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DISTRIBUTION OF LYSOSOMAL ENZYME ACTIVITY IN CORTICAL CELLS OF THE KIDNEY AFTER SUBCAPSULAR TRANSPLANTATION OF ALLOGENEIC SPLEEN CELLS

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Following injection of allogeneic spleen cells beneath the renal capsule of mice irradiated in a dose of 650 R structures of the renal cortex were damaged. This effect was closely connected with the function of macrophages and activation of the lysosomal enzymes.

KEY WORDS: macrophages; lysosomes; renal cortex; local graft versus host reaction; acid phosphatase.

In recent years considerable attention has been paid to the study of reactions of cellular immunity, which play an important role in widespread pathological processes such as allergic states [1, 2, 17], autoimmune diseases [3, 12], and phenomena of tissue incompatibility [6, 11]. In some context new models have been developed in order to study these reactions in greater detail and depth. One such model is the recently suggested local renal graft versus host reaction [13, 14].

Considering the important role of lysosomal enzymes in the formation of reactions of cellular immunity [4, 8, 9], and also the fact that the histochemical distribution of the enzymes and the degree of their activity can provide an indication of the various functional states of the cell, including injury to it [5, 10], it was decided to study the distribution of activity of the lysosomal marker enzyme acid phosphatase (AcP) in the kidney cells during the local graft versus host reaction.

EXPERIMENTAL METHOD

Adult C57 Bl mice (H-2^b histocompatibility locus) were used as donors and adult CBA mice (H-2^k) as recipients. The recipients were irradiated on the RUM-11 apparatus under the following conditions: voltage 180 kV, current 10 mA, filters 0.5 mm Cu and 1 mm Al, skin-focus distance 60 cm, dose rate 10 R/min. The total dose of irradiation was 650 R. An extraperitoneal (lumber) approach was made to the left kidney under ether anesthesia 24-36 h after irradiation and 35×10^6–45×10^6 allogeneic donors' spleen cells in 0.1-0.2 ml medium No. 199 were injected beneath its capsule.

The cell suspension from the donor mouse was obtained by pressing spleen tissue (after first incising the capsule) through a Kapron filter in medium No. 199 at 0-4°C. The number of viable cells was determined by the trypan blue test (80%).

Animals undergoing a mock operation and mice into which syngeneic or disintegrated allogeneic spleen cells were transplanted were used as the control. Sections through the kidneys of the control and experimental animals were stained with hematoxylin–eosin, with Methyl Green and pyronine by Brachet's method, and for AcP by Gomori's method.

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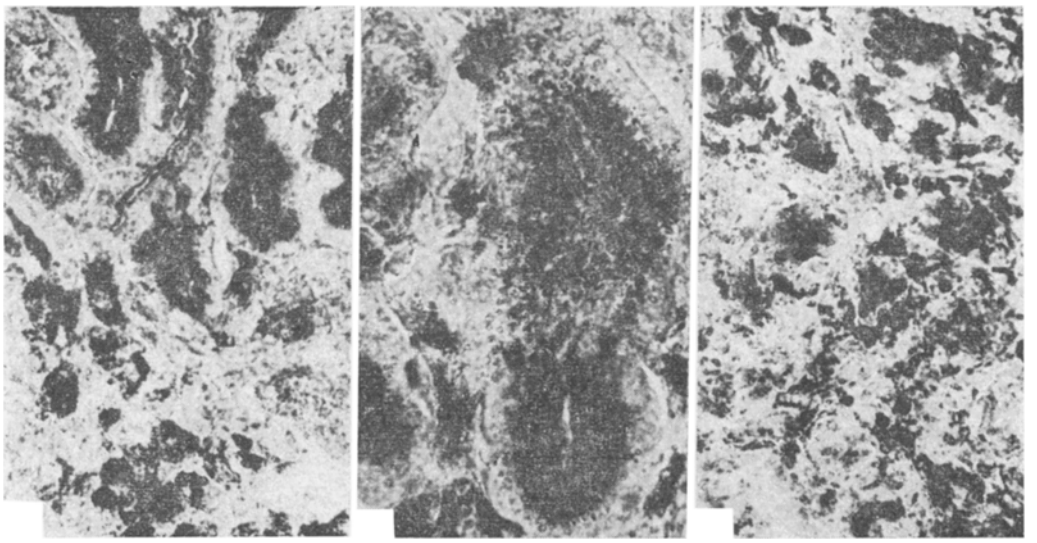

Fig. 1. Distribution of lysosomal enzyme activity in cells of the renal cortex 10-11 days (a, b) and 14-15 days (c) after cell transplantation: a) invasion of parenchyma of renal cortex by monocytes with high AcP activity (200 ×); b) injury to part of a proximal convoluted tubule. AcP (+) monocytes visible next to damaged epithelial cells (400 ×); c) numerous AcP (+) infiltrating cells in renal cortex on 14th-15th day of experiment. Disintegration of structural complexes of the kidney and weakening of AcP activity in cells of those complexes (300 ×).

EXPERIMENTAL RESULTS

On the sixth to seventh day after injection of allogeneic lymphocytes beneath the kidney capsule a thin layer consisting mainly of ground monocytes of different sizes, most of them with high or moderate AcP activity, was discovered.

"Tongues" of invasion of monocytes deep into the renal cortex were discovered in sections through the kidneys of the experimental animals 10-11 days after transplantation (Fig. 1a). In this phase, besides lymphocytes at different stages of maturation, large numbers of cells of macrophage type, giving off processes and with cytoplasm entirely stained black in the Gomori reaction, also were detected among the infiltrating cells.

The infiltrating cells accumulated around the glomeruli and in the peritubular spaces, causing injury to structures of the renal cortex. This injury was most severe in the proximal convoluted tubules. In the cytoplasm of these tubules a homogeneous distribution of drop-like granules was replaced by a polymorphic and irregular scattering of granules (Fig. 1b).

On the 14th-15th day of the experiment damage to the renal cortical parenchyma reached a high level of severity; numerous foci of infiltration were seen in the peritubular spaces, in the lumen of the small vessels, and around the glomeruli (Fig. 1c). A sharp decrease in AcP activity was observed in the cells of the necrotic tubules and also of the glomeruli. Many degenerating cells also were seen among the others in the foci of infiltration.

After subcapsular transplantation of allogeneic immunocompetent cells, differing in the H-2 histocompatibility locus, into the kidney of an immunologically weakened (irradiated) animal, an invasive-destructive process thus develops in the cortex of the kidney which is not obseved after transplantation of syngeneic or disintegrated allogeneic cells. Most infiltrative monocytes are known to be of the donor's genotype [13, 14]. Starting from the sixth to seventh day of the experiment many immature pyroninophilic cells of blast type were seen in the focus of infiltration, possible evidence of the immunological maturation of immunocompetent cells of donor origin [14, 15].

It is important to note that in the immediate vicinity of concentrations of monocytes with high AcP activity, disturbances in the distribution of AcP activity were observed in the cytoplasm of the adjacent epithelial cells, leading ultimately to their necrosis. This process was clearly defined on the ninth to tenth day of the experiment, when many macrophages with a high level of lysosomal enzyme activity were discovered among the infiltrating cells. According to data in the literature [7, 16], such cells may be the effector elements of cellular

immunity. A close connection is thus observed between the spatial proximity of the mixed population of macrophages and lymphocytes with cells of the renal parenchyma, activation of lysosomal enzymes in the contacting cells, and cell injury.

It can also be postulated that the greater vulnerability of the proximal convoluted tubules than of the other renal cortical structures is determined by the highest content of lysosomes and their enzymes in the epithelial cells of these tubules, which makes them most resistant to the harmful action of the donor's effector cells.

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