# Functional Activity of Macrophages against *Yersinia pseudotuberculosis* Plasmid Variants

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 128, No. 10, pp. 429-432, October, 1999
Original article submitted April 23, 1999

Functional activity of macrophages against *Yersinia pseudotuberculosis* plasmid variants carrying 45, 48 and 82, and 82 MD plasmids, and plasmid-free variants was studied. The phagocytic capacity of macrophages was inversely related to plasmid-associated virulence of Yersinia and correlated with morphological parameters of phagocyte stimulation. However, phagocytes show no bactericidal activity towards these strains, which indicates incompleteness of phagocytosis.

**Key Words:** *macrophage; Yersinia pseudotuberculosis; plasmids*

Complete phagocytosis of avirulent bacteria by reticuloendothelial cells at the initial stage of infection determines its uneventful course; by contrast, incomplete phagocytosis of virulent forms is a cause of unfavorable outcome of the disease [1,2,4,7]. The presence in *Y. pseudotuberculosis* of a virulence plasmid with a molecular weight (mol. weight) of 42-47 MD common for all *Yersinia* encoding 7 toxic Yop proteins of the outer bacterial membrane [6], determines the resistance of these microorganisms to phagocytosis. YopE protein selectively paralyzes neutrophil microfilaments, disturbing phagosome formation around the digested bacterium, while protein YopA exerts a cytolytic effect on peripheral blood leukocytes [5,9].

We investigated the effect of plasmid variants of *Y. pseudotuberculosis* on functional activity of macrophages (MP).

## MATERIALS AND METHODS

Primary culture of peritoneal MP from outbred albino mice was prepared as described previously [10]. Cell concentration was brought to $10^6$/ml with medium 199 containing 5% fetal calf serum (Vektor, Kol'tsovo) without antibiotics (Ya. K. Korn's modification, 1988). For MP adhesion, cell suspension in penicillin flasks (2 ml) with cover slides was incubated for 45 min at 5% $CO_2$ and 37°C. Nonadherent cells were washed twice with the same medium and left at 37°C for 3 days. The quality of culture was evaluated by phase contrast microscopy.

Phagocytosis objects were 1) virulent strain with 45 MD virulence plasmid, No. 500-995; 2) virulent strain with 48 and 82 MD plasmids, No. 3260 and its isogenic variants; 3) low virulent strain with 82 MD plasmid, No. 1182-3260; and 4) avirulent plasmid-free strain, No. 1184-3260.

MP adhered to slides were fixed after 30- and 120-min contact with bacteria, after which the phagocytic number (PN, number of phagocytosed bacteria) and phagocytic index (percentage of phagocytes) were evaluated. Apart from quantitative parameters, lifetime phagocytosis of bacteria by MP was studied under phase contrast microscope.

Intracellular bactericidal activity of MP was evaluated as described previously [3,7]. Bacterial suspension was added to phagocyte monolayer (10 per cell), incubated for 30 min at 37°C and 5% $CO_2$, and washed with medium 199 to remove free bacteria. In half of samples phagocytes were lyzed with 2 ml sterile distilled water (10 min), transferred to immunological multiwell plate (0.02 ml+0.18 ml medium 199), mixed thoroughly, and inoculated (0.02 ml) onto meat-peptone agar. The rest samples were treated likewise after 4-h incubation. Bacteria were cultured for 2 days and the number of

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colonies was counted. The data were expressed as a ratio: $(N_x-N_0)/N_0\times100$, where $N_x$ is the number of viable bacteria after 30-min contact and $N_0$ number of viable cells after 4-h contact. The mean value was estimated from 3 experiments.

## RESULTS

After long-term (2 days) culturing without stimulators, MP acquired a round, flattened, and spindle-like shape with thin cytoplasmatic processes (taken as intact). Stimulated MP looked like maximally adhered to the glass with nuclei occupying $^1/_3$ of the cytoplasm and with increased content of vacuoles. These cells (90.0±6.7% per sample) were observed after 30-min incubation of phagocytes with plasmid-free Yersinia strains (Fig. 1, *a*). Bacteria were found mainly intracellularly. The number of activated MP after their contact with single-plasmid strain (82 MD) was 76.0±6.8%, with strain containing 45 MD plasmid 34.0±2.7%, and with

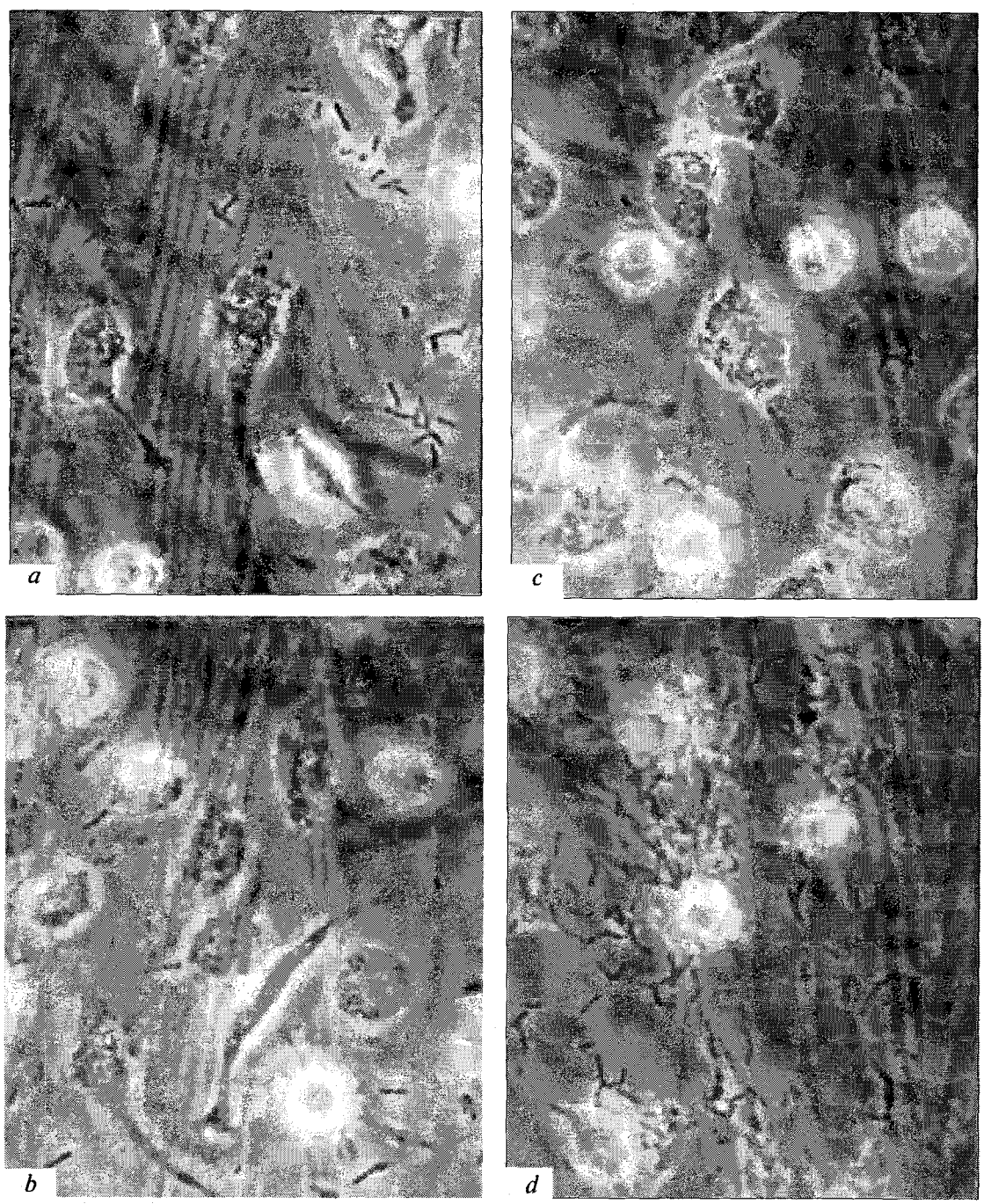


**Fig. 1.** Macrophage absorption of *Y. pseudotuberculosis* plasmid variants after 30- (*a, b*) and 120-min (*c, d*) incubation. Phase contrast microscopy, ×2500. *a, c*) strain 1182-3260, 82 MD plasmid; *b, d*) strain 3260, 48 and 82 MD plasmids.

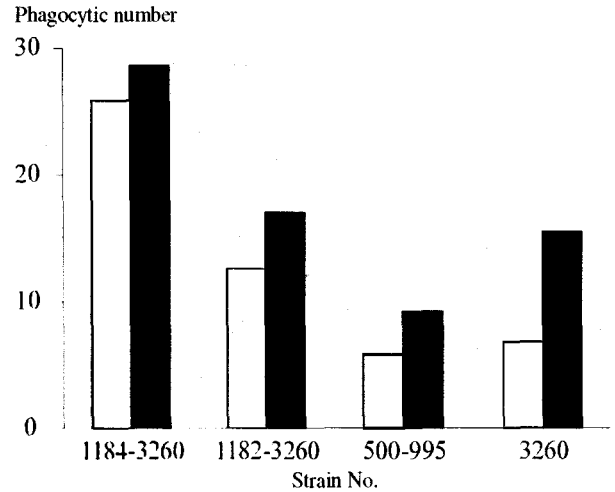


**Fig. 2.** Phagocytic activity of macrophages towards *Y. pseudotuberculosis* plasmid variants after 30-min (light bars) and 120-min (dark bars) exposure.

two-plasmid (48 and 82 MD) strain 21.0±1.8%. In the latter case, destroyed MP (fluorescent) constituted 5.0± 0.4% (Fig. 1, *b*).

After 120-min contact of phagocytes with plasmid-free and single-plasmid (82 MD) variants of *Y. pseudotuberculosis*, the number of bacteria outside the cells was scanty and there were almost no damaged phagocytes (Fig. 1, *c*). Two other strains caused almost complete destruction of cells and intensely multiplied in the medium (Fig. 1, *d*).

Evaluation of the phagocytosis parameters showed that MP more actively phagocytosed plasmid-free low-virulent *Y. pseudotuberculosis* bacteria, which correlated with cell activation parameters. After 30 and 120-min contact, the PN was 23.7±1.9 and 26.8±2.95, respectively (Fig. 2). For bacteria with 82 MD plasmid these values were 10.9±1.45 and 15.57±1.85, respectively. These values significantly ($p<0.01$) differed from phagocytosis parameters of strains with 45 and 48 MD virulence plasmids. For strain with 45 MD plasmid, PN after 30 and 120-min incubation was 3.44±0.27 and 7.84±0.58, respectively. For two-plasmid strain, the PN was 4.53±0.5 and 13.64±1.9, respectively. The experiment was repeated twice with similar results with other strains with the same plasmid characteristics.

Despite the absorption capacity of MP towards all *Y. pseudotuberculosis* strains, we revealed no bactericidal activity of phagocytes: bactericidal activity index had negative values from 94 (two-plasmid strain) to 99% (plasmid-free strain). Our data on the number of CFU were of special interest. After 4-h incubation the number of CFU was 670.0±88.5 for the two-plasmid strain, 413.0±39.0 for strain with 45 MD plasmid, 189.0±12.7 for strain with 82 MD plasmid, and 126.0± 11.2 for plasmid-free strain. Therefore, the bactericidal activity of MP is higher towards low-virulent strains.

As we previously mentioned, secretion of toxic Yop proteins of the outer Yersinia membrane is due to 45-47 MD virulence plasmid. Transport of YopE and YopH proteins through MP membrane depends on bacterial secretion of other proteins, namely YopB and YopD [11]. Other scientists [9] report that these proteins inhibit fusion of phagosomes with lysosomes in MP during phagocytosis of *Y. pseudotuberculosis*. The relationship between MP phagocytic activity towards *Y. pseudotuberculosis* and their plasmid-associated virulence confirms the reports of other authors. Our results indicate that Yersinia with 45-48 MD plasmid more actively suppress phagocytic capacity of MP. Presumably, deactivation occurred before MP contact with microorganisms, due to secretion of Yop proteins by *Y. pseudotuberculosis* into extracellular space. Decreased bactericidal activity of MP towards all plasmid variants of Yersinia suggests that the phagocytic resistance of these bacteria depends not only on plasmid, but also on their genetic characteristics [4]. Incomplete phagocytosis in yersiniasis can be caused by functional insufficiency of reticuloendothelial cells despite preserved capacity to capture bacteria. This is in line with other bacteriological studies showing that only few phagocytosed bacteria were destroyed after absorption by MP, while most were retained viabile and multiplied in MP causing cell degeneration and death.

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