CHANGES IN LYSOSOME ACTIVITY AFTER REJECTION OF A HETEROTOPICALLY IMPLANTED HEART IN MICE

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After allogeneic implantation of the heart of newborn C57BL/6 mice subcutaneously in the pinna of CBA mice increased acid phosphatase activity and activation of the Golgi apparatus took place on the 3rd day after the operation. Later numerous lysosomes appeared in the cardiac myocytes of the graft, and on the 7th-9th day after implantation their membrane disintegrated. Simultaneously during histological investigation foci of loss of acid phosphatase activity were observed, together with diffuse brown staining of the cytoplasm. After isologous implantation (CBA-CBA) no signs of destruction of the lysosome membranes or liberation of enzymes into the cytoplasm was observed.

KEY WORDS: implantation; heart; lysosomes; rejection.

Experimental observations and clinical investigations have shown [2-4, 6, 8, 12, 15] that during allergic and autoallergic processes the activity of the lysosomal enzymes in the blood and tissues rises sharply; some workers associate the destruction of the cells arising in certain forms of allergy with damage to the lysosomal membranes and the release of enzymes into the cytoplasm [1, 5]. It was accordingly decided to investigate the character of changes in the lysosomes following rejection of the implant.

EXPERIMENTAL METHOD

Free grafting of the heart in mice [12] was used as the experimental model. The recipients were 80 CBA mice and the donors were C57BL/6 mice. In the control group isologous implantation (CBA-CBA) was performed. A separate control group consisted of 20 newborn C57BL/6 mice. The hearts of the newborn animals were grafted subcutaneously into the pinna of adult mice. As previous investigations [9] showed, in implantation by this method the recipient's blood vessels grew into the implant, and rejection of the graft was observed on the 9th-11th day. In isologous implantation the grafted heart exhibited bioelectrical activity (and in all the experiments this was used to determine the viability of the graft) for several months. The grafted heart was removed 3, 5, 7, and 9 days after both heterologous and isologous implantation and subjected to histochemical examination for acid phosphatase by Burstone's method in frozen cryostat sections and to examination (after fixation of the material in 3% glutaraldehyde solution and postfixation in 1% buffered osmic acid, embedding in Araldite, and cutting of sections on the LKB-8800 Ultratome) in the EMV 100L electron microscope under a magnification of 20,000× (the sections were stained with lead hydroxide and uranyl acetate). The hearts of the newborn mice were investigated in the same way at the above-mentioned times after birth and also on the first day.

EXPERIMENTAL RESULTS

The experiments showed fairly high acid phosphatase activity in the myocardium of the newborn mice (Fig. 1A); the reaction product was uniformly distributed in the myocardium as large granules. After 3 days the number and size of these granules increased a little and remained constant throughout the subsequent period of investigation (Fig. 1B).

On the third day after heterologous implantation uniform deposition of the reaction product for acid phosphatase was found in the myocardium; granules of this product were very small, smaller than in the newborn

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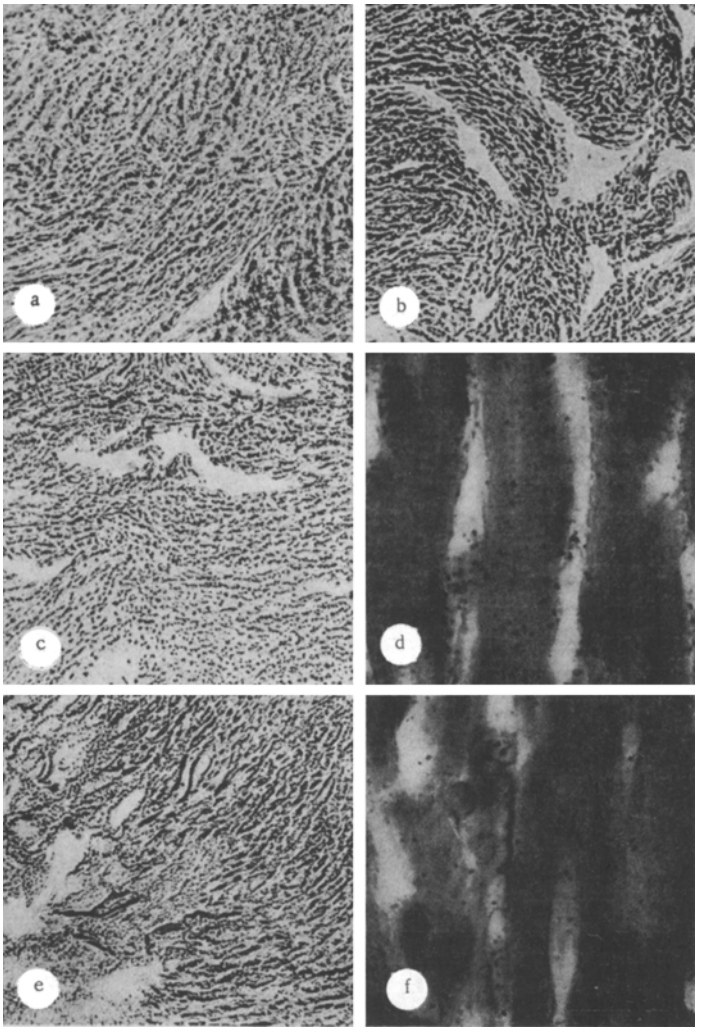

Fig. 1. Changes in acid phosphatase activity during course of rejection of heterografted heart. Reaction for acid phosphatase (magnification: a, b, c, e 80×; d, f 200×). a) Myocardium of newborn mouse; b) myocardium of mouse 9 days after birth; c) myocardium of implanted heart 3 days after transplantation. Deposition of reaction products as numerous tiny granules; d) myocardium of implanted heart 5 days after grafting. Formation of conglomerates of granules of enzyme reaction product; e) myocardium of implanted heart 7 days after implantation. Focal demonstration of enzyme activity; f) myocardium of implanted heart 9 days after grafting. Enlargement of regions of deposition of enzyme reaction product.

mice (Fig. 1C). The activity of the enzyme was increased a little 5 days after implantation, and in some places deposition of the product of the enzymochemical reaction was observed as large granular conglomerates (Fig. 1D). After 7 days (Fig. 1E) the number of these large conglomerates increased, whereas in some parts of the myocardium the activity of the enzyme was sharply reduced. After 9 days these phenomena were intensified (Fig. 1F); most of the preparation was free from granules of reaction product and stained diffusely a pale brown color.

Electron-microscopic investigation after heterografting showed only a few small lysosomes in the myocardium 3 days after implantation. Meanwhile the number of vesicles of the Golgi apparatus was considerably greater than in the heart of a newborn mouse 3 days old (Fig. 2A). After 5 days (Fig. 2B) some increase was found in the number of lysosomes in the cardiac myocytes compared with the 3rd day. The lysosomes were

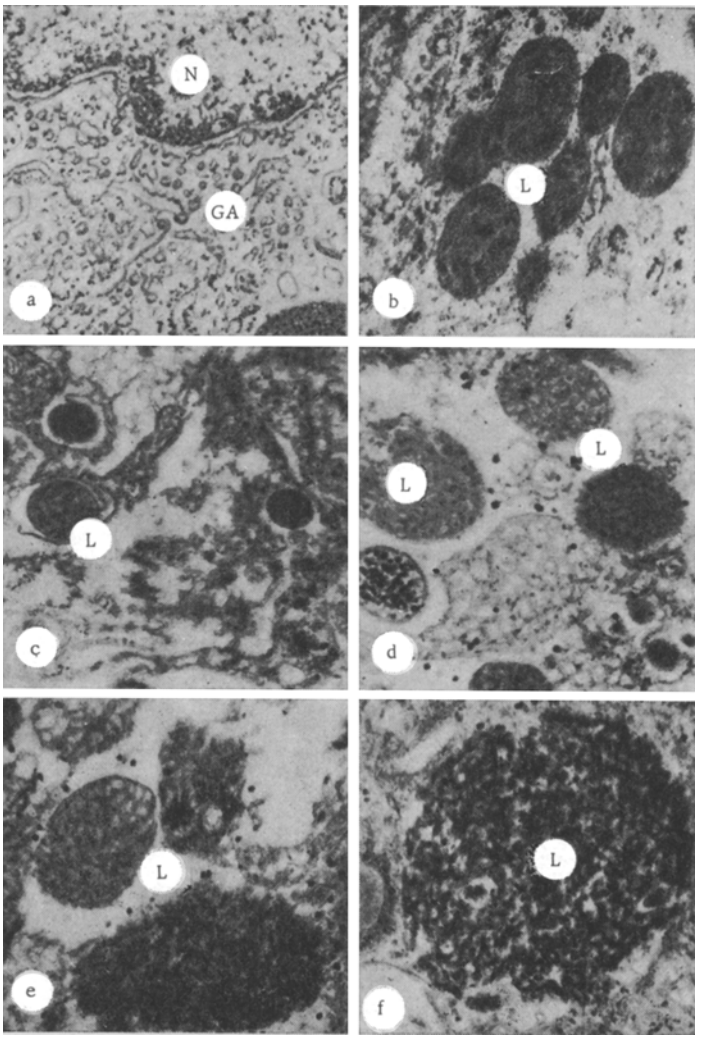

Fig. 2. State of myocardial ultrastructures during rejection of heterografted heart. Electron-micrographs of myocardium (20,000×). L) Lysosomes; N) nucleus; GA) Golgi apparatus. a) Activation of Golgi apparatus 3 days after transplantation; b) lysosomes in graft 5 days after transplantation; c) many small lysosomes in implant 5 days after grafting; d) destruction of membrane of some lysosomes in implant 7 days after grafting; e) destruction of membranes of most lysosomes 9 days after grafting; f) appearance of "giant" lysosomes with destroyed membrane 9 days after grafting.

mainly small, and were sometimes arranged in groups. All the lysosomes were primary, some had a pale matrix, but all had a clear membrane. After 7 days (Fig. 2C) the number of small lysosomes increased. Meanwhile large areas of cytoplasm contained no lysosomes. In some lysosomes destruction of the membrane (Fig. 2D) and liberation of the contents into the cytoplasm were observed. Signs of destruction of other cell organelles were noted. After 9 days the number of lysosomes in the myocardium was a little lower than at the previous times of investigation, but they were considerably enlarged and gross destruction of the membranes was observed in nearly all of them (Fig. 2E). Individual "giant" lysosomes (Fig. 2F) with a completely destroyed membrane were seen. Marked destruction of the subcellular structures was present in the cardiac myocytes.

On the 3rd day a moderate number of lysosomes with a distinct membrane was found in the myocardium of the newborn animals; this picture persisted throughout the period of investigation.

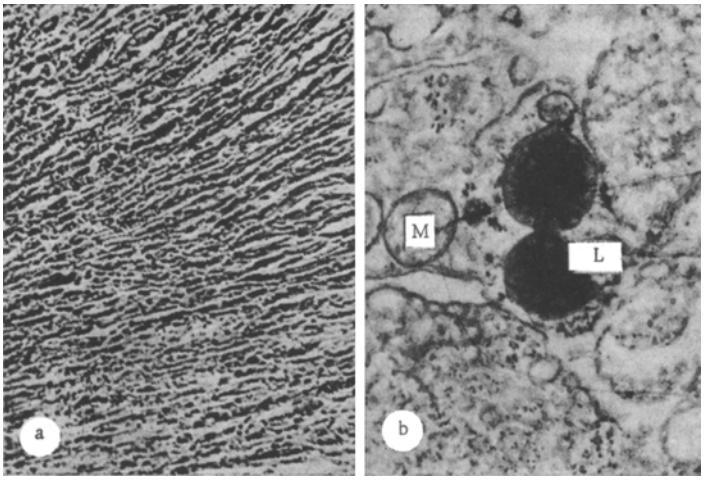

Fig. 3. Reaction for acid phosphatase (a) and lysosomes in myocardium (b) of implanted heart 9 days after isografting (magnification: a) 80; b) 20,000). M) Mitochondria; L) lysosomes.

In the case of isografting, on the third day after the operation, just as in the animals of group 1 at the same period, high acid phosphatase activity was observed with deposition of the reaction products as tiny granules, uniformly distributed in the muscle fibers. At later times of observation these granules were a little larger but remained uniformly distributed in the cytoplasm (Fig. 3A). On electron-microscopic investigation on the third day after the operation activation of the Golgi apparatus and the appearance of a certain number of small lysosomes were observed. Later the number of lysosomes was greater than in the heart of the newborn mice but less than after heterografting. The membrane of all the lysosomes remained distinct and intact at all times of the investigation (Fig. 3B).

After birth definite evolution of the lysosomal apparatus takes place in the myocardium, evidently in connection with the development and reorganization of metabolism of the cardiac myocytes in the early postnatal period [7], and expressed, in particular, as some increase in the number of lysosomes starting from the third day of extrauterine life. In the case of both hetero- and isografting, on the third day after the operation activation of the Golgi apparatus was observed and was expressed histochemically as increased acid phosphatase activity. This may have been the result of increased "assembly" of lysosomes on account of the entry of various substances into the graft from the surrounding exudate and tissues of the recipient. Substrates formed as a result of damage to the graft itself during the operation and to changes in its metabolism during the first days after the operation, when the grafted myocardium received its nutrition through diffusion from one tissue into another, may also have played an important role in this process.

A characteristic feature distinguishing the state of the ultrastructure of the myocardium in the later stages of observation after heterografting was destruction of the lysosome membranes, the discharge of their contents into the cytoplasm, and autolysis of the cardiac myocytes. Meanwhile after isografting the normal ultrastructure of the myocardial cells was restored. This difference suggests that the disturbance of the lysosomal apparatus revealed after heterografting was due to the development of immunologic conflict, more especially because the histological changes in the graft at these times were characteristic of a well-marked rejection reaction [11].

The mechanisms of destruction of the lysosome membranes may be connected with injury to them by antibodies or immune complexes formed in such cases, absorbed on the membranes, that produce the "assault" outpouring of lysosomal enzymes into the cell cytoplasm [9, 15]. The view that the enzymes are liberated into the cytoplasm is confirmed both by the destruction of the lysosomal membranes and appearance of their granules in the cytoplasm, observed under the electron microscope, and by the results of histochemical investigation (diffuse pale brown staining of the cytoplasm of the 7th and 9th days after implantation of the heart).

On the other hand, destruction of the lysosomes may be connected with changes in myocardial metabolism resulting from thrombosis of the small vessels (since diffusion of nutrients into the implant at this stage takes place not from one tissue to another, but from the recipient's blood vessels which have grown into the graft),

of infiltration by monocytes, and of marked edema observable at this stage of the reaction, i.e., it does not necessarily depend on the direct action of immunologic factors on the lysosomal membranes. In both cases, however, enzymes entering the cytoplasm from the lysosomes induce processes of autolysis, which may play a role in the pathogenesis of rejection of the grafted heart.

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AMYLOID TRANSFER FROM A SYNGENEIC GRAFT OF AMYLOID SPLEEN IN INTACT AND AMYLOID MICE

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The investigation was carried out on male CBA mice using the casein model of amyloidosis. After simultaneous transplantation of fragments of spleen from intact and amyloid donors beneath the capsule of opposite poles of the kidney into intact and amyloid recipients, deposits of amyloid both in the endogenous spleen and in the graft from intact donors were found in 40% of intact animals. In amyloid recipients under observation for periods of between 5 days and 6 months, deposits of amyloid in the intact graft were observed in only 5% of cases. It is postulated that amyloidosis is "transferred" through migration of cells participating in amyloid formation and that this mechanism is inhibited in animals with amyloidosis.

KEY WORDS: experimental amyloidosis; "transfer" of amyloidosis; transplantation.

Several investigators have described the development of amyloidosis in syngeneic recipients after injection of a suspension of spleen cells or transplantation of fragments of spleen from an amyloid donor – the

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