EFFECT OF DILYSOSOMAL MACROPHAGES ON DEVELOPMENT AND OUTCOME OF PNEUMONIA CAUSED BY THE AGENT OF ENZOOTIC ABORTION IN EWES

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Repeated intranasal injection of the bacterial lipopolysaccharide pyrogenal leads to the accumulation of numerous macrophages packed with lysosomes of leukocytes in the hilar and apical zones of the lungs in mice. Dilysosomal macrophages increase the level of resistance of the mice to the agent of enzootic abortion in ewes (AE) and thus convert a lethal infection into a nonlethal disease.

KEY WORDS: leukocytic lysosomes; macrophages; resistance.

The agent of enzootic abortion in ewes (AE) is a member of the ornithosis – lymphogranuloma –trachoma (OLT) group of organisms with which it shares the properties of their life cycle [3, 7]. After intranasal infection of mice the agent of AE multiplies in the macrophages of the lungs and is destroyed in leukocytes [8].

The object of this investigation was to study whether the breakdown products of leukocytes, ingested by macrophages, affect the development and outcome of pneumonia induced in albino mice by the agent of AE.

EXPERIMENTAL METHOD

The method of subculture and isolation of the agent of AE from the vitelline membranes of 7-day chick embryos was the same as for the agent of ornithosis [3]. The virus-containing yolk suspension in physiological saline with phosphate buffer (pH 7.2-7.4) in a dilution of 1:10 was freed from yolk by centrifugation and used to infect mice.

Repeated intranasal injection of the bacterial lipopolysaccharide pyrogenal caused the accumulation of numerous macrophages containing their own and leukocytic lysosomes in the lungs of mice [4]. Experiments were carried out on 120 noninbred male albino mice (weighing 10-12 g), divided into two groups. Before administration of the agent of AE the mice of group 1 received pyrogenal in doses of 0.3, 0.75, and 1.5 μg in 0.03 ml physiological saline over a period of 2 days at intervals of 24 h by intranasal injection. The animals of the second (control) group received physiological saline. The experimental and control mice were infected intranasally under superficial ether anesthesia with the agent of AE (1.5 LD_{50} per mouse). The survival rate was calculated in 25 mice. The remaining animals were used for morphological and virological tests.

The content of the agent of AE in the lungs of the mice was determined by the usual method: Tenfold dilutions from 10^{-1} to 10^{-6} were prepared from the original 10% suspension. With each dilution of the suspension four mice were infected intranasally. The experimental results were analyzed over a period of 18 days. The titer of the agent of AE was determined as LD_{50} using the method of Reed and Muench. For the

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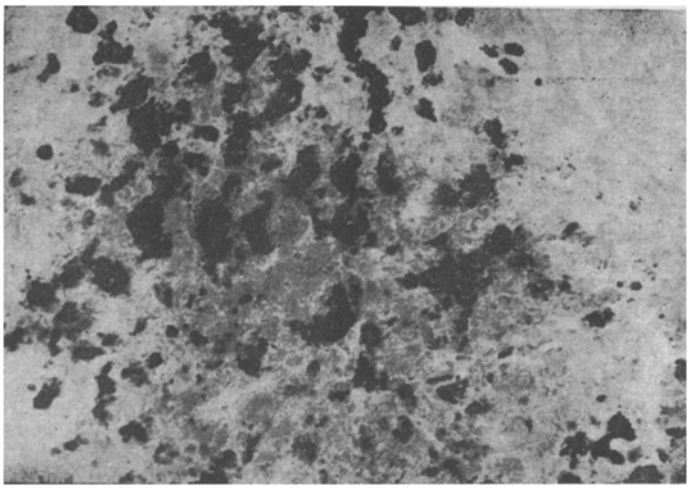

Fig. 1. Accumulation of numerous dilysosomal macrophages in apical region of the lung 24 h after intranasal injection of 0.3, 0.75, and 1.5 g pyrogenal (stained with Sudan and α-naphthol by Goldman's method, 620 ×).

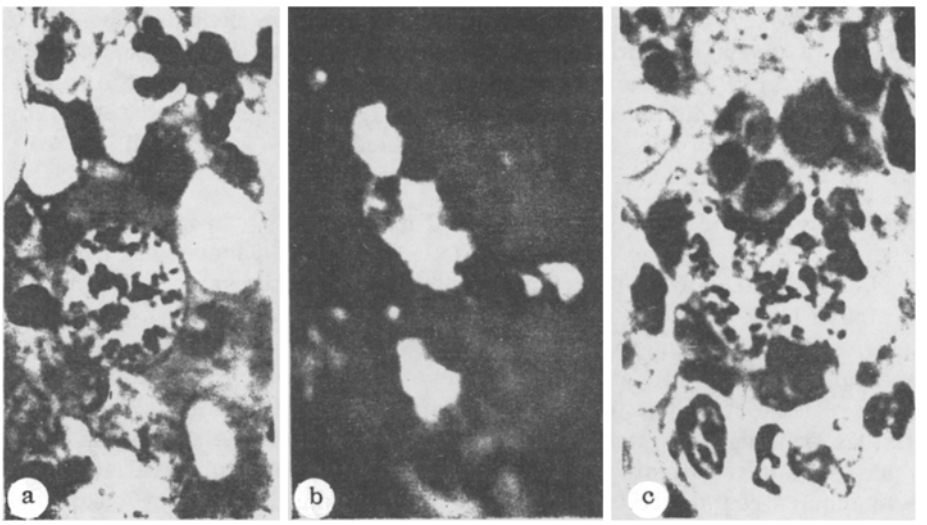

Fig. 2. Colonies of agent of AE in lung macrophages: a) large colony of agent of AE; 4 days after infection (stained with azure II–eosin, 1900 ×); b) specific fluorescence of antigen in alveolar macrophages; 4 days after infection (direct Coons' method, 1100 ×); c) accumulation of leukocytes close to elementary bodies of agent of AE liberated from disintegrated macrophage; 4 days after infection (stained with azure II–eosin, 1900 ×).

histological tests the lungs of the mice were fixed in Stive's fluid and embedded in paraffin wax. Sections were stained with azure II–eosin. For elective detection of leukocytes and leukocytic lysosomes the lungs were fixed with 10% formalin and frozen sections were stained with Sudan and α-naphthol by Goldman's method. Cryostat sections were treated with fluorescent antiornithosis pigeon serum containing antibodies against the agent of AE. The material was examined in the ML-2 luminescence microscope in blue-violet light with 40 × (water immersion) and 90 × (oil immersion) objectives.

EXPERIMENTAL RESULTS

Repeated intranasal injection of the bacterial lipopolysaccharide pyrogenal in increasing doses led to the development of a quickly subsiding aseptic inflammation in the lungs of the mice with a leukocytic response, breakdown of the leukocytes, phagocytosis of remnants of the leukocytes by macrophages, and the accumulation of dilysosomal macrophages containing their own and leukocytic lysosomes (Fig. 1).

Of 25 experimental mice infected with the agent of AE not one died, but in the control group 10 of 25 mice died. On the second day the titer of the agent of AE in the lungs of the experimental mice was lower than in the control by 0.28 log LD_{50}, and on the fourth to sixth day the difference was 1.26-1.0 log LD_{50} in 0.03 ml.

Microscopic examination 48 h after infection revealed macrophages in the lungs of the control mice containing colonies of the agent of AE. The colonies consisted of tightly packed small coccoid particles staining blue with azure II and brightly luminescent after treatment with antiornithosis fluorescent serum (Fig. 2a, b). On the second to fourth day some of the infected cells were destroyed and the liberated elementary bodies induced local (focal) accumulation of leukocytes (Fig. 2c). Elementary bodies stained with varied intensity, evidently indicating their death, were found in small numbers in the cytoplasm of the leukocytes. At the same time, leukocytes which had ingested elementary bodies of the agent of AE themselves often had clear signs of destruction and their remnants were phagocytosed by macrophages. No colonies of the agent of AE were found in macrophages containing remnants of leukocytes.

If the outcome of the pneumonia was favorable the inflammatory process was localized chiefly in the hilar and apical regions of the lungs. In mice which died or were killed in a state of agony, pneumonia affected several lobes of the lungs.

In the mice in which accumulation of dilysosomal macrophages in the lungs had been induced previously the pneumonia showed no tendency to progress. The inflammatory process was localized in the hilar and apical regions of the lungs, the destination of most of the intranasally injected agent of AE where dilysosomal macrophages had accumulated. During the period of formation of inflammatory foci (second to fourth day) the number of colonies of the AE agent in the experimental mice did not exceed 65-100 per histological section, compared with 500-600 in the control. Elementary bodies of the AE agent liberated from the disintegrated cells were phagocystosed by leukocytes. However, the leukocytic response subsided rapidly and by the end of the sixth day after infection the number of leukocytes in the capillaries of the alveolar septa of the experimental mice did not differ significantly from normal. On the 9th-12th day macrophages with oxidase-positive granulation and lipid particles, stained orange with Sudan III, were present in the inflammatory foci. No colonies of the AE agent were present in these foci.

The facts described above show that dilysosomal macrophages can modify the course of pneumonia induced by the agent of AE and can convert a lethal infection into a disease with a mild course. Differences in the fate of agents of the OLT group in different macrophages of the same inflammatory focus have frequently been mentioned [6, 9, 10]. The mechanism of this phenomenon has not yet been explained. According to the present investigation changes in the properties of macrophages arise after phagocytosis of remnants of leukocytes and of leukocytic lysosomes. These observations are in harmony with the view that breakdown products of leukocytes participate in the defensive reactions of the organism against pathogenic bacteria [1]. Besides fragments of nuclei and leukocytic lysosomes, the cytoplasm of macrophages also acquires histones and lysosomal cationic proteins with antibacterial and antiviral activity [2, 5]. The ability of macrophages to acquire resistance to the agent of AE after phagocytosis of leukocytic lysosomes is evidently one mechanism of cellular resistance in inflammatory foci in infected processes caused by agents of the OLT group.

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