DISTURBANCE OF PHAGOCYTOSIS BY ALVEOLAR MACROPHAGES DURING THERMAL TRAUMA

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Disturbances of function of the lung macrophages are of fundamental importance because inflammatory changes in the lungs are the commonest complication of burns and resistance of the lung tissue to infection largely depends on the integrity of the protective function of local macrophages.

There have been few investigations of alveolar macrophages (AM) in thermal trauma, both in the West [6, 8] and the USSR [1-4], and nearly all have been concerned with changes in the functional properties of AM in the early stage (up to 7 days) after burning.

The aim of this investigation was a dynamic study of function of AM after thermal trauma starting from the first day and continuing until convalescence.

EXPERIMENTAL METHOD

Experiments were carried out on 75 chinchilla rabbits weighing 3000-3500 g. Intact animals served as the control. An open flame burn of the IIIB degree and covering 15-18% of the body surface was inflicted on the previously shaved skin of the back and the lateral surface of the chest of the experimental series of animals. The rabbits were killed by air embolism 1, 7, 14, and 30 days after burning. AM were obtained by pulmonary lavage with specially prepared Hanks' solution (not containing antibiotics), cooled to 4°C, by the method in [7] in the writer's modification. The ingestive and digestive power of the cells was studied against *Staphylococcus* (Museum Strain No. 600). The following parameters of phagocytosis were studied by methods described previously [2, 5]: phagocytic activity of the macrophages (PAM) — the percentage of active macrophages; phagocytic number (PN) — the mean number of microorganisms ingested by one macrophage; the index of completeness of phagocytosis (ICP). Characteristics of cell metabolism were studied by determination of activity of several enzymes in them: succinate dehydrogenase (SDH), malate dehydrogenase (MDH), acid phosphatase (AP), and their metabolites — glycogen (Gl) and neutral lipids (NL). An ultrastructural study of isolated AM also was undertaken.

EXPERIMENTAL RESULTS

Analysis of the results shows that thermal trauma leads to a disturbance of phagocytosis. Although the total number of AM obtained from the lungs 24 h after burning did not change significantly, marked inhibition was observed of all the parameters of phagocytosis (Fig. 1). Depression of phagocytic activity was reflected in a considerable (by more than half) decrease ($P < 0.0001$) in the number of active cells capable of ingesting microorganisms (PAM = 33 ± 3%). The ingestion process was disturbed to such a degree that only solitary microorganisms were ingested (PN = 1.8 ± 0.2), and even these were actively multiplying in AM because of disturbance of their digestion. The index of completion of phagocytosis was reduced by two-thirds (ICP = 0.6 ± 0.1), and in all cases phagocytosis was incomplete. Meanwhile no significant changes were yet observed in the ultrastructure of AM. Only swelling of individual mitochondria could be noted. Lysosomes were mainly primary, with only a few secondary lysosomes. Activity of intracellular enzymes (SDH, MDH) was only a little depressed, AP activity remained high, and the Gl and NL content differed only a little from that in the control animals. After 7 days a tendency was noted for the number of AM to increase ($P > 0.05$), and an increase

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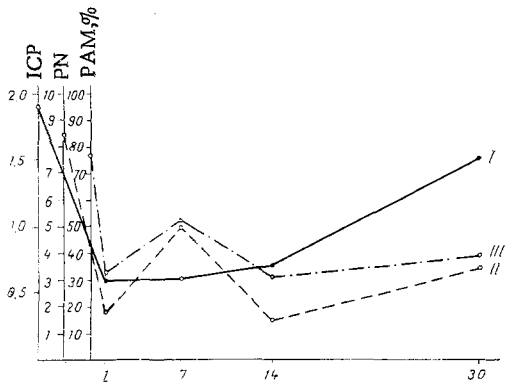

Fig. 1. Dynamics of phagocytic function of AM in burned animals. I) ICF; II) PN; III) PAM.

was observed in the number of smaller and younger cells. Some recovery of phagocytic function of AM was observed, as shown by a tendency for the number of active cells to increase (PAM = 52 ± 5.6%) compared with the previous time of investigation, and an increase in the phagocytic number (PN = 5.0 ± 0.4; $P < 0.05$). Nevertheless phagocytosis, as before, remained incomplete (ICP = 0.6 ± 0.08). Meanwhile changes also progressed in the ultrastructure of the mitochondria, with a marked increase (almost twofold) in the number of primary lysosomes and an increase also in the number of secondary lysosomes (Fig. 2a). Cytochemically, a marked increase in AP activity was found, to a higher value than at any other time of investigation, whereas SDH and MDH activity was reduced and the Gl and NL content also.

After 14 days the number of AM obtained from the lungs was lower ($P < 0.01$ than at any other time of the investigation or initially. The youngest lymphocyte-like forms of AM, with the smallest size, little cytoplasm, and a high nucleoplasmic ratio, appeared for the first time. In addition, there were many fragmented cells. At this time the phagocytic activity of AM showed a second significant fall ($P < 0.001$), to the level observed 24 h after burning (PAM = 31 ± 3.5%, PN = 15 ± 0.2, ICP = 0.7 ± 0.1). Swelling of most mitochondria was observed accompanied by marked transparency of the matrix, almost total disappearance of the cristae, and loss of clarity of the outlines of the outer double membrane (Fig. 2b). The number of secondary lysosomes was increased, they contained remnants of phagocytosed particles and intracellular organelles, and in some cells they reached a considerable size, to form residual bodies. The outlines of AM were smooth, with no outgrowths for pseudopodia. The disturbances of cell metabolism were recorded cytochemically: marked inhibition of activity of respiratory enzymes (SDH, MDH), and even a fall in AP activity, accompanied by a sharp decline in the Gl and NL content.

A considerable increase ($P < 0.01$) in the number of AM, which was several times higher than initially, was observed in animals in the stage of convalescence 1 month after the beginning of the experiment. A study with the light microscope revealed many small lymphocyte-like forms. Meanwhile large multinuclear cells appeared for the first time. The process of phagocytosis still remained disturbed, as shown by low values of PAM and PN (39 ± 3.4% and 3.4 ± 0.5 respectively), but the increase in ICP to 1.4 ± 0.2 is evidence that the digestive power of the cells was gradually being restored. Heterogeneity of the ultrastructural organization of AM was found: Some cells had the usual structure, in others marked signs of intracellular regeneration were observed (Fig. 2d); many undifferentiated forms characterized by pale cytoplasm, containing solitary mitochondria and lysosomes, also were found. Cytochemical investigation revealed a high Gl and NL content. Activity of SDH, MDH, and AP differed in different cells: Besides AM with high activity of all these enzymes, there were some cells with a much reduced enzyme activity.

Thermal trauma thus leads to substantial and prolonged disturbance of phagocytosis, which begins immediately after birth, continues until the period of convalescence and, as the writers showed previously [2], is independent of the object of phagocytosis. Under these experimental conditions the first and strongest depression of the phagocytic response was found 24 h after burning. It was manifested as blocking of both ingestive and digestive power of AM, in agreement with the results of other investigations [1, 4, 6]. The mechanism of this block is complex and still awaits study, but in the writers' view, this is primary inhibition of phagocytosis developing in the period of shock as a manifestation of general inhibition of biological

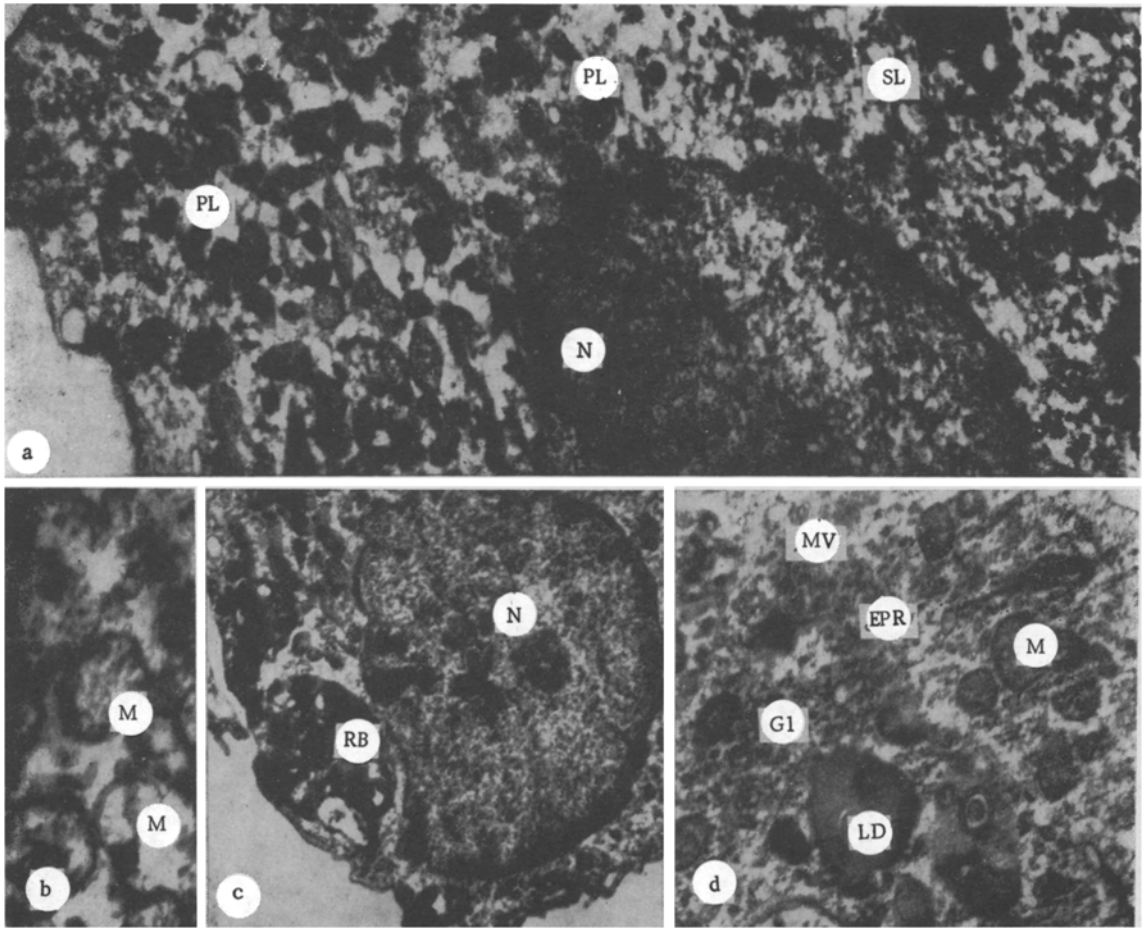

Fig. 2. Changes in AM ultrastructure after thermal trauma; a) 7th day after burning: increased number of lysosomes in cytoplasm of macrophage; N) nucleus, PL) primary lysosomes, SL) secondary lysosomes, 12,000 ×; b) 14th day after burning: swelling of mitochondria (M), translucency of their matrix, destruction of cristae and loss of clarity of outlines of outer membrane, 23,000 ×; c) same time: residual bodies (RB) in cytoplasm of macrophage, 14,000 × ; d) 30th day after trauma: restoration of macrophage structure, high glycogen (G1) content and many microvesicles (MV) and structures of endoplasmic reticulum (EPR) in cytoplasm; mitochondria (M) have usual structures; LD) lipid drops, 17,000 ×.

processes in the cell in a severe stress situation. At the next stage of the investigation phagocytic function recovers somewhat, but the increasing dystrophic changes, manifested as structural disturbances of the intracellular organoids and leading to inhibition of cell metabolism, cause a second fall of activity of phagocytic function, which may be regarded as the result of an energy-deficiency state, developing in the cell. Not until 1 month after burning (during convalescence) is a picture of complete phagocytosis observed for the first time. Even then, however, PAM was reduced by half and PN by more than half compared with normal. In the writers' view this is connected with the appearance of a large number of insufficiently differentiated AM, with defective phagocytic function.

It can be concluded from the facts described above that primary blocking of the function of ingestion and digestion of antigen by AM during the period of burn shock and the prolonged disturbance of this function in the subsequent stages after thermal trauma are among the mechanisms leading to the development of an immunodeficiency state that is characteristic of burns.

The functional capacity of AM thus undergoes substantial disturbances under the influence of burn trauma, expressed to different degrees at different times after burning and continuing until the period of convalescence. The mechanism of disturbance of the phagocytic function of AM differs at different times after trauma. Immediately after burning primary inhibition of phagocytosis is observed, at the height of burn pathology cell metabolism is depressed, leading to an energy-deficiency state, and during the period of convalescence many undifferentiated forms of macrophages with reduced phagocytic ability appear.

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