AUTOIMMUNE PROCESSES IN ANIMALS INJECTED WITH HOMOLOGOUS HEART ANTISERUM

N. M. Mazina, M. I. Undritsov,
V. D. Akhnazarova, M. S. Ruseva,
and T. V. Mel'nikova

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Prolonged administration of homologous serum containing cardiac and streptococcal antibodies gives rise to physiological and morphological disturbances of the myocardium. The electrocardiogram is modified, vasculitis and diffuse proliferative cell reaction develop, and focal collections of cells resembling an Aschoff–Talalaev granuloma appear. If administration of the serum continues, profound degenerative changes develop in the myocardium, and immunomorphological responses of the spleen and lymph glands are depressed.

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Attempts to simulate the autoimmune mechanism of rheumatic carditis experimentally have been made by injecting animals with homogenate of homologous heart tissues together with streptococci [1, 4, 5, 7, 11, 12], and also by injecting heterologous cytotoxic cardiac sera in small doses [2, 3, 6].

The object of the present investigation was to make a physiological, immunological, and morphological analysis of autoimmunogenesis and changes in the cardiovascular system produced experimentally by the action of homologous heart antiserum.

EXPERIMENTAL METHOD

The animals (15 rabbits) of group 1 received intravenous injections of homologous serum containing cardiac antibodies (titer of complement-fixing antibodies 1:20-1:40, latex-agglutinating 1:10,000-1:20,000, and hemagglutinating 1:16-1:32). The animals of group 2 (15 rabbits) received the same serum mixed with heat-killed streptococcal vaccine. The serum was injected in doses of 2-3 ml twice a week in a 6-week cycle, with intervals between cycles of 1.5-2.5 months. Streptococcal vaccine was injected twice a week in a dose of 250 million bacterial cells. All the experimental animals were subdivided into three series: in series I the animals received one cycle of injections, in series II three cycles, and in series III eight cycles. The control group (10 rabbits) received serum of intact animals in accordance with the same scheme. The experimental and control animals were tested immunologically every 10-12 days by the complement fixation reaction in the cold (CFR), the latex-agglutination test (LA), and Boyden's agglutination of tanninized erythrocytes test (PHR). A result assessed at not less than ++ was regarded as positive. Animals not containing antibodies in their blood were chosen for the experiments. The electrocardiogram (ECG) was recorded periodically. After sacrifice of the animals the organs were examined morphologically, using histochemical, histological, and electron-microscopic methods. Altogether, 30 animals were investigated morphologically and 120 immunologically.

EXPERIMENTAL RESULTS

Virtually no antibodies were found in the blood of the control animals receiving serum of healthy rabbits, but pathomorphological reactions were found in the tissues and other organs. Cardiac antibodies appeared in low concentration in the blood of the experimental animals at the end of the first cycle of immunization (CFR 1:10, PHR 1:4, LA 1:10,000). The titers rose gradually to reach a maximum on the 40th-50th day after the end of the cycle (1:20-1:40, 1:8-1:32, 1:20,000-1:40,000, respectively). However, the highest titers were observed at the same times in the animals of series II (1:40-1:160, 1:32-

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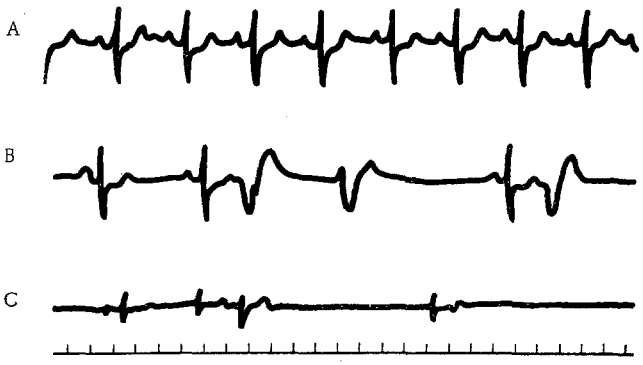

Fig. 1. ECG of normal rabbit (A), after three cycles of injection of homologous heart antiserum (B), and after seven cycles of injection of homologous heart antiserum (C).

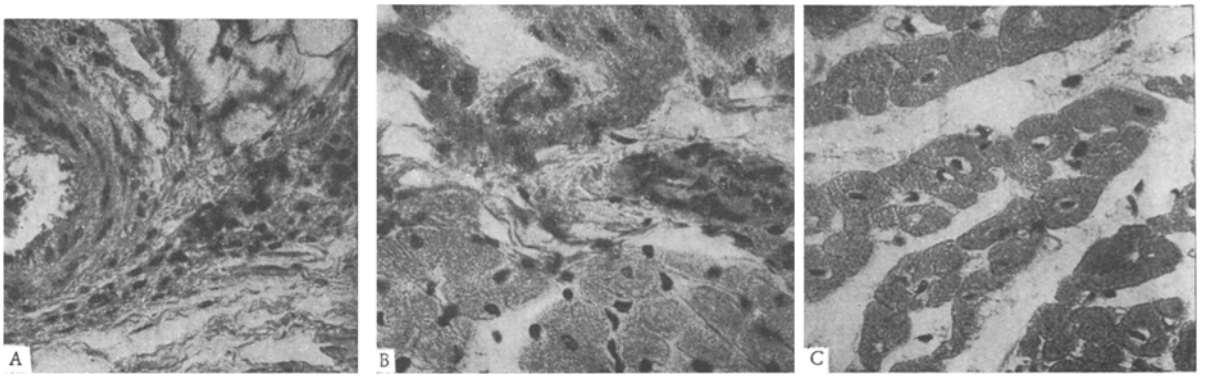

Fig. 2. Morphological changes in myocardium of experimental rabbits. A) Perivascular granuloma with large hyperchromic histiocytes; B) productive vasculities in myocardium, perivascular edema; C) degeneration of myocardium with development of perinuclear and intermuscular edema. Hematoxylin—eosin, 400 ×.

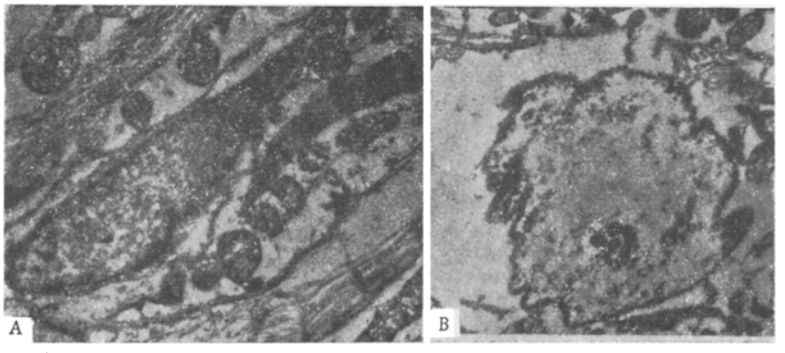

Fig. 3. Electron-microscopic changes in myocardium of experimental rabbits. A) Degeneration of myofibrils and mitochondria of myocardium; B) modified nucleus of muscle cell (irregular shape, disappearance of chromatin, and enlargement of perinuclear space). Uranyl acetate, 21,000 ×.

1:128, 1:80,000–1:120,000). In the animals of series III, despite repeated injections of cardiac antiserum, a gradual decrease in the antibody concentration in the blood was observed, down to the level of their concentration in the animals of series I, and sometimes even lower. Meanwhile, streptococcal antibodies

were detected in the blood of the experimental animals, but in lower titers (lower by 1-2 orders). The dynamics and intensity of antibody formation were of the same pattern when determined in the animals by the three serological methods.

No significant differences could be found between the immunologic and morphological reactions of the animals of groups 1 and 2. A study of the ECG of the animals in series I showed no appreciable changes except an increase in the sensitivity to adrenalin, indicating the development of sympathicotonia (Fig. 1A). In the ECG of the animals of series II disturbances of excitability and conductivity appeared, as shown by an increase in voltage of the T wave and in the length of the P—Q interval, changes in position of the isoelectric line, and the appearance of ventricular extrasystoles (Fig. 1B). Changes in the ECG in series III consisted of a sharp decrease in voltage of the QRS wave, lengthening of the P—Q interval, deepening of the S wave, and single ventricular extrasystoles (Fig. 1C). A sharper decrease in voltage of the QRS wave was observed in the experiments in which serum was injected mixed with streptococcal vaccine.

The results of morphological investigations on the animals of series I showed the development of changes in the cardiovascular system reflecting a state of sensitization, namely proliferation of the vascular endothelium, focal proliferation of the endothelium of the valves, and, in some cases, the appearance of small collections of lymphocytes and histiocytes in the myocardium. Groups of plasma cells were seen in the spleen, the regional lymph glands, and also in the kidneys and heart. In the animals of series II, proliferative cell reactions of the connective tissue were more intensive, with the appearance of groups of cardiohistiocytes and cells with a vesicular nucleus in the valve. In some cases focal collections of cells were observed in the myocardium, with a perivascular or subendocardial distribution. These clusters were similar to the specific Aschoff — Talalaev granuloma (Fig. 2A). Productive vasculitis developed in the blood vessels (Fig. 2B). In series III degenerative changes of muscle cells were constantly found, with signs of perinuclear edema and homogenization of the cytoplasm, and also areas of lysis of muscle fibers without a histiocyte reaction (Fig. 2C). The nuclei of most muscle cells have become irregular in shape and were deficient in chromatin. Tests for respiratory enzymes revealed areas of myocardium with an uneven distribution and a decreased content of formazan granules, evidence of depression of cell respiration. In the animals of series III, proliferative cell reactions in the heart were less marked than in the animals of the preceding series; reactions indicative of disorganization of the connective tissue were more pronounced. In the spleen and lymph glands, emptying of the follicles of cells were observed, accompanied by absence of a plasma-cell reaction.

The results of the electron-microscopic study of the myocardium in animals receiving homologous serum over a long period (series III) revealed considerable changes. The myofibrils became thinner, and developed vacuoles and a granular structure. In many places the muscle fibers were split apart or completely destroyed. The intercalated disks were broken up. Lipid inclusions were present between the myofibrils, indicating degenerative changes in the muscle cells. The arrangement of the mitochondria was disturbed, and the mitochondria themselves showed degenerative changes: the double outline of the membrane was disturbed, the cristae were partially destroyed, and vacuoles appeared in many mitochondria (Fig. 3A). Changes in the shape of the nuclei, disappearance of chromatin, and a marked increase in size of the perinuclear spaces were found in the muscle fibers and connective-tissue cells (Fig. 3B).

The dynamics of antibody formation in the experimental animals suggests that injection of homologous cytotoxic serum stimulates mechanisms of autoimmunogenesis. This is confirmed by the fact that the concentration of heart antibodies was higher in the serum of the recipient animals than of the donors. The uniform pattern of the results of the immunologic tests is evidence of the consistency of the immunologic response of the experimental animals. The appearance of streptococcal antibodies in the animals of group 1 receiving only cardiac antiserum without streptococci may have been brought about because the heart tissue contained components also present in streptococci [8-10]. The cytotoxic action of the injected sera could have helpted to bring about this manifestation of the antigenic properties of these components in the body.

Morphological observations with the optical and electron microscopes constantly revealed degenerative changes in the myocardium, most marked in rabbits receiving cardiac antiserum for long periods. Changes in the structure of the mitochondria were evidently responsible for the decrease in content or activity of respiratory enzymes in the myocardium. The ECG gave evidence of profound changes developing in the myocardium: a sharp decrease in amplitude of the QRS waves. Comparison of the morphological and serologic data showed that they were in agreement and reflected fluctuations in the intensity of

immunogenesis during the period of the experiments. In the first two series of the investigation, for instance, highest titers of antibodies were observed and the plasma-cell reaction in the spleen and lymph glands, and also in the heart, kidneys, and lungs, was correspondingly widespread. Proliferative cell reactions of granulomatous type were clearly seen in the heart of these animals. In the experiments of series III, serologic, immunological, and morphological reactions were, on the other hand, slight in intensity, and the tendency to form granulomatous cell clusters was only slight. The results of these experiments support the view of those workers [13] who consider that granulomatous cell reactions in the heart are a specific manifestation of the intensity of immunogenesis. The results described above further demonstrate the important role of autoimmune mechanisms in the pathogenesis of rheumatic heart disease.

LITERATURE CITED

1. V. D. Akhnazarova, in: Common and Distinguishing Features of the Pathogenesis and Clinical Picture of Rheumatic Fever and Other Collagen Diseases [in Russian], Moscow (1966), p. 8.
2. A. G. Beglaryan and L. M. Khai, Arkh. Pat., No. 2, 11 (1961).
3. D. A. Biryukov, V. I. Ioffe, A. I. Strukov, et al., in: Problems in General Immunology [in Russian], Moscow (1959), p. 20.
4. A. S. Kaplanskii, G. V. Orlovskaya, and A. A. Tustanovskii, Vopr. Revmat., No. 2, 3 (1961).
5. L. D. Krymskii, G. E. Perchikova, and I. Ya. Uchitel', Éksperim. Khir, No. 4, 44 (1958).
6. N. A. Levkova, Arkh. Pat., No. 2, 40 (1957).
7. I. M. Lyampert, O. N. Gryzlova, L. V. Beletskaya, et al., Vopr. Revmat., No. 3, 92 (1964).
8. N. M. Mazina and I. I. Rassokhina, Vopr. Revmat., No. 1, 16 (1966).
9. A. A. Tustanovskii, in: Contemporary Problems in Rheumatology [in Russian], Moscow (1965), p. 34.
10. A. A. Tustanovskii and I. I. Rassokhina, in: Current Problems in Rheumatology. Scientific Survey, No. 1 [in Russian], Moscow (1965), p. 3.
11. M. I. Undritsov, N. M. Mazina, V. D. Akhnazarova, et al., Vopr. Revmat., No. 4, 20 (1964).
12. P. Cavelti and E. Cavelti, Arch. Path., 44, 119 (1947).
13. P. Miescher and K. O. Vorlaender (editors), Immunopathologie in Klinik und Forschung, Stuttgart (1957).