MORPHOLOGY AND PATHOMORPHOLOGY

Rheumatoid Course of Humoral (Vascular) Rejection after Heart Allotransplantation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 142, No. 9, pp. 337-340, September, 2006
Original article submitted January 30, 2006

Analysis of planned endomyocardial biopsy specimens of heart allotransplants from 22 recipients revealed signs of humoral type rejection (slight, medium, and severe) presenting as fixation of IgG, IgM, and complement components (C3, C4d) in 61 of 63 sections. Permanent presence of rejection signs attests to rheumatoid course of this process.

Key Words: *humoral rejection; allotransplant; heart; human*

Adequate evaluation of the results obtained in organ transplantation studies is important for modern clinical practice and helps to select methods for effective suppression of the immune response and eliminate disorders developing as the reaction of rejection of the transplanted organ.

Modern protocol of immunosuppression after heart allotransplantation (cyclosporine, methylprednisolone, azathioprine) largely suppresses the rejection reaction and provides long-term functioning of the transplanted organ [3,10,13]. Activated T cells and antibodies to HLA antigens (elements of cellular and humoral immunity) act as effectors of the immune reaction. Immunosuppression largely modulates T cell component, preventing, and, in case of crisis development, suppressing cellular type rejection due to sensitivity of the T-cell biological cycle to cyclosporine, which eventuates in cessation of these cells proliferation. The function of B cells and, hence, humoral rejection, then becomes a priority problem. The data of regular studies of material obtained at planned endomyocardial biopsy of the heart allotransplant indicate that signs of humoral rejection are more incident than signs of cellular reaction [2,4,5]. This is true for the early and late periods after organ transplantation. It is partly due to the fact that antibodies to HLA antigens play the leading role in the development of the transplant vascular diseases often leading to death of the transplanted organ [4].

The aim of this study was immunohistochemical detection of the signs of humoral (vascular) rejection in the material obtained at endomyocardial biopsy, location of these signs, and evaluation of their severity and dissemination in the allotransplanted heart tissues.

MATERIALS AND METHODS

Orthotopic allotransplantation of the heart was carried out at Institute of Transplantology and Artificial Organs by surgeons headed by V. I. Shumakov, Member of Academy of Medical Sciences, and Prof. E. N. Kazakov.

The material was collected during 63 planned endomyocardial biopsies of the heart allotransplants in 22 recipients (4 women and 18 men, mean age

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0007-4888/06/1423-0363

36.6 years). Biopsy was carried out weekly during the first 1.5 months after transplantation, monthly during the first 6 months, every other month during months 7-12 postoperation, every 3 months during the 2nd year, every 4 months during the 3rd year, and every 6 months starting from the 4th year. Biopsy samples were taken in the period of 1 year to 15 years after transplantation.

Cryostat sections (5 μ) were made in a cryostat microtome (Microm HM505E) at -20 to -25°C. Some sections were stained with hematoxylin and eosin for morphological evaluation of the degree of cellular rejection. Unfixed sections were treated routinely for immunofluorescence assay [1]. For direct immunofluorescence, the sections were treated with F(ab')2 fragments of antibodies to IgG, IgM, IgA, labeled with fluorescein isothiocyanate (FITC; Dako cytomation). For indirect immunofluorescence, the sections were treated with monoclonal antibodies to C3 complement component (Dako), C4d complement fragment (Quidel), HLA-ABC (ICO-53) and HLA-DR (ICO-1) (Medbiospektr). FITC-labeled monoclonal antibodies to mouse immunoglobulin served as second antibodies. Fibrin was detected using FITC-labeled monospecific serum to fibrin or fibrinogen (N. F. Gamaleya Institute of Epidemiology and Microbiology).

RESULTS

Morphological examination of sections showed complete absence of cellular rejection signs (lymphocytic infiltration of myocardial tissues) in 41 cases and slight infiltration (1A-1B degree [8]), in 22 cases. The degree of humoral rejection was evaluated by the number of capillaries with walls containing immunoglobulin with or without complement components [2,4]. The presence of 5-15% capillaries with immunoglobulin in their walls was regarded as slight humoral rejection, up to 25% as moderate, and more than 25% as severe rejection. In addition to immunoglobulin (Fig. 1, *a*) and complement components (Fig. 1, *b*) in capillary walls, the expression of HLA antigens on the endothelium and the presence of fibrin in capillary walls or interstitial tissue (Fig. 1, *c*) were taken into consideration as signs of capillary damage. The presence of fibrin in the interstitial space is a reliable marker of rejection crisis, but it characteristic of both humoral and cellular rejection. Therefore, detection of C4d complement component resistant to catalysis was an important finding; it was characterized as a humoral rejection marker [6,7,11].

Some authors [9] noted that signs of humoral rejection of the heart allotransplant are most often observed during the first 6-8 weeks after the organ transplantation. Numerous factors associated with transplantation causing disorders in tissues stimulate natural defense mechanisms, in particular, humoral component of the immunity. Immune complexes participating in resorption of degradation products can form during reparation. For this reason analysis of the material collected during the first biopsies shows fixation of immunoglobulins and complement in a variety of myocardial structures, including the cardiomyocyte sarcolemma and capillary wall.

Immunosuppressive therapy inhibits the transition from synthesis of early immunoglobulin IgM to late immunoglobulin IgG [4]. As a result, immune complexes containing IgM were more incident in capillary walls (in 50 of 63 specimens). The presence of IgG alone (without IgM) was noted in only 2 cases, simultaneously with IgM in 23 cases. Capillary walls contained C4d complement component and IgM (but not IgG) simultaneously in 30 cases. Long intervals between planned biopsies (even 1 month) decrease the probability of detecting the immunomorphological picture of rejection reaction at the peak of its development. The immune complexes degrade and their components are washed out from tissues. During regeneration the endothelium surface is released from foreign protein ballast. Thus released immune complex granules are sometimes detected in the myocardial interstitium [9]. In our study immune complexes or their components were detected in deep intimal layers of small arteries, on their elastic membrane (Fig. 1, *d*), in cardiomyocyte sarcolemma (Fig. 2, *a*), and in the interstitium. The location of immune complexes is changing also during humoral rejection after transplantation of other organs. In kidney allotransplants, fixation of stable humoral rejection marker C4d complement fragment is usually detected not on the peritubular capillary surface, but in the basal membrane of these vessels [12]. When the immune complexes are desquamated from the cell surface, they naturally migrate with tissue fluids. The immune complexes from the connective tissue, associated with capillaries, can be translocated into the cardiomyocyte sarcolemma. Presumably, imbibition of the sarcolemma by immune complexes is a result of the pumping function of transverse tubules of the sarcomers participating in cardiomyocyte metabolism.

IgG was detected in cardiomyocyte sarcolemma in 41 specimens, C4d complement component in 14, C3 in 4, and IgM in 1 specimen.

The heart allotransplant endocardium was studied in 15 biopsy specimens. Histocompatibility

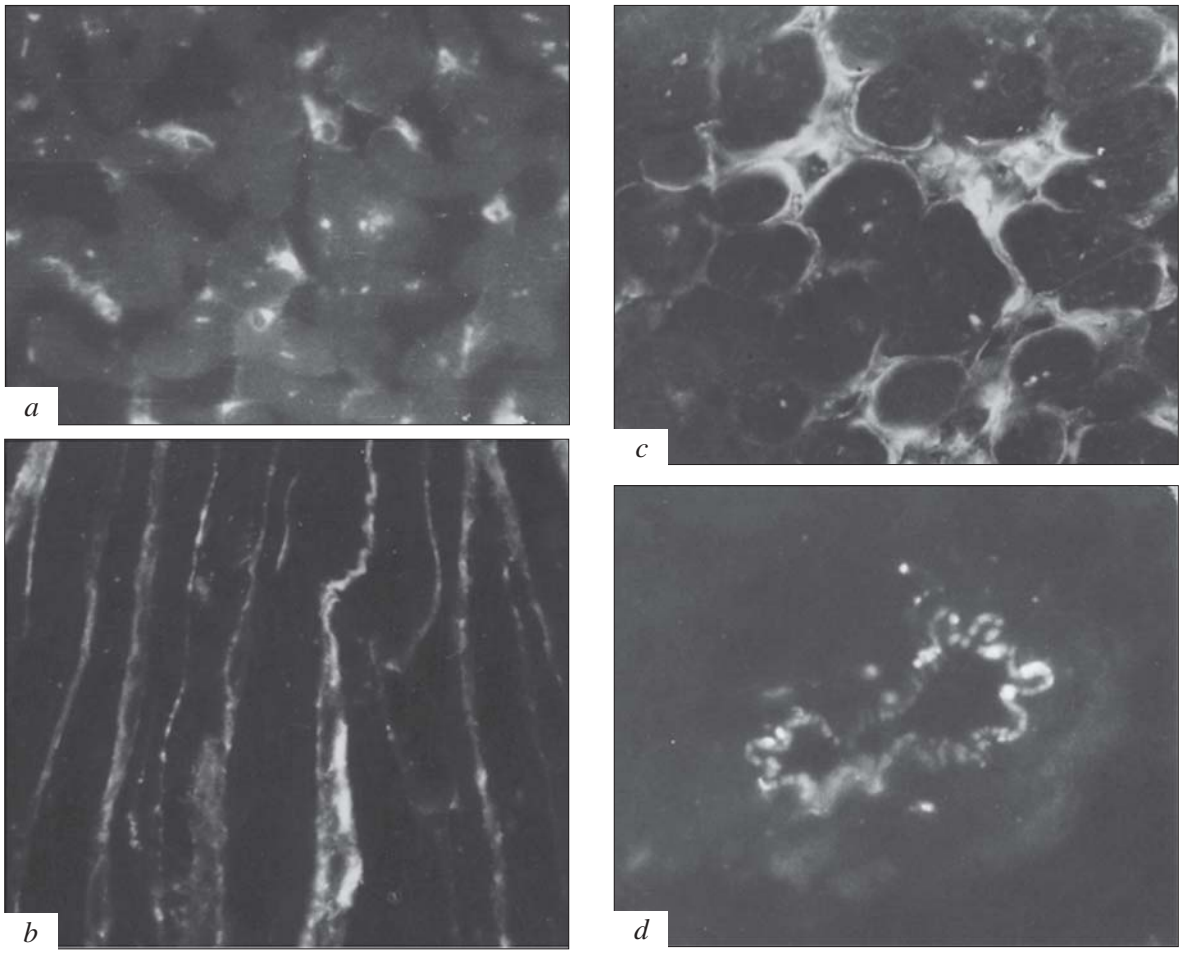

Fig. 1. Cryostat sections of heart allotransplant. Immunofluorescence, ×400. *a*) IgM fixation of myocardial capillary endothelium; *b*) deposition of C4d complement component in capillary walls; *c*) fibrin precipitation in the allotransplant interstitial connective tissue; *d*) fixation of C4d complement component in the intima of a small myocardial artery.

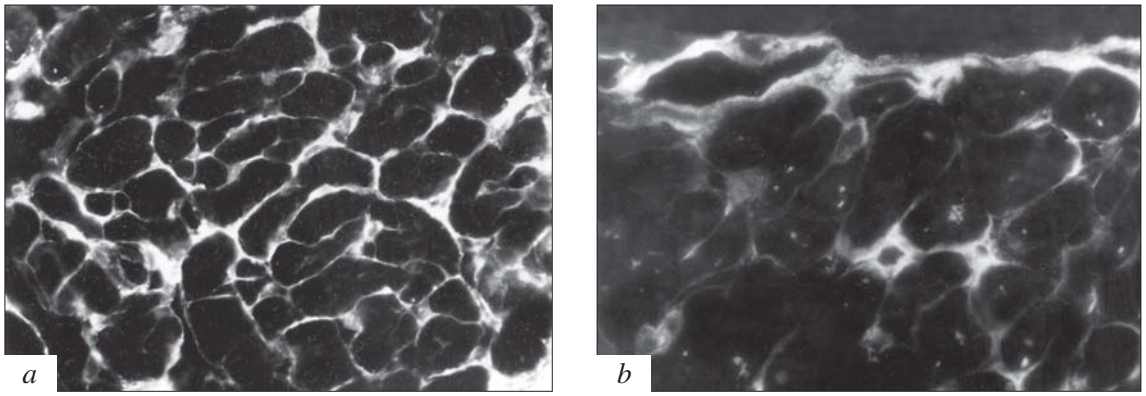

Fig. 2. Cryostat sections of heart allotransplant. Immunofluorescence, ×400. *a*) IgG deposit in myocardial sarcolemma and interstitium; b) fibrin deposit in endocardial lymph capillary walls (above) and myocardial blood capillaries (below).

antigens were expressed on the endothelium of endocardial lymph capillaries, similarly as on all nuclear cells. As a result, isolated IgG fixation in the endocardial lymph vascular walls was detected in 8 cases, IgM in 5 cases, and C4d complement fragment in 8 cases. Simultaneous fixation of IgG and C4d and of IgM and C4d was detected in 3 specimens. Fibrin deposition was noted in the same structures in some cases (Fig. 2, *a*).

Hence, the results indicate permanent presence (period of observation 1-15 years after transplantation) of immunohistochemical signs of vascular

rejection of different degree in the myocardium and endocardium of heart allotransplant. This is true for signs of acute crisis stage and for periods between the peaks of the reaction. Permanent detection of immunomorphological signs of humoral rejection in heart allotransplant tissues indicates a rheumatoid course of the process.

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