INTRAVITAL LUMINESCENCE-SEROLOGIC STUDY OF INTRACELLULAR PROCESSES DURING PHAGOCYTOSIS OF BRUCELLAS BY MACROPHAGES

M. Ya. Korn and L. P. Shuvalov

UDC 612.112.3:576.851.42

The ingestion of a heterologous brucellar luminescent antiserum by cultures of macrophages from intact and immune guinea pigs and its accumulation in the cytoplasmic granules (secondary lysosomes) and its entry into phagocytic vacuoles (phagosomes) were studied. The accumulation of antiserum in the phagosomes was judged from the appearance of specific luminescence of the phagocytosed brucellas. Accumulation of luminescent antigen–antibody complex in the cytoplasmic granules also was investigated at various time intervals after phagocytosis of brucellas treated with brucellar luminescent serum. No difference was found between macrophages from intact and immune animals as regards the character of pinocytosis of the luminescent sera. Differences between macrophages were found in the rate of passage of the antisera from the lysosomes into the phagosomes and the rate of formation of luminescent granules after phagocytosis of brucellas treated with luminescent antiserum.

KEY WORDS: macrophages; brucellas; antibodies; phagocytosis; pinocytosis.

Recent investigations [7, 9-11] have shown differences between immune and normal macrophages. However, it is not fully clear whether the differences depend on changes in the cells themselves or whether they are connected with cytophilic and pinocytotic antibodies from the surrounding medium. The possibility of pinocytosis of immunoglobulins by macrophages and their intracellular interaction with phagocytosed antigens were demonstrated by Chakhava and Tsatsenkina [6]. Gill and Cole [8], by intravital observations of phagocytosis of streptococci treated with luminescent antibodies found that the luminescent complex accumulates in vacuoles of the macrophages. By use of a technique of color luminescence-phase contrast microfilming from the screen of an electron-optical intensifier [1, 2], it was found that after absorption of microorganisms (STI anthrax vaccine) treated with luminescent serum by macrophages, luminescent granules appear in the cytoplasm of the macrophages.

TABLE 1. Intracellular Reaction of Luminescent Brucellar Serum with Phagocytosed Spheroplasts (M ± m)

Group of guinea pigs	No. of animals	No. of macrophages (%) with cytoplasmic granules	No. of macrophages (%) with specific luminescence of spheroplasts
Normal	9	84,1±3,6	15,9±3,6
Immunized	8	60,3±4,8	39,7±4,8
p		<0,01	<0,01

In the present investigation, brucellar infection was used as the model to study the dynamics of absorption of specific luminescent immune sera by the macrophages of intact and immune animals and also the passage of pinocytosed antibodies from pinocytotic vacuoles into phagosomes containing the phagocytosed antigen, and the fate of the luminescent antigen–antibody complex after phagocytosis of brucellas treated with luminescent serum.

EXPERIMENTAL METHOD

Experiments were carried out with cultures of peritoneal macrophages obtained in the usual manner from

Laboratory of Brucellosis and Group for Scientific Motion Picture Documentation, N. F. Gameleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR P. A. Vershilova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 2, pp. 70-73, February, 1975. Original article submitted February 13, 1974.

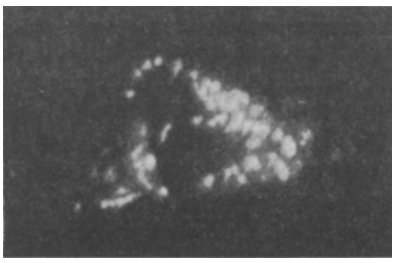

Fig. 1. Pinocytosis of luminescent antibrucellar serum by macrophage. Brightly luminescent cytoplasmic granules. Here and in Fig. 2: ML-2 microscope, objective 90 × (oil immersion), ocular homal 3 ×.

9 intact and 8 immunized guinea pigs. Immunization was carried out by a single dose of 2 billion bacterial cells of the vaccine strain Brucella abortus BA 19.

Peritoneal macrophages after culture for 3-5 days were incubated with rabbit antibrucellar serum labeled with fluorescein isothiocyanate. For this purpose, 0.2 ml of serum in a dilution of 1:10 was added to tubes containing the cells. After incubation for 2 h at 37°C the macrophages were washed with medium No. 199 to remove the excess of antiserum and infected with spheroplasts obtained from Br. abortus, strain 870, biotype 6, by the method of Ostrovskaya and Tolmacheva [5] at the rate of 10 brucellas per cell.

Experiments also were carried out in which a culture of macrophages was incubated with spheroplasts treated with luminescent antiserum. After washing to remove the excess of antiserum, antibodies bound with brucellar antigen remained on the surface of the bacteria. These cells could be regarded as an antigen-antibody complex that does not dissociate in the first stages of intracellular digestion [8].

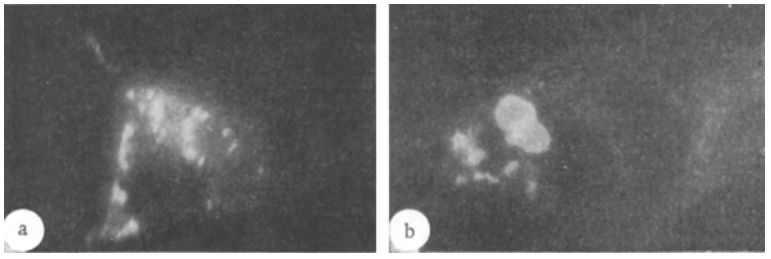

Fig. 2. Change in character of luminescence of brucellar spheroplasts when phagocytosed by a macrophage after preliminary pinocytosis of luminescent antibrucellar serum: a) 30 min after phagocytosis. Numerous bright cytoplasmic granules surrounding spheroplasts. Weak luminescence of spheroplasts; b) 2 h after phagocytosis. Reduction in number of cytoplasmic granules and increase in luminescene of spheroplasts.

TABLE 2. Dynamics of Behavior of Brucellar Antigen–Antibody Complex in Culture of Peritoneal Macrophages of Immune and Intact Guinea Pigs (% of macrophages, M ± m)

Group of guinea pigs	No. of animals	Time of investigation			
		1 h			3 h
		L_c	$L_c - L_g$	L_g	L_c
Normal	9	76,8±9,3	23,2±9,3	0	69,4±4,6
Immunized	8	81,4±7,8	18,6±7,8	0	40,9±9,4
P		>0,05	>0,05		<0,05

Group of guinea pigs	No. of animals	Time of investigation				
		3 h		24 h		
		$L_c - L_g$	L_g	L_c	$L_c - L_g$	L_g
Normal	9	29,3±4,4	1,3±1,3	22,8±6,1	50,9±7,5	26,3±4,3
Immunized	8	54,2±7,8	4,9±2,5	6,8±5,2	19,6±7,4	73,6±11,6
P		<0,05	>0,05	>0,05	<0,01	<0,01

Note: L_c) luminescence of complex; L_c-L_g) luminescence of complex and granules; L_g) luminescence of granules.

The preparations were studied in the ML-2 luminescence microscope 1, 3, and 24 h after contact of the cells with the phagocytosed object. Coverslips with the cells underneath were placed on slides. The capillary space between the coverslip and the slide was filled with medium No. 199 without serum.

EXPERIMENTAL RESULTS

After incubation of the culture of macrophages with luminescent antibrucellar serum about 35% of the cells contained pinocytosed protein which, under high power, appeared as bright green pinpoint granules in the perinuclear zone (Fig. 1).

When pinocytosis was assessed from the number of active macrophages and intensity of luminescence of the granules no differences were found in the experimental and control groups.

On subsequent incubation of macrophages for 2 h with spheroplasts, phagocytosis and specific luminescence of the phagocytosed bacterial cells were observed (Fig. 2a, b). Simultaneously with an increase in the number of luminescent spheroplasts and in the intensity of their luminescence, the luminescence of the cytoplasmic granules gradually weakened. This process took place more rapidly in macrophages from immunized animals (Table 1).

In the experiments of series II the intracellular behavior of the phagocytosed brucellar antigen–antibody complex in macrophages from normal and immunized animals was studied.

On incubation of a cell culture with spheroplasts treated with luminescent antiserum the following findings were observed. After incubation for 1 h, the macrophages contained mainly only spheroplasts luminescent at the periphery, and only in a small percentage of macrophages were cytoplasmic granules found also. After 3 h, the number of cells containing both luminescent bacteria and granules increased. At the same time, macrophages with a sharply reduced intensity of luminescence of phagocytosed bacterial cells and with bright luminescence of the cytoplasmic granules was noted. By 24 h, the percentage of these macrophages had increased considerably. This process took place particularly intensively in a culture of cells from immunized animals (Table 2).

The results are clear evidence that heterologous specific luminescent antiserum undergoes active pinocytosis by normal and immune macrophages; immunization has no effect on the rate of pinocytosis or on the character of distribution of the ingested protein, which accumulates in the cytoplasm as granules corresponding in size and location to secondary lysosomes, containing both luminescent protein and enzymes. The passage of luminescent antibodies from these lysosomes into a phagosome containing phagocytosed antigen discovered in this investigation and the differences between macrophages from intact and immune animals are interesting from this point of view.

The use of luminescence microscopy and phase-contrast microscopy with illumination from the side showed that spheroplasts adsorbed on macrophages are not luminescent; this is evidence of the absence of luminescent antibodies in the surrounding medium and on the surface of the macrophages.

A previous investigation showed [3] that a luminescent complex containing acridine orange, possibly a complex of acridine orange with RNA [4], also passes from secondary lysosomes into phagosomes. This indicates that the function of the lysosomes in the phagocytic cell is not limited to the intracellular digestion of the phagocytosed antigen.

Differences between macrophages of intact and immune animals as regards the rate of destruction of phagocytosed spheroplasts containing luminescent antigen-antibody complex on their surface also were found, and luminescent granules which, on the basis of their morphology and arrangement, can be classed as secondary lysosomes, appeared in the cytoplasm. However, the mechanism of the passage of the luminescent complex from lysosomes into phagosomes requires further study.

LITERATURE CITED

1. M. Ya. Korn and M. A. Grigor'eva, in: Proceedings of the 15th All-Union Congress of Microbiologists, Epidemiologists, and Infectious Diseases Specialists [in Russian], Vol. 2, Moscow (1970), p. 161.
2. M. Ya. Korn and M. I. Butslov, in: Proceedings of the 25th Congress of the International Association of Scientific Motion Pictures [in Russian], Kiev (1971), p. 5.
3. M. Korn, in: N. K. Luzio and K. Flemming (Editors), The Reticuloendothelial System and Immune Phenomena, New York (1971), p. 73.

4. M. Ya. Korn, in: Problems in Radiation Immunology and Microbiology [in Russian], Moscow (1972), p. 9.
5. N. N. Ostrovskaya, P. A. Vershilova, and T. A. Tolmacheva, Zh. Mikrobiol., No. 4, 108 (1972).
6. O. V. Chakhava and T. N. Tsatsenkina, Byull. Éksperim. Biol. i Med., No. 9, 55 (1970).
7. R. V. Blanden et al., J. Exp. Med., 124, 585 (1966).
8. F. A. Gill and R. M. Cole, J. Immunol., 94, 898 (1965).
9. G. B. Mackaness, J. Exp. Med., 120, 105 (1964).
10. G. B. Mackaness, J. Exp. Med., 129, 973 (1969).
11. D. J. Ralston and S. S. Elberg, J. Bact., 96, 24 (1968).