MORPHOLOGICAL AND FUNCTIONAL CHANGES IN MAST CELLS AS A PROTECTIVE RESPONSE TO THERMAL TRAUMA

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Mast cells are the first structures to take part in the protective response in the affected area after thermal burns. The highly sulfated mucopolysaccharides liberated by these cells on degranulation assist in the regulation of trophic homeostasis and in the formation of leukocytic infiltration.

The functional activity of the mast cells is one of the factors determining the course of protective and regenerative processes at the site of injury [1, 12, 13]. According to some reports, in aseptic inflammation the primary protective barrier is formed by lymphocytes and leukocytes, while connective-tissue elements, especially mast cells, become activated only on the 3rd-4th day after the beginning of the process [3, 7].

The object of this investigation was to study the role of mast cells and other protective elements in the dynamics of development of the inflammatory process at the site of injury in experimental burns.

EXPERIMENTAL METHOD

A standard steam burn was inflicted on an epilated area of the dorsal region of 36 experimental albino rats. The animals were sacrificed 1, 3, 6, 12, 24, 48, and 72 h after the burn. A group of tissues from the side of injury was taken for histological and histochemical investigation: the skin, subcutaneous cellular tissue, and the adjacent muscles. The mast cells also were investigated in films taken from the subcutaneous cellular tissue at the site of the burn and in an area of healthy tissue. The material was fixed in 10% neutral formalin by Carnoy's method and in cold acetone. The secretions and films were stained with azure II-eosin, with 0.5% aqueous solution of toluidine blue in Michaelis buffer, pH 5.0, and with toluidine blue and Schiff's reagent by the method of Nepomnyashchikh and Kalacheva [5]. Alkaline phosphatase was determined by Gomori's method [10]. Mast cells were counted in the films in 10 fields of vision (320 ×). The ratio between the number of degranulated mast cells and the total number of mast cells was expressed by the coefficient of degranulation.

EXPERIMENTAL RESULTS

In intact rats, in the dorsal region where burns were inflicted on the experimental animals, mast cells were found around the small blood vessels. The cells were oval and compact, with a slight degree of metachromasia (Fig. 1a, b).

Total destruction of the epidermis and partial destruction of the superficial part of the dermis were observed in the affected area 1 h after burning. Individual mast cells with marked degranulation, giving rise to bright red metachromasia, were arranged around the deformed skin appendages. At the base of the dermis and in the subcutaneous cellular tissue, around the dilated blood vessels and capillaries, the

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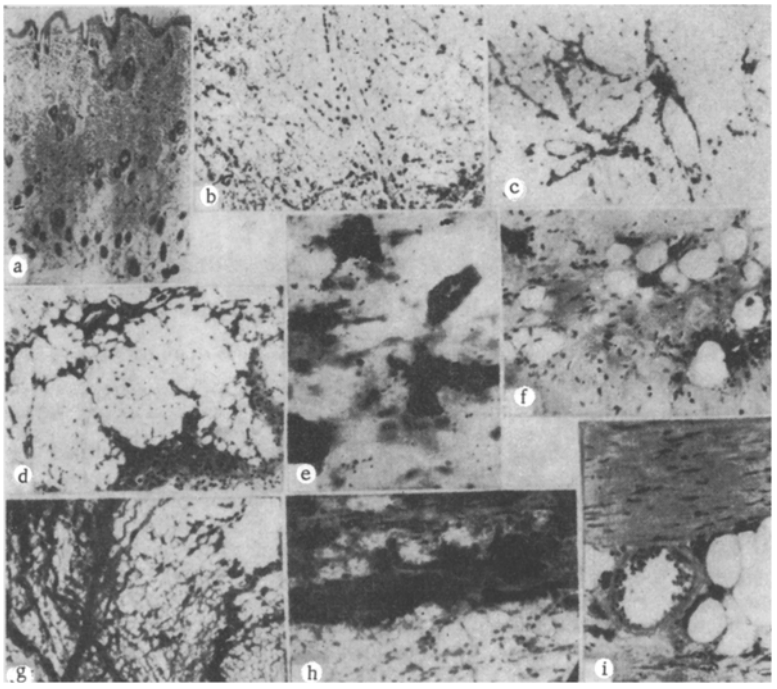

Fig. 1. Changes in mast cells during development of inflammatory changes in dermis and subcutaneous cellular tissue (a, b) in intact rats, and c) 1 h; d, e) 3 h; f, g) 6 h; h) 12 h; and i) 24 h after burning. Toluidine blue; magnification: a, c, d) 72 ×; f, i) 320 ×. Films, magnification: b, g) 72 ×; e, h) 320 ×.

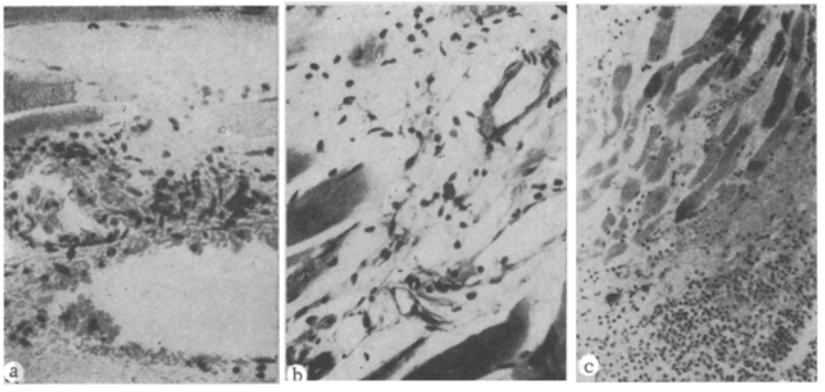

Fig. 2. Formation of neutrophilic infiltration in focus of injury: a) 6 h; b) 12 h; c) 24 h after burning. Toluidine blue, magnification: a, b) 320 ×; c) 160 ×.

mast cells were considerably enlarged and showed marked granulation of the cytoplasm and metachromasia. Some cells were degranulated (Fig. 1c).

At the base of the dermis and in the loose subcutaneous connective tissue all the mast cells were enlarged 3 h after injury, and some were degranulated (Fig. 1d). PAS-positive granules were found among the granules of the mast cells, mostly in the peripheral part of the zone of dissemination (Fig. 1e). The enlarged mast cells in the muscle tissue were granulated and showed bright metachromasia. Individual lymphocytes and fibroblasts with metachromatic cytoplasm were found around the small blood vessels. These cells and the endothelium of the capillaries gave a weak reaction for alkaline phosphatase (Fig. 3a).

In the deep layers of the dermis 6 h after burning the number of mast cells was increased; they were very large in size and, as a rule degranulated. Small groups of blood cells, including solitary

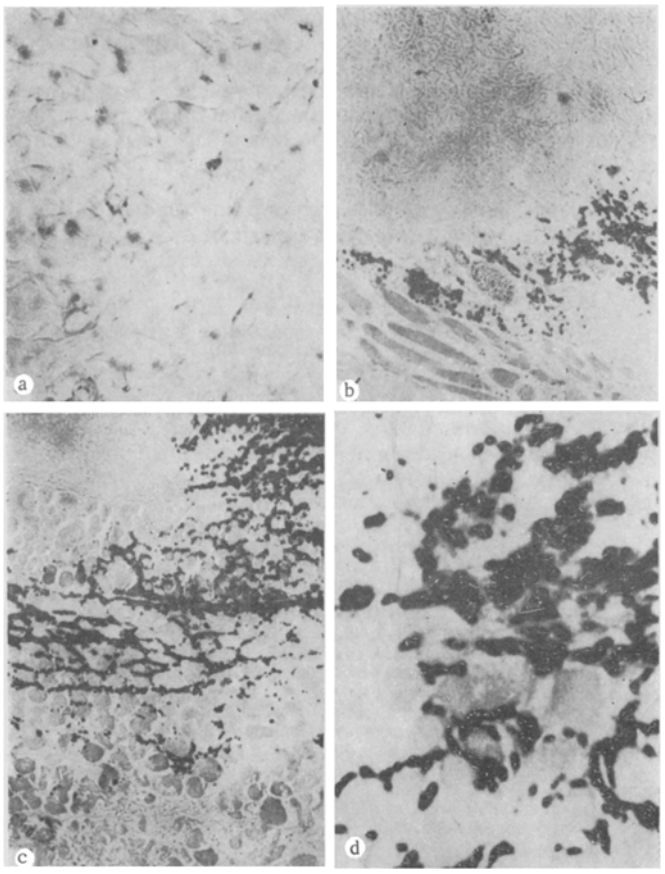

Fig. 3. Activity of reaction for alkaline phosphatase in cells at site of thermal injury: a) 3 h; b) 24 h; c, d) 72 h after burning. Gomori, magnification: a, d) 320 ×; b, c) 72 ×.

neutrophils, were observed around the dilated blood vessels in this area (Fig. 1f). Degranulated mast cells with intensive metachromasia sometimes formed clusters around the vessels in the subcutaneous cellular tissue (Fig. 1g). Vacuolation was clearly distinguishable in the cytoplasm of these cells. A few granules and the vacuolated cytoplasm gave a positive PAS reaction. In the superficial groups of muscles, clusters of blood cells and individual fibroblasts, with weakly PAS-positive cytoplasm, appeared around the small blood vessels (Fig. 2a). Alkaline phosphatase was present in the cytoplasm of some neutrophils and lymphocytes, and also in the capillary endothelium in the intermuscular spaces.

At the base of the dermis and in the subcutaneous cellular tissue, the mast cells were completely degranulated 12-24 h after burning. Their metachromatic granules infiltrated the subcutaneous cellular tissue (Fig. 1i). Some granules gave a positive PAS reaction. In the muscles 12 h after burning the neutrophilic infiltration was increased in size and localized in the ground substance of the deep, uninjured layers of muscles (Fig. 2b). The neutrophil barrier extended into the subcutaneous cellular tissue 24 h after burning (Fig. 2c). Mast cells were rarely seen among the muscle fibers. Beyond the accumulation of neutrophils in the muscles, fibroblasts and PAS-positive fibrils were visible. An active reaction for alkaline phosphatase was seen in the neutrophils and other blood cells (Fig. 3b).

Some of the mast cells were distintegrated 48-72 h after thermal injury. Vacuolated areas of cytoplasm were observed in the degranulated cells. Granules of mast cells infiltrating the subcutaneous cellular tissue were strongly metachromatic, and gave a negative PAS reaction. Many neutrophils also appeared destroyed and their cytoplasm gave a weakly positive PAS reaction. In the superficial layers of the muscles, the mast cells also were degranulated. Their granules were PAS-negative, with bright metachromasia. Fibroblasts and other connective-tissue cells located beyond the zone of neutrophilic infiltration showed absence of metachromasia. The cytoplasm of these cells was weakly PAS-positive. At this period of observation, activity of the reaction for alkaline phosphatase was considerably increased in the neutrophils, the capillary endothelium, the blood cells, and connective tissue (Fig. 3c), and in the neutrophils the enzyme also could be detected in the cell nuclei (Fig. 3d).

In films taken from the subcutaneous cellular tissue in the region of the burn from 3 to 72 h after its infliction, complete degranulation of the mast cells was observed both along the course of the dilated vessels and at a distance from them. Nearly all the mast cells 24-72 h after burning were disseminated, so that it was impossible to count them exactly. A statistically significant increase in the coefficient of degranulation was observed, however, 1 h after burning and it continued until the end of the period of observation. A characteristic feature of the mast cells in the films was the weak degree of metachromasia for the compact forms and the intensive metachromatic staining of the granulated and degranulated cells. PAS-positive granules began to appear 1 h after burning in the granulated and degranulated mast cells, and in some specimens their number reached 40%.

Data in the literature show that mast cells, a morphological index of the reaction to stress [2, 8], react like nerve receptors to the slightest sign of pathogenic factors in the tissues [4]. The highly sulfated

mucopolysaccharides which they excrete can restore the normal blood circulation [11] and also facilitate access of protective blood cells to a focus of injury [13], and stimulate the enzymic [6] and phagocytic [9] activity of the neutrophils.

These concepts are in agreement with the results of the present investigation indicating a sharp increase in functional activity of the mast cells during the first hour after burning. By liberating highly sulfated mucopolysaccharides, the mast cells on the one hand play an essential role in the regulation of trophic homeostasis during the first hours after thermal injury and, on the other hand, they promote the formation of neutrophilic infiltration and stimulate the phagocytic activity of the cells. Other evidence in support of these views is given by the fact that the barrier of neutrophilic infiltration, with an active reaction for alkaline phosphatase in the cell nuclei, is formed in a burn wound 24-72 h after trauma.

The first structures to take part in protective processes at the site of a burn are thus the mast cells, which play an essential role in the subsequent formation of the response reactions to the burn.

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