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Specific Features of the Effect of "Murine" Toxin and Antigen of *Yersinia pestis* Fraction I on Cells of Plague-Sensitive Animals

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Effects of *Yersinia pestis* capsular antigen and toxin on the cells of experimental animals are described. The antigen of bacterial fraction I enhanced the oxidative burst of white mice peritoneal leukocytes but suppressed the activity of guinea pig leukocytes. The effect of "murine" toxin was quite the contrary. Moreover, the effects of the toxin on the phosphorylation of leukocyte membrane and cytosol proteins of various origin differed, this correlating with fluctuations in the activity of tyrosine phosphatase.

Key Words: *plague; capsular antigen, "murine" toxin*

Yersinia pestis pFra plasmid with a molecular weight of 65 MD determines the synthesis of two products: "murine" toxin-exotoxin (MT) forming at 28°C and fraction I antigen (FIA) accumulating on the cell surface as a mucous layer at 37°C. Studies of the contribution of these substances to the development of plague infection have been carried out for a long time, but many aspects of the mechanism of their modulating effect on cells of the immune system of experimental animals (white mice and guinea pigs) are still not clear. MT (LD_{50} 0.6 to 1.25 μg) is known to be lethal for white mice, whereas guinea pigs are little sensitive to much higher doses. Conversely, FIA, a potent immunogen for white mice, rats, and monkeys, causes "immunoparalysis" in guinea pigs when used in the same doses [7,9].

In this research we compared the effects of FIA and MT on peritoneal leukocytes and macrophages of white mice and guinea pigs *in vitro*.

MATERIALS AND METHODS

Preparations of FIA capsular antigen isolated from *Y. pestis* [3] and toxin from *Escherichia coli* strain

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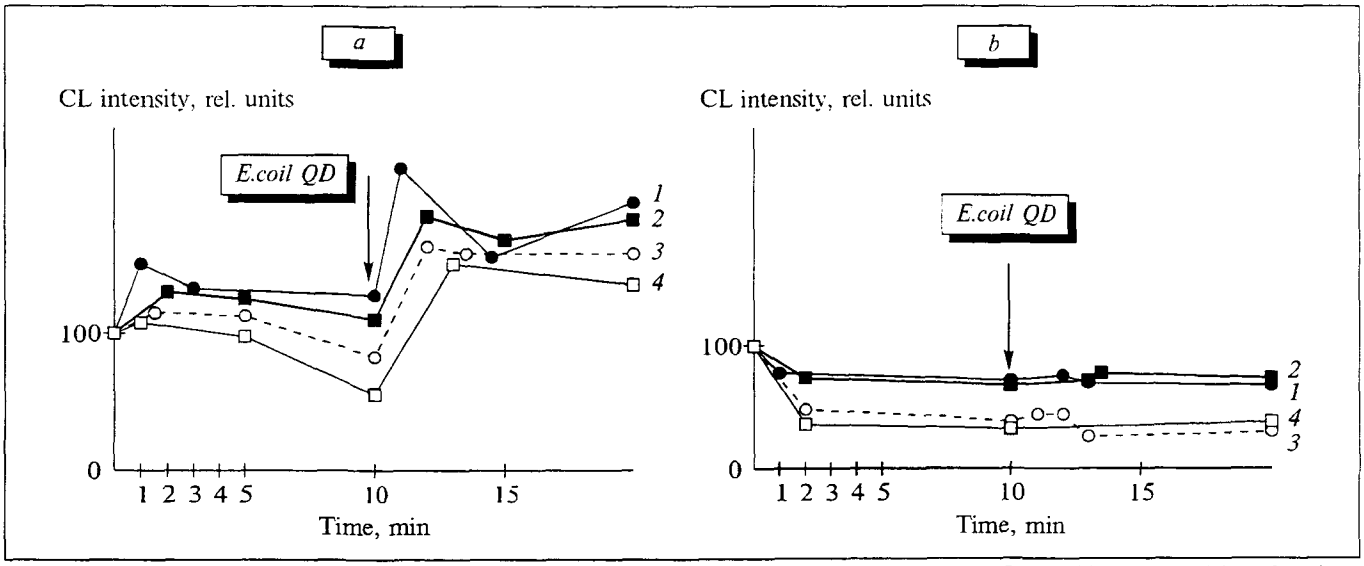

Fig. 1. Effects of different concentrations of *Y. pestis* capsular antigen on the intensity of CL of white mouse (*a*) and guinea pig (*b*) leukocytes. *1*) 0.1; *2*) 1; *3*) 10; *4*) 100 μg/ml.

OH5α carrying recombinant plasmid with cloned MT gene were used in experiments. MT preparations from *Y. pestis* and *E. coli* were identical in physicochemical and serological characteristics. The molecular weight of the toxin as shown by electrophoretic mobility data was 61 kD, and its LD_{50} for white mice 0.6 to 1.2 μg.

Luminol-dependent chemiluminescence (CL) during exposure to FIA and MT was recorded on peritoneal leukocytes and macrophages of outbred white mice and guinea pigs as described elsewhere [1].

Phosphorylating and dephosphorylating activities of the toxin were assessed on membrane and cytosol fractions of peritoneal macrophages prepared as described previously [2]. Proteinase activity was recorded using ATP after a previously described method [8] in our modification, and tyrosine phosphatase activity was measured using O-phospho-L-tyrosine as substrate.

Electrophoresis of membrane and cytosol fractions was carried out in 10% polyacrylamide gel with 0.1% sodium dodecylsulfate.

Autoradiograms were made after a 5-day exposure of dried gel plates with RM-B x-ray film. Protein was measured after Hartree [6].

RESULTS

Luminol-dependent CL of peritoneal leukocytes and macrophages of white mice and guinea pigs varied in response to treatment with FIA in concentrations of 0.01 to 100 μg per sample. The addition of 100-10 μg capsular antigen to a sample with white mouse leukocytes boosted CL intensity to 125-150%. The response developed rapidly, the maximal CL values being observed between the first and second minute after the addition of the agent. Reduction of the concentration to 1.0-0.01 μg was paralleled by a negligible increase of CL to 105-110% followed by its drop to 85-50%. Subsequent addition of 10^7 live *E. coli* QD 5003 cells to assess the potential function of leukocytes caused a marked increase of CL intensity (Fig. 1, *a*).

In contrast to the case with white mice, the CL response of guinea pig peritoneal leukocytes declined to 45-30% under the influence of FIA. The same effect was observed after addition of the agent in a dose of 1.0 to 0.01 μg, the reduction of CL in this latter case being, however, negligible and reaching just 70% of the initial level over 10 min of observation. After the addition of *E. coli* QD 5003 suspension to a sample with FIA-treated guinea pig leukocytes, no CL response was observed (Fig. 1, *b*). Hence, *Y. pestis* capsular FIA exerted different effects on the test cells: in doses of 100 to 10 μg it increased the functional activity of peritoneal leukocytes of white mice but suppressed the activity of guinea pig leukocytes.

A study of CL of peritoneal leukocytes and macrophages of white mice and guinea pigs under the influence of *Y. pestis* MT in doses of 0.06, 0.6, and 9 μg per sample showed an opposite effect of MT on the leukocytes: in the indicated concentrations it boosted the metabolic burst of guinea pig peritoneal leukocytes but depressed the functional activity of murine cells (Fig. 2, *a*, *b*).

No reliable differences in CL intensity in white mice and guinea pigs were observed in response to treatment with FIA and MT. These data are in line with previous reports [5] about a close

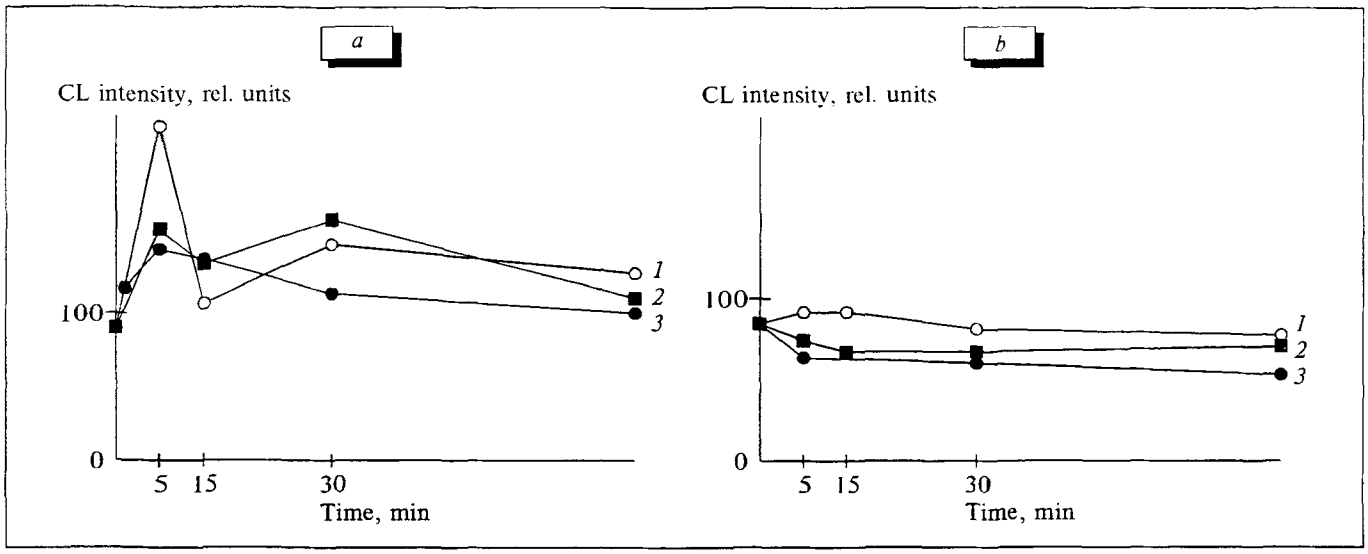

Fig. 2. Kinetics of CL of white mouse (*a*) and guinea pig (*b*) leukocytes exposed to *Y. pestis* toxin. *1*) 0.06; *2*) 0.6; *3*) 9 μg/ml.

correlation between CL and the formation of reactive oxygen species in leukocytes. A clear-cut positive correlation between the studied processes in macrophages may be absent when the metabolc response is associated with both oxygen-dependent and oxygen-independent reactions. Leukocytes, and, specifically, polynuclears, are believed to be the primary target of toxin action. The detected unusual effects of FIA and MT in physiological concentrations on cells of experimental animals may be regarded as evidence of individual features in the pathogenesis of plague in white mice and guinea pigs with an equally high sensitivity to this infection.

Protein phosphorylation in eukaryotic cells is an important process in the pathways of signal transduction between bacterium and host cell, and may be conducive to internalization of bacteria [10].

In this connection we attempted to study MT-boosted phosphorylation-dephosphorylation processes of target cell proteins. MT in a dose of 16 μg per sample was found to stimulate the phosphorylation of numerous proteins with molecular weights of 45 to 90 kD in the membrane and cytosol fractions of guinea pig peritoneal macrophages, the total kinase activity in membranes and soluble components of cells increasing by, respectively, 220 and 125% in this case. In mouse leukocytes, on the other hand, the efficacy of high-molecular protein phosphorylation was decreased, which was paralleled by a reduction of proteinkinase activity to 65% and of soluble fraction activity to 70%.

Measurements of tyrosine phosphatase activity in cell fractions exposed to *Y. pestis* MT showed that the above-mentioned 65% inhibition of phosphorylation in membrane fractions of white mice leukocytes correlated with a 50% increase of tyrosine phosphatase activity. This trend was observed in the cytosol as well: a 70% reduction of protein phosphorylation was associated with a 30% increase of tyrosine phosphatase activity. In guinea pig leukocytes intensified phosphorylation went along with an 18% increase of tyrosine phosphatase activity in the membranes and a 316% increase in the cytosol. The findings indicate that MT is capable of exerting different effects on the cascade of reactions associated with its interactions with the target cell, these differences being determined by the species appurtenance of a sensitive animal.

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