ANTIBODIES AGAINST HEART TISSUE IN TRANSPLANTATION OF XENOGENEIC AORTIC VALVES

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During transplantation of valves in patients with rheumatic fever and acquired heart defects a marked increase in the level of antibodies against the components of the muscle fiber and connective tissue of the heart was observed by the use of the direct immunofluorescence method. The immune response varied depending on the method of treatment of the graft.

After transplantation of aortic xenogeneic valves into a patient, in some cases late degenerative changes take place in the valve tissue leading to inadequacy of the graft. Some workers under these circumstances have not observed cellular rejection reactions. No circulating antibodies likewise have been found [5, 6, 10]. Recently, however, reports have been published in which the causes of complications of this type have been attributed to immunological reactions of the recipient to the graft. These investigations have revealed collections of immunocompetent cells at the boundary between the donor's and recipient's tissues [3, 4]. Bound γ-globulin has been found by the immunofluorescence method in the valve tissue [8, 9]. There is evidence of the toxic action of the recipient's serum on the donor's fibroblasts [11].

The agents now used to treat valves (4% formaldehyde solution, γ-ray irradiation in a dose of 3 Mrad) have been shown not to abolish completely the antigenic activity of the valves [2]. The exception to this rule is the conservation method suggested by Carpentier et al. [3]. The results of experiments on animals have shown that treatment of valves by this method virtually completely suppresses their antigenic activity [2].

The object of this investigation was to study the immune response in patients with rheumatic fever and acquired heart defects after transplantation of xenogeneic (hog) aortic valves treated in different ways (with 4% formaldehyde solution, by γ-ray irradiation, or by a mixture of sodium metaperiodate and glutaraldehyde).

EXPERIMENTAL METHOD

The sera of patients with rheumatic fever and acquired heart defects were studied before and after operations for transplantation of the valves at intervals of two weeks on the average until five months after the operation. The patients were subdivided into three groups depending on the method of treatment of the transplanted valve: group 1) 4% formaldehyde solution (four patients), group 2) γ-ray irradiation (five patients), group 3) sodium metaperiodate with glutaraldehyde by Carpentier's conditioning method (eight patients). The control group consisted of the sera of patients into whom a synthetic ball valve was grafted (seven patients). In addition the sera of healthy donors were tested. The sera were preserved by freezing to −20°C and were tested in dilutions of 1 : 4-1 : 6.

Antibodies against heart tissues were studied in sections of normal bovine and porcine heart tissue. Pieces of tissue taken from the wall of the left ventricle were frozen at −70°C. Sections 4 μ in thickness

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TABLE 1. Intensity of Reactions with Components of Muscle Fiber and Connective Tissue in Patients with Rheumatic Fever after Transplantation of Valves

Intensity of reactions	With components of muscle fiber			With components of connective tissue		
	before operation	at time of maximal reaction	at end of investigation	before operation	at time of maximal reaction	at end of investigation
++++, +++	1	3	–	1	13	2
++	3	9	2	–	5	2
+	9	7	7	5	3	9
±, –	9	3	13	16	1	9

Note. Numbers show number of patients; total 22.

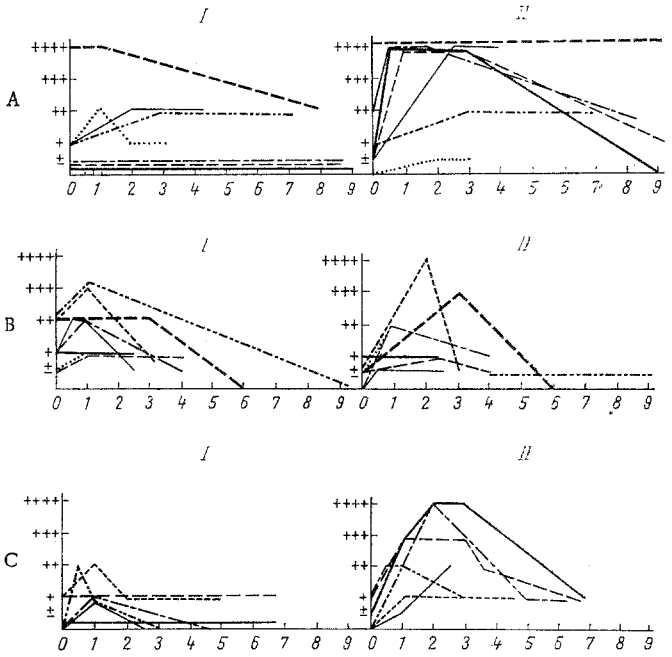

Fig. 1. Reactions with components of muscle fiber (I) and connective tissue (II) in patients with rheumatic fever after transplantation of xenogeneic valves treated with formaldehyde solution and γ rays (A), of xenogeneic valves treated by Carpentier's conditioning method (B), and of synthetic valves (C). Each curve represents the study of sera of one patient over a period of time; O) time of operation. Abscissa, time after transplantation (in months); ordinate, intensity of luminescence.

were cut in a cryostat at −20°C and were used unfixed. Four of the sera were tested on tissue sections of the human heart (biopsy material: tissue from the auricle of the heat of a person with blood group O, obtained during commissurotomy).

The investigations were carried out by the indirect immunofluorescence method using pure antibodies against human γ-globulin labeled with fluorescein isothiocyanate. The technique of preparing the pure antibodies and the treatment of the preparations were described previously [1]. The specimens were examined with the ML-2 luminescence microscope with a 40× objective and Homal 3× ocular. The reactions were assessed by intensity: ++++ very bright luminescence, +++ bright luminescence, ++ moderate luminescence, + weak luminescence, ± very weak luminescence, – absence of luminescence.

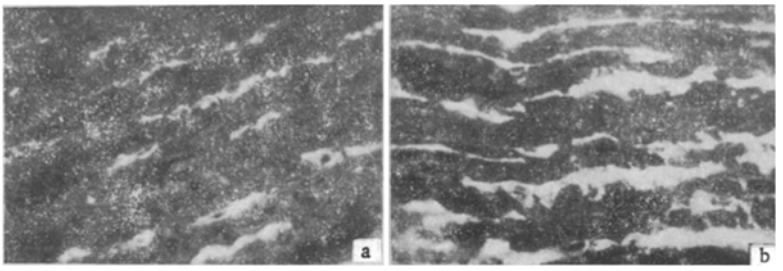

Fig. 2. Reactions of sera of patients with rheumatic fever after transplantation of valves treated with formaldehyde solution and γ rays with tissue sections of bovine (a) and porcine (b) heart; a) luminescence of cells of interstitial connective tissue; b) diffuse luminescence of interstitial tissue.

EXPERIMENTAL RESULTS

The sera of the patients before operation, when tested on sections of bovine and porcine heart tissue, as a rule reacted weakly with the components of the muscle fiber and connective tissue (except one serum). After transplantation of the valves the intensity of the reactions with heart tissue increased sharply in all groups. The maximal intensity of luminescence was observed between the second and eighth weeks, when most of the sera reacted with heart tissues, especially with the connective tissue, giving results of ++, +++, and ++++ (Table 1). The intensity of luminescence fell appreciably toward the end of the investigation.

The sera reacted with the majority of elements of the myocardial muscle fiber. Antibodies were discovered most frequently to discs and sarcoplasm, less frequently to sarcolemma and the intercalary discs of the muscle fiber. Reactions with the connective tissue of the heart were localized chiefly in the region of the cells, and diffuse staining of the interstitial tissue was seen less commonly.

The results of tests of the patients' sera after transplantation of xenogeneic valves treated with formalin and γ rays are shown in Fig. 1A. In both groups the reactions, especially with connective tissue, reached high intensity after transplantation (+++, ++++), remaining at this level for a long time (8-9 months). In the group in which valves treated with formalin were transplanted, the sera of two of the four patients were taken in the late stages (six months after the operation). The sera of one of them reacted both with the muscle fiber and with the connective tissue. The sera of another patient reacted very weakly with heart tissue. These sera are not shown on the graph.

As a rule, during parallel tests of the sera on the sections of bovine and porcine heart the results were identical. However, the sera of four of the nine recipients, starting from 2.5-3.5 months after transplantation of the valve, stained the whole of the interstitial connective tissue (by ++++) in the sections of porcine heart tissue, whereas usually fluorescence of only individual cells was observed on the sections of bovine heart (Fig. 2).

After transplantation of valves treated by the conditioning method into the recipients of group 3, their sera reacted with heart tissue a little weaker than those of the two previous groups (Fig. 1B). Only one patient's serum reached very strongly with connective tissue components. By 4 months the intensity of the reactions with muscle fiber and connective tissue as a rule was reduced; in some cases the sera ceased to react with heart tissue. Parallel tests of the sera on sections of bovine and porcine heart yielded identical results.

In the control group (synthetic prosthesis) the recipients' sera also reacted with the components of the muscle fiber and connective tissue of the heart. The sera of two patients reacted very strongly (++++) with connective tissue. The intensity of fluorescence reached a maximum after 3-4 weeks; sometimes the intensity of the reaction increased for 2 months and then fell gradually until 4-5 months (Fig. 1C). Parallel tests of the sera on sections of bovine and porcine heart yielded identical results.

Some sera from recipients of the different groups, which reacted intensively with tissue sections of the animals' heart and, in particular, with the connective tissue, were tested on tissue sections from the human heart. Reactions were found in these experiments with different elements of the muscle fiber; the sarcolemma, sarcoplasm, discs, and intercalary discs (by ++, +++, and ++++). However, none of the sera reacted with the components of the interstitial connective tissue.

The sera of healthy persons, when tested on tissue sections of the heart, did not react or reacted only weakly with elements of the myocardium.

An increase in the level of antibodies against components of the heart tissue was thus observed in patients with rheumatic heart disease after valve transplantation irrespective of whether a biological or a synthetic prosthesis was grafted. Antibodies against heart tissue are known to be found after the operations of commissurotomy and cardiotomy [7, 12], and it is possible, therefore, that the elevation of the antibody level after transplantation of the valves was due to some degree to the operation. The spectrum of antibodies against components of the muscle fiber was the same in the patients after transplantation as in patients with rheumatic fever in the actue period of the disease, when the sera were tested on tissue sections of bovine heart [1]. As a rule the sera reacted with the discs and sarcoplasm, but luminescence of the sarcolemma and intercalary discs of the myocardium was observed less frequently. However, after transplantation of the valves antibodies against elements of the connective tissue were found in a higher percentage of cases. The sera of 21 of the 24 recipients reacted with connective tissue, and in some cases the intensity of the reactions was considerable (+++, ++++).

In all groups the reactions with heart tissue reached maximal intensity by the 3rd-4th or, sometimes, the eighth week. However, the intensity and duration of these reactions varied from one group to another. The reactions lasted longest in the group in which the recipients were grafted with valves treated with γ rays. When valves treated by the conditioning method were transplanted, the sera ceased to react with the heart tissue 3-5 months after the operation.

In most cases the antibodies found reacted equally with tissue sections of bovine and porcine heart, evidently on account of antibodies against antigens common to these two species. However, in the groups of patients into which xenogeneic valves treated with γ rays and formalin were transplanted, an immune response was observed to specific porcine antigens, evidently located in the region of the interstitial connective tissue. This is shown by the more intensive and extensive staining of the interstitial tissue in the sections of the porcine heart with sera obtained 2.5-3.5 months after transplantation. A reaction to these antigens was not given by patients grafted with valves treated by Carpentier's method. The character of the immune response to the graft thus differed depending on the method of treatment of the valve. Immune reactions were less intensive after transplantation of a valve treated by the conditioning method, when, as experiments have shown, a sharp decrease in the antigenic properties of the tissue is observed [2].

In the group of patients with a synthetic prosthesis the reactions of the sera with heart tissue were similar to those observed after transplantation of xenogeneic valves treated by Carpentier's method. It is not yet clear why antibodies reacting with bovine connective tissue and not reacting with human connective tissue should be formed after transplantation of a synthetic valve. A similar phenomenon has been observed during the study of sera from patients with rheumatic fever in the active period of the disease [1]. On this basis it was postulated that human and bovine connective tissue contain identical antigenic components, but the bovine antigens are located on the surface of the connective-tissue structures while in man they are hidden. Trauma of the fibrous ring during fixation and operation of the prosthesis evidently leads to exposure of these components, and this stimulates antibody formation.

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