted cultured pig aortic valves. — A cultured pig aortic valve was sutured in the thoracic descending aorta of 47 adult mongrel dogs with surgically induced aortic insufficiency (6). 32 dogs were excluded from study due to surgical death or infection. The remaining 15 valves were obtained at intervals between 4 and 142 days.

Each organ or tissue was homogenized in saline to extract the soluble antigens. A part of each extract was stored at —20° C to be used as antigens when required. The other part of each extract was mixed 1:1 with Freund's adyuvant and was used to immunize rabbits following the technique of TORMO (19). Anti-pig valve, anti-pig heart and anti-dog heart sera were thus obtained. The absorption of serum proteins and other organ antigens was carried out using 1 volume of antigen for 5 volumes of immuneserum. The mixture was incubated for 1 hour at 37° C and for 18 hours at 4° C. Finally it was centrifuged and the supernatant was used as the absorbed serum if the absorption controls were negative by immunoelectrophoresis and Ouchterlony.

The antigenic analysis of each group of valves was determined by immunoelectrophoresis in agar gel following the micromethod of SCHEIDEGGER (17) modified by MARTÍNEZ-RESA *et al.* (11).

* LYG medium. Köln University. Germany.

Results

Fresh pig aortic valve. — The homogenate from the fresh pig aortic valves when tested against the different immunesera gave the following results: Against the anti-pig valve serum 9 antigenically different proteins were detected which are the total valve antigens (Fig. 1.1). Testing it with the anti-pig heart serum 5 precipitation bands appeared with the exact mobility as five of the 9 proteins already detected (Fig. 1.2). Testing it against the anti-dog heart the same five bands appeared (Fig. 1.3). Testing it against the anti-pig valve serum absorbed with dog valve only 2 precipitation bands were detected (Fig. 1.4). Tested against the anti-pig valve serum absorbed with dog valve and cultured pig valve no antigens were observed (Fig. 1.5).

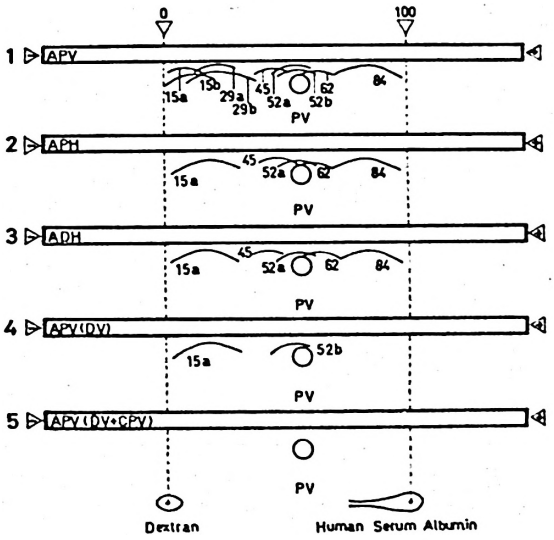

Fig. 1. *Antigenic analysis of the fresh pig aortic valve.*
The valve (PV) is placed in the well and the different immunesera in the trough: 1. Anti-pig valve (APV). 2. Anti-pig heart (APH). 3. Anti-dog heart (ADH). 4. Anti-pig valve absorbed with dog valve [APV (DV)]. 5. Anti-pig valve absorbed with dog valve and cultured pig valve [APV (DV + CPV)]. The different precipitation bands are numbered according to their electrophoretic mobility.

Dog aortic valve. — The cross reaction between the dog valve homogenate and the anti-pig valve serum gave 7 different antigens (Fig. 2.1). Five of them reappeared when the dog valve was tested against the anti-dog heart (Fig. 2.2).

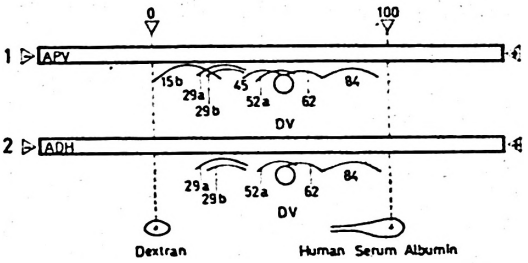

Fig. 2. *Antigenic analysis of the dog aortic valve.*
The dog valve (DV) is faced to anti-pig valve (APV) and anti-dog heart (ADH) immunesera.

Cultured pig aortic valve. — The cultured pig aortic valve homogenate when tested against anti-pig valve serum gave only 6 of the precipitation bands observed in the fresh pig valve (Fig. 3.1). When tested against the anti-pig valve immuneserum, absorbed with dog valves, 2 bands appeared (Fig. 3.2). Finally when tested against anti-dog heart serum 4 of the 6 bands could be observed (Fig. 3.3).

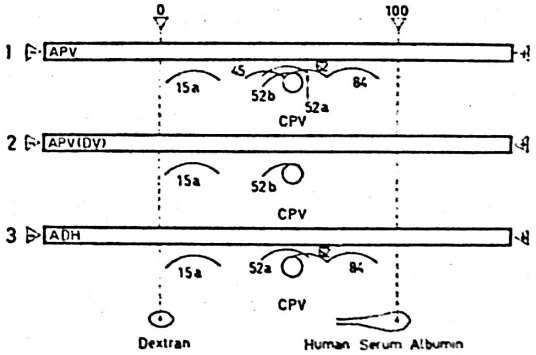

Fig. 3. *Antigenic analysis of the cultured pig valve.*
The cultured pig valve (CPV) is placed in the well and the different immunesera in the trough: 1. Anti-pig valve (APV). 2. Anti-pig valve absorbed with dog valve [APV (DV)]. 3. Anti-dog heart (ADH).

Implanted cultured pig aortic valve. — The homogenate of all the cultured pig aortic valves, previously implanted in the abdominal muscles of the dog, gave the following results: Tested against the anti-pig valve serum 8 antigenically different proteins appeared (Fig. 4.1). All of these, except two, were present in the normal dog valve (Fig. 4.2), and all but one were common with the dog's heart (Fig. 4.3).

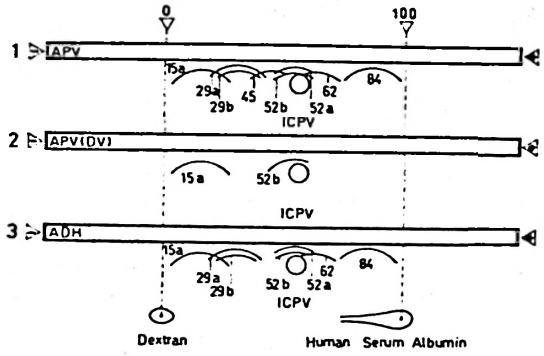

Fig. 4. *Antigenic analysis of the implanted cultured pig valve.*
The implanted cultured pig valve (ICPV) is placed in the well and the different immunesera in the trough: 1. Anti-pig valve (APV). 2. Anti-pig valve absorbed with dog valve [APV (DV)]. 3. Anti-dog heart (ADH).

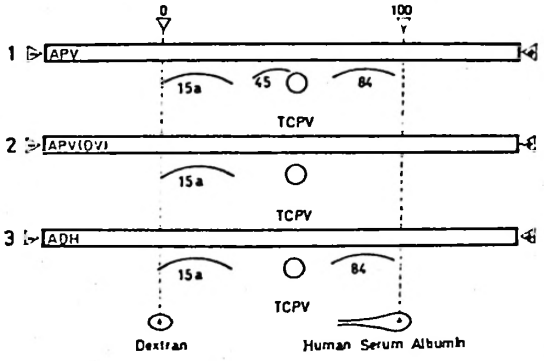

Fig. 5. *Antigenic analysis of the transplanted cultured pig valve.*
The transplanted cultured pig valve (TCPV) is placed in the well and the different immunesera in the trough: 1. Anti-pig valve (APV). 2. Anti-pig valve absorbed with dog valve [APV (DV)]. 3. Anti-dog heart (ADH).

Transplanted cultured pig aortic valve. The 15 transplanted valves into the descending aorta of the dog were homogenated. Only three distinct bands appeared when tested against the anti-pig valve serum (Fig. 5.1). Two of them were antigens also present in the dog valve (Fig. 5.2). When tested against the anti-dog heart two different proteins appeared (Fig. 5.3).

Discussion

We have only found nine different antigens in the fresh pig aortic valve. Although these might not be the only ones, they are certainly antigenic under the conditions of this study and may therefore be relevant to xenograft rejection (Table I).

Seven of the nine antigens were also present in the dog aortic valve. Two of them (the 15a and 52b) were not found in the dog. It does not mean that these are absent, but rather that their concen-

Table I. *Different antigens (mobility of the bands in the first vertical column) detected in the fresh pig aortic valve.*
(PV), dog aortic valve (DV), cultured pig valve (CPV), implanted cultured pig valve (ICPV) and transplanted cultured pig valve (TCPV). A reduction in the number of antigens can be observed when the valve is cultured and transplanted.

Antigens	PV	DV	CPV	ICPV	TCPV
15 a	+		+	+	+
15 b	+	+			
29 a	+	+		+	
29 b	+	+		+	+
45	+	+	+	+	
52 a	+	+	+	+	
52 b	+		+	+	
62	+	+	+	+	
84	+	+	+	+	+
Total	9	7	6	8	3

tration was too small to be detected by immunoelectrophoresis.

Culturing the valve in a maintenance medium resulted in an antigenic loss. Three of the nine antigens isolated in the fresh valve had dissappeared (15b, 29a, 29b). However, the two antigens found in the pig valve and not present in the dog (15a and 52b) were still present after culture.

URIEL (20) has described a similar process of antigenic simplification with other tissues subjected to tissue culture. Probably the reduced cell activity decreases the protein synthesis and it is possible, at least theoretically, that a reduction in antigen formation will follow. The washing out action of the change of medium must also be considered.

When the cultured valve was implanted, we found paradoxically that the valve acquired two new antigens common with the dog heart muscle. This may well be due to a faulty technique of implant removal because the firm adhesion to the surrounding tissues of the dog makes it impossible to separate completely the graft.

In the cultured valves positioned in the descending aorta, only three antigens were detected (15a, 45, 84). It seems as if the hemodynamic positioning of the graft, subjected to a continous wash out by the blood stream, decreases the number of its antigens. These three antigens have been a constant finding in all valves, whether fresh or cultured, implanted or transplanted, and probably indicates that they are the most important in the valve but not the most important immunologically since two of them are also present in the dog.

This hypothesis of «antigenic simplification» occurring after culture or transplantation of pig valves is supported by the morphological studies and particularly by the titres of haemaglutinating antibodies measured in the serum of grafted animals, which will be reported elsewhere. It is felt that this hypothesis might have some valuable application in other fields of transplantation.

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