ROLE OF LEUKOCYTE BREAKDOWN PRODUCTS IN INCREASING THE RESISTANCE OF MACROPHAGES TO ORNITHOSIS VIRUS

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The dynamics of formation of macrophagal granulomas in the liver after intravenous infection of albino mice with ornithosis virus was studied. During granuloma formation the macrophages pass through a stage of reutilization of breakdown products of leukocytes and they become resistant to ornithosis virus. Together with remnants of leukocytes, histones and cationic proteins with high antiornithosis activity in vitro may also enter the cytoplasm of the macrophages. The increased resistance of macrophages to the virus after reutilization of the leukocyte breakdown products is evidently one of the mechanisms of resistance formation in inflammatory foci associated with ornithosis.

KEY WORDS: ornithosis; macrophages; histones; resistance to infection.

Previous investigations showed that ornithosis virus propagates in macrophages and dies in granulocytes. The suggestion has been made that local leukocytic responses during ornithosis infection are evidence of protection and are aimed against the agent [4-6].

In this investigation relations between leukocytes and macrophages were studied in inflammatory foci in experimental ornithosis, and the antiornithosis activity of total leukocytic histone and of lysosomal cationic proteins was examined.

EXPERIMENTAL METHOD

Male albino rats (weight 14-16 g) and guinea pigs (weight 250-300 g) were used. The mice were infected by intravenous injection of 1 LD_{50} of ornithosis virus. Guinea pigs received an injection of 1 ml of allantoic fluid containing $3 \cdot 10^{0.2}$ LD_{50} ornithosis virus for chick embryos (strain V) by cardiac puncture. The animals were killed at different times (from 1 to 12 days) after infection. The lungs, liver, and spleen were examined morphologically. Histological sections were stained with azure II-eosin and with Sudan and α-naphthol by Goldman's method, and treated with immune fluorescent serum by the direct Coons' method [7]. The content of virus in the liver and spleen of the animals was determined by titration of a suspension of these organs in albino mice weighing 8-9 g. The titer of virus was measured in LD_{50} units by analysis of the data by the method of Reed and Muench. The number of macrophages containing colonies of ornithosis virus and the number of mononuclear granulomas were counted in sections through the liver 16 mm^2 in area, stained with azure II-eosin.

For testing the antiornithosis activity of subcellular fractions of the leukocytes, leukocytes from sterile peritoneal exudate of rabbits were used [3]. The ET_{20} fraction, with a high concentration of nonenzymic cationic proteins [8], was isolated from leukocytic lysosomes. Total leukocytic histone was obtained from DNP of the leukocytes by extraction of the basic proteins with 0.4 N H_2SO_4. After extraction, the proteins were precipitated with 10 volume of cold acetone. The precipitate was centrifuged at 2000g for 30 min, washed twice with

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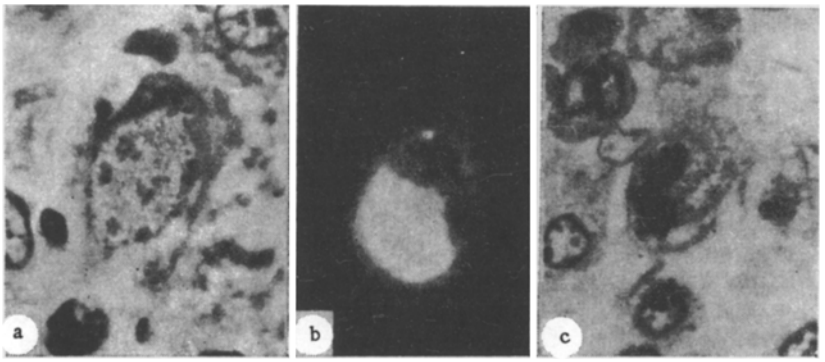

Fig. 1. Ornithosis virus in Kupffer cells of liver (a, b) and in cytoplasm of mitotically dividing macrophage (c): a) stained with azure II–eosin, 1500 ×; b) direct Coons' method, 1500 ×; c) azure II–eosin, 1700 ×.

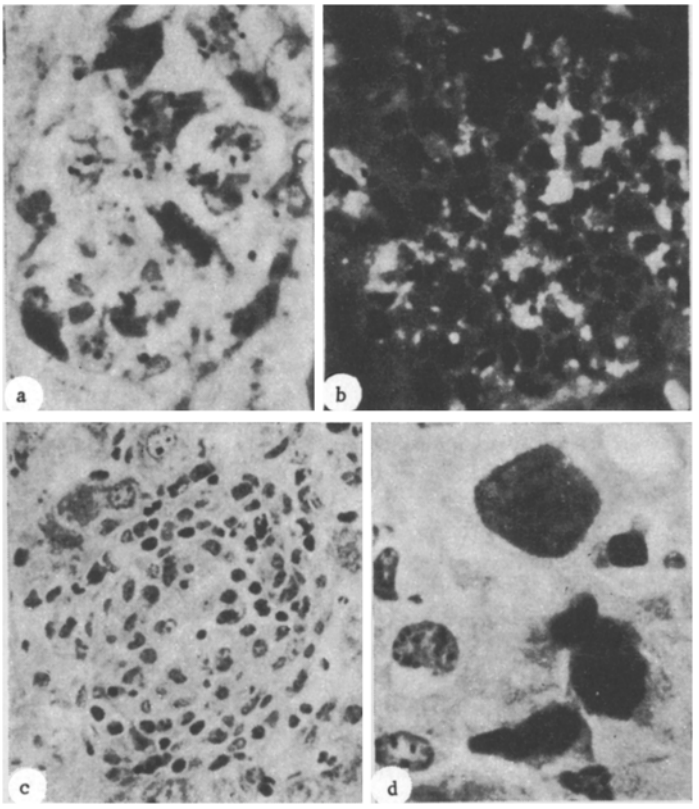

Fig. 2. Focal changes in the liver in experimental ornithosis: a) particles of ornithosis virus inside and bebetween leukocytes; azure II–eosin, 1700 ×; b) specific fluorescence of virus in focus of leukocytes; direct Coons' method, 700 ×; c) macrophagal granuloma 7 days after infection; azure II–eosin, 500 ×; d) the same specimen; ornithosis virus in Kupffer cells outside granulomas; azure II–eosin, 1500 ×.

acetone, and dried in vacuo. The mean yield of histones from leukocytic DNP was 8.4 mg/10^9 cells.

EXPERIMENTAL RESULTS

Examination of the albino mice revealed the severest lesions in the liver. Starting from the second day, colonies of ornithosis virus, staining blue with azure and brightly luminescent after treatment with fluorescent

TABLE 1. Resistance of Macrophages to Ornithosis Virus

Period of experiment (in days)	Conc. of virus in liver and spleen (LD_{50} in 0.03 cm^2)	Number of granulomas	No. of colonies of ornithosis virus in liver macrophages	
			outside granulomas	inside granulomas
1	—	—	1	—
2	3,33	—	6	—
3	—	—	16	—
4	4,9	8	63	—
7	7,16	85	146	2
8	—	67	73	2
9	4,66	94	81	2
12	0,5	72	2	—

serum, were found in the cytoplasm of the Kupffer cells (Fig. 1a, b). On the third day some of the infected cells were destroyed and liberation of the virus immediately gave rise to a local (focal) accumulation of leukocytes. The leukocytes phagocytosed the elementary bodies of ornithosis virus. However, the virus in the leukocytes showed no signs of multiplying, and the intensity of its staining varied, evidently reflecting its death. Meanwhile the leukocytes which had ingested the elementary bodies of ornithosis virus themselves often showed clear features of destruction.

Focal concentrations of leukocytes appeared in different parts of the hepatic lobules and they differed in size, often reaching 200 μ in diameter. In some concentrations particles of the virus could sometimes be seen inside the leukocytes and between them (Fig. 2a, b). In the larger concentrations many leukocytes, as well as individual hepatocytes in this area, were destroyed, in which case small foci of necrosis appeared; no ornithosis virus could be found in such situations.

As the infectious process developed, the number of focal lesions in the liver rose rapidly: 10-20 in an area of 16 mm^2 on the third day, 70-80 on the fourth day, and 130-140 on the seventh day. Meanwhile, definite changes were observed in the cell composition of the foci themselves. Starting from the fourth day the number of leukocytes and the quantity of their breakdown products decreased and mononuclear cells of the macrophage type appeared instead of them. The cytoplasm of the macrophages contained phagocytosed fragments of nuclear chromatin and leukocytic granules, giving a positive reaction for oxidase. The formation of macrophagal granulomas was complete by the seventh day, when the content of virus and the number of infected cells in the liver reached their maximum. The formed granulomas consisted of macrophages with the appearance of epitheloid cells. By this time the number of macrophages and cells of polyblast type in the liver had increased considerably. Mitotic figures could be seen in many of them. Sometimes mitoses also were found in macrophages containing the virus (Fig. 1c).

As Table 1 shows, the macrophages in the granulomas became resistant to ornithosis virus although the intact cells did not. Colonies of the virus were found in many Kupffer cells and free macrophages lying close to the granulomas, but in the macrophages of the granulomas themselves ornithosis virus was found exceptionally rarely (Fig. 2c, d).

Similar results were obtained during the investigation of guinea pigs. Ornithosis virus could be seen 36 h after infection in the cytoplasm of the Kupffer cells. After 60 h focal lesions of liver tissue infiltrated by polymorphs, many of them in a state of disintegration, were found. Cells of macrophage type with phagotosed remnants of the leukocytes and with their granules were found in the same region. Among the leukocytic debris sometimes scattered particles of the virus could be seen. At the end of the fifth day macrophagal granulomas consisting of epitheloid cells were found in the liver. No virus was present in these granulomas but some cells contained remnants of leukocytes.

To determine whether the breakdown products of leukocytes possessed antiornithosis activity experiments were carried out in vitro with total leukocytic histone and the fraction of lysomal cationic proteins (ET_{20}). Each preparation in a concentration of 250 μg/ml was mixed in 0.85% NaCl solution, pH 6.2, with 1 LD_{50} of ornithosis virus and the mixture was incubated at 37°C for 2 h. Ornithosis virus was kept at the same temperature and pH values without leukocytic substances. Neutralization of the virus was judged from the survival rate of albino mice (weighing 10-12 g) after intranasal injection of 0.04 ml of liquid incubation medium containing a mixture of histones and ornithosis virus. Statistical analysis of the experimental results was carried out by the chi-square method [1]. Of 20 mice receiving a mixture of total leukocytic histone and the virus, two (10%) died. The same result was obtained with the ET_{20} fraction, with a high concentration of nonenzymic cationic proteins, isolated from the total acid-soluble lysosomal extract. The mortality of the control mice from the same dose of virus was 95-100%.

The results show that during formation of the ornithosis granuloma the macrophages pass through a stage of reutilization of breakdown products of leukocytes and become resistant to ornithosis virus. The mechanism of this phenomenon requires further study. Besides remnants of leukocytes, the cytoplasm of the macrophages also contains histones and lysosomal cationic proteins, possessing antibacterial and antiviral activity [2].

Ornithosis virus is neutralized in vitro by very low concentrations of total leukocytic histone or of lysosomal cationic proteins (the ET_{20} fraction). The ability of macrophages to become resistant to ornithosis virus after reutilization of leukocytic breakdown products is evidently one mechanism of the formation of resistance in inflammatory foci in ornithosis.

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