Structural and Molecular Reorganization of Cardiomyocytes in Transposition of the Main Vessels

A. M. Volkov, E. E. Kliver, E. L. Lushnikova*, P. M. Larionov, D. V. Subbotin, D. S. Sergeyevichev, and L. M. Nepomnyashchikh*

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Studies of myocardial autopsy specimens from infants (0-12 months) with transposition of the main vessels showed the formation of a complex of compensatory adaptive, degenerative, and destructive changes, manifesting at a tissue level in cardiomyocyte heterogeneity and formation of cardiosclerosis zones. Cardiac myosin synthesis was replaced by synthesis of skeletal myosin, which was detected at the molecular level. Clinically it manifested in the progress of heart failure. Hyperplastic processes (intensive polyploid transformation of the nuclei) play an important role in heart remodeling in patients aged over 6 months. The findings of immunohistochemical and fluorescent studies seem to be prognostically important and provide more accurate data on the pathological processes in the myocardium at the initial stages of heart disease development starting from birth.

Key Words: *transposition of main vessels; cardiomyocyte reorganization; fluorescence; immunohistochemistry*

New data on the proliferative processes in the myocardium of adult mammalians and humans were published in recent years [8,13]. This was due to intensive development of new immunofluorescent methods for detection of marker proteins precisely identifying cardiomyocytes among other heart cells and simultaneously detecting the appearance of specific proteins involved in DNA replication in these cells [3,12]. An important problem of the present stage of cardiosurgery development is the search for approaches to accurate diagnosis, for the most informative signs in the treatment of the complex, heretofore considered inoperable, congenital heart diseases (CHD), one of which is transposition of the main vessels (TMV).

Difficulty of treating these diseases is determined by the need to correctly evaluate the potentialities of cardiac ventricles in provision of the adequate cardiac output [4,5]. This evaluation should be based on determination of possible ranges for age periods favorable for effective correction of heart disease. By the present time, the relationships between physiological growth of the heart and development of compensatory myocardial hypertrophy, particularly for infancy, are not yet quite clear. It is known that ventricular function at rest and during extra stress depends on hemodynamic and autonomic factors promoting the progress of structural changes [6,9,11]. Experimental findings indicate that age is a very important factor for evaluation of the type, rate, and functional characteristics of myocardial reactions to pressure overloading [7,10,14]. Surgical correction is to be carried out during the optimal phase of heart growth, this promoting normalization of ventricular function and elimination of the unfavorable impact of CHD for other vital organs.

We studied the potentialities of compensatory adaptive reorganization of cardiomyocytes in cardiosurgical patients aged 0-12 months with TMV in order to develop the prognostic criteria.

E. N. Meshalkin Institute of Circulatory Pathology, Rosmedtechnologies, Novosibirsk; *Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk, Russia. ***Address for correspondence:*** pathol@soramn.ru. E. E. Kliver

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MATERIALS AND METHODS

The study was carried out on myocardial specimens collected at autopsy of 68 patients with TMV, aged under 1 year, dead during the postoperative period, and of 10 age-matched infants dead from non-cardiac causes. The TMV patients were divided into 2 groups by anatomical variants. Group 1 included patients with the so-called common TMV form: TMV with atrial septal defect and intact ventricular septum (n=37: 19 infants aged under 1 month, 18 aged 1-6 months). Group 2 were TMV patients with atrial septum and ventricular septum (VSD) defects (n=31; 7 patients aged under 1 month, 13 aged 1-6 months, and 11 aged 6-12 months).

Myocardial specimens were fixed in 4% formalin solution in phosphate buffer, washed in distilled water, and processed in cryoprotectors (sucrose solutions: 2 h in 5%, 2 h in 10%, and 12 h in 15% solution). Fluorometry of histological preparations was carried out under an Axioskop 40FL microscope with an AxioCamHRc camera. A total of 20 computer images (Zeiss Plan-Neofluar objective, ×40) of each histological preparation were obtained at 24°C. The images were then processed using Axio Vision 3.1 software (Carl Zeiss).

Fluorometry of the myocardium was carried out using fluorescent probes: ethidium bromide and chlorotetracycline. Myocardial sections were stained with ethidium bromide (5×10^{-3} g/liter) in phosphate buffer (pH 7.4; 5 min, 25°C). Staining with chlorotetracycline (2.6×10^{-2} g/liter) was carried out in PBS (pH 7.4; 1 min, 25°C). Fluorometry of histological sections was then carried out (at 510-523 nm absorption and 595-605 nm emission for ethidium bromide and 400 nm absorption and 520 nm emission for chlorotetracycline). Skeletal myosin was detected in myocardial specimens by immunohistochemical methods with antibodies Monoclonal Anti-Skeletal Myosin, FAST, Clone MY-32, and FITC-conjugated second antibodies.

The results were processed using Microsoft Excel 2000 software. The significance of differences in the means was evaluated by Student's t test. The differences were considered significant at $p<0.05$.

RESULTS

The hemodynamics in group 1 TMV patients was characterized by a bidirectional ejection. Its volume in case of isolated shunting at the atrial level depended on atrial stretching, pressure difference in the atria during different phases of the cardiac cycle, on the size of atrial defect, and difference in the lesser and greater circulation resistance. Since the systemic and pulmonary circulations are disconnected in TMV, the main compensatory mechanism is an increase in circulating blood volume, which leads to plethora in the lesser circulation [1]. The functional loading of both ventricles is virtually the same in this anatomical variant of TMV, which is confirmed by the results of myocardium staining with ethidium bromide.

The hemodynamics in the other group of TMV patients was characterized by two communications at the levels of atrial and ventricular septa (atrial septal defect and VSD), this improving blood mixing at the ventricular level due to cross-ejection. If the VSD is small, the lesser circulation pressure does not increase much; if the defect is large, it is leveled in both circulations, which leads to development of high pulmonary hypertension and progressive hypoxemia [2,7]. The functional loading of both ventricles is greater in this anatomical variant of TMV because of a greater blood volume than in group 1.

Panoramic fluorescent microscopy of ethidium bromide-stained preparations showed drastic reduction of the fluorescent label intensity in patients aged under 6 months in comparison with the control (Fig. 1, *a*, *b*). The incorporation of ethidium bromide in cardiomyocytes was significantly higher in patients aged over 6 months (Fig. 1, *c*). We think this indicates predominance of cardiomyocytes with diploid nuclei in the hearts of infants aged under 6 months; in patients older than 6 months, heart remodeling is associated with predominating polyploid transformation of the nuclear material and subsequent development of cell hypertrophy. Ethidium bromide intercalation in the double-stranded DNA during this period caused more intensive fluorescence. It is noteworthy that the level of polyploidization was significantly higher in left-ventricular *vs.* right-ventricular cardiomyocytes.

Evaluation of the dynamics of chlorotetracycline probe fluorescence showed significant reduction of its intensity in myocardial specimens from patients with congenital heart diseases (Fig. 2, *b*) in comparison with the controls (Fig. 2, *a*). As chlorotetracycline binds the intramitochondrial Ca^{2+} ions, forming a fluorescent complex, the cardiomyocyte fluorescence intensity indirectly reflects functional activity of the mitochondria (presence or absence of injuries in them). The detected decrease in chlorotetracycline fluorescence intensity in cardiomyocytes of children with TMV can be due to reduced accumulation of Ca^{2+} ions in the mitochondria and to reduction of the percentage or counts of mitochondria in cardiomyocytes as a result of hypertrophy of these cells or for some other reasons. One of these reasons can be cardiosclerosis development, clinically manifesting by predominating symptoms of heart failure.

Hypertrophy is associated with not only changes in myocyte volume (size), but in their molecular ge-

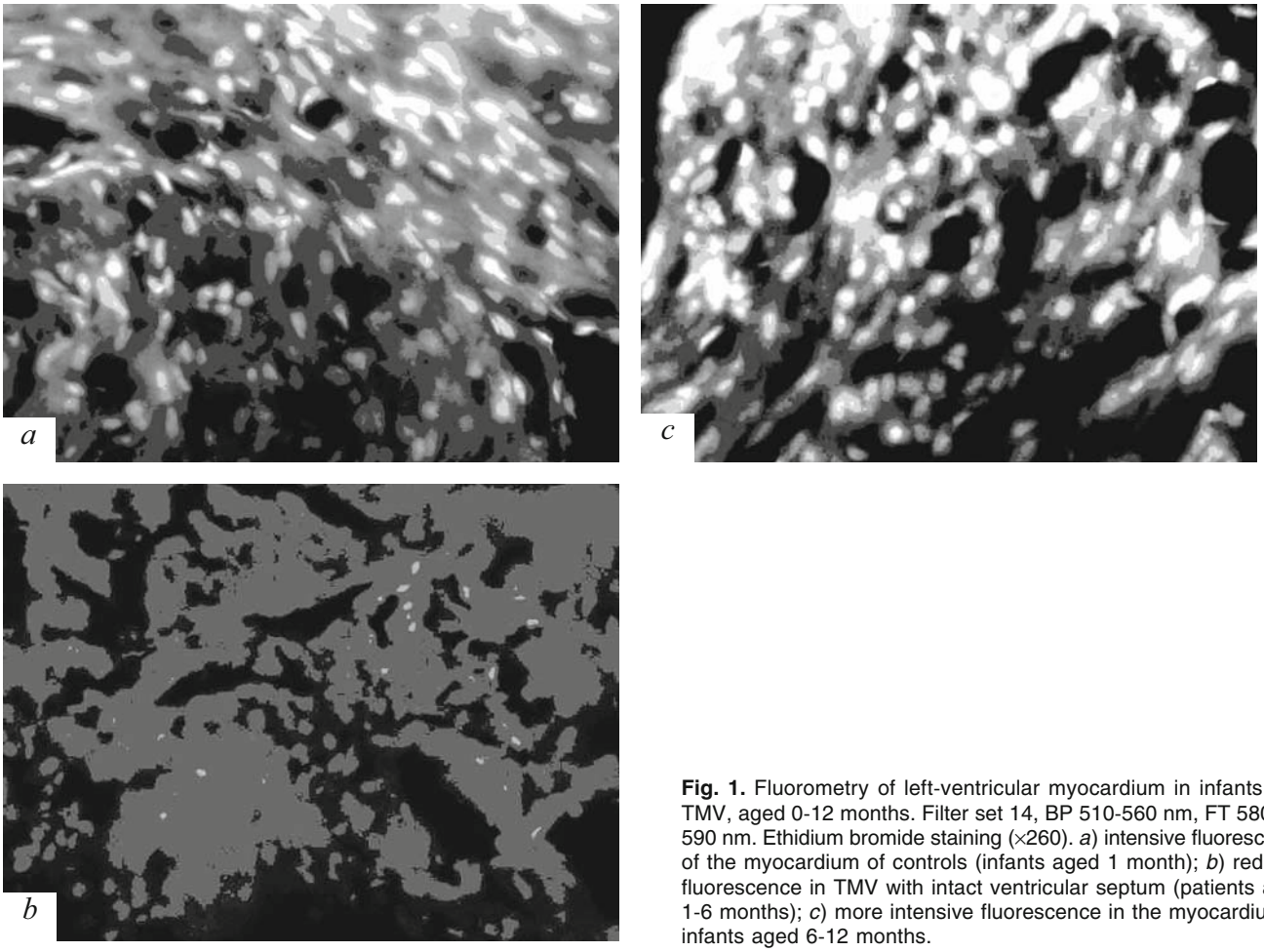

Fig. 1. Fluorometry of left-ventricular myocardium in infants with TMV, aged 0-12 months. Filter set 14, BP 510-560 nm, FT 580, LP 590 nm. Ethidium bromide staining (×260). *a*) intensive fluorescence of the myocardium of controls (infants aged 1 month); *b*) reduced fluorescence in TMV with intact ventricular septum (patients aged 1-6 months); *c*) more intensive fluorescence in the myocardium of infants aged 6-12 months.

netic phenotype as well. Expression of some isoforms of contractile proteins, characteristic of fetuses, was observed in cardiomyocytes under conditions of hemodynamic overloading. For example, suppression of the α-myosin heavy chain gene was associated with replacement of cardiac myosin gene synthesis by the synthesis of skeletal myosin gene. Immunohistochemical findings indicated intensification of skeletal myosin synthesis in the myocardium of TMV patients (Fig. 3). All this led to inhibition of the hypertrophic filaments'

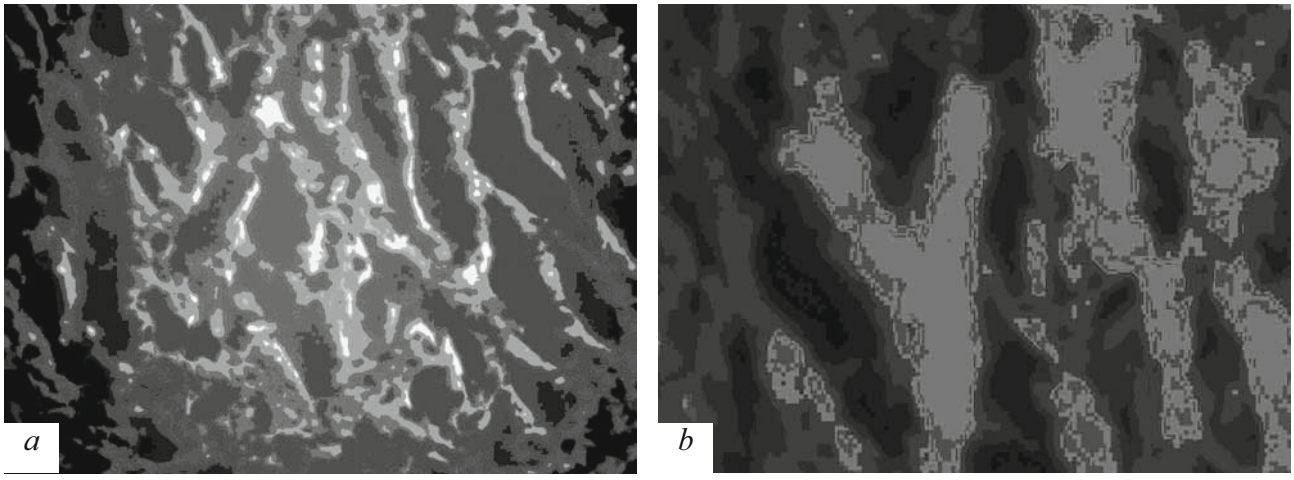

Fig. 2. Intensity of chlorotetracycline incorporation in left-ventricular myocardial specimens of infants aged 0-12 months. Filter set 05, BP 395-440 nm, FT 460, LP 470 nm. Chlorotetracycline staining (×260). *a*) intensive fluorescence in controls (infants aged 1-6 months); *b*) reduced fluorescence intensity in the myocardium of infants with TMV aged 6-12 months.

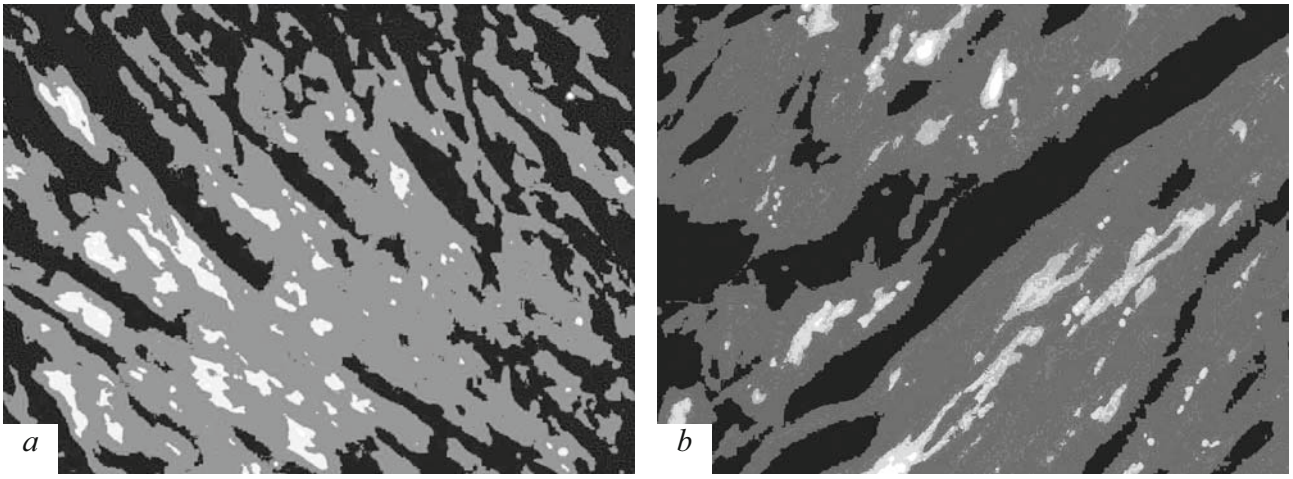

Fig. 3. Immunohistochemical evaluation of skeletal myosin in myocardial specimens of infants with TMV aged 0-12 months. Filter set 09, BP 395-440 nm, FT 460, LP 470 nm (×260). *a*) control; *b*) TMV and intact ventricular septum in infants aged 1-6 months.

contraction. Hypertrophy is also associated with stimulation of other genes, including some early growth regulators, heat shock response and growth factor (transforming growth factor-β) genes, and the atrial natriuretic factor gene. The latter factor is a peptide hormone; by regulating the blood pressure and saline release by the kidneys, it relieves the hemodynamic overloading.

Hence, a complex of compensatory adaptive, degenerative, and destructive changes develops in the myocardium of infants with TMV aged under 1 year. At the tissue level, it manifests in cardiomyocyte heterogeneity and formation of cardiosclerosis zones. At the molecular level, the synthesis is switched over from cardiac to skeletal myosin, this clinically manifesting by augmenting symptoms of heart failure. Hyperplastic processes, associated with intensive nuclear polyploidization, play an important role in heart remodeling in patients aged over 6 months. Reduced level of summary calcium ions in cardiomyocytes of TMV patients indicates (according to fluorometry) disorders in oxidative phosphorylation and the progress of heart failure. Cardiac to skeletal myosin synthesis switch-over in cardiomyocytes of infants with TMV reflects the development of compensatory adaptive reaction in response to chronic hypoxia associated with CHD as in this case energy expenditures for the contraction/relaxation processes are reduced. This reorganization of cardiomyocytes and some molecular mechanisms of heart failure development in infants with TMV aged under 1 year should be taken into consideration in surgical correction of CHD.

REFERENCES

1. D. V. Adkin, S. F. Gnusaev, and V. N. Ilyin, *Pediatriya*, No. 3, 88-91 (2002).
2. L. A. Bokeriya and S. V. Gorbachevskii, *Grudn. Serdech.-Sosud. Khir.*, No. 3, 31-34 (1996).
3. R. Bolli, *N. Engl. J. Med.*, **346**, No. 1, 55-56 (2002).
4. A. R. Castaneda, *Acta Chir. Austriaca*, **25**, 80-82 (1993).
5. A. R. Castaneda, G. A. Trusier, M. H. Paul, *et al.*, *J. Thorac. Cardiovasc. Surg.*, **95**, No. 1, 14-28 (1998).
6. T. de Lange, *Oncogene*, **21**, 523-540 (2002).
7. S. Isoyama, J. Y. Wei, S. Izumo, *et al.*, *Circ. Res.*, **61**, No. 3, 337-345 (1987).
8. A. S. Khan, C. D. Lynch, D. C. Sane, *et al.*, *J. Gerontol.*, **56**, B364-B371 (2001).
9. Y. Liu, E. Cigola, W. Cheng, *et al.*, *Lab. Invest.*, **73**, NZ5 6, 771-787 (1995).
10. Z. Mallat, P. Fornes, R. Costagliola, *et al.*, *Gerontology*, **56**, M719-M723 (2001).
11. D. Orlic, J. Kajsutra, S. Chimenti, *et al.*, *Proc. Natl. Acad. Sci. USA*, **98**, No. 18, 10,344-10,349 (2001).
12. R. N. Re, *Am. J. Cardiol.*, **60**, 100-104 (1987).
13. K. Reiss, W. Cheng, A. Ferber, *et al.*, *Proc. Natl. Acad. Sci. USA*, **93**, No. 16, 8630-8635 (1996).
14. T. Scholzen and J. Gerders, *J. Cell. Physiol.*, **182**, No. 3, 311-322 (2000).