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Effect of Metalloorganic Immunomodulators on Susceptibility to Plague Toxin

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The use of immunomodulators (IM) is being increasingly extended to the treatment of various pathological states. Reports have appeared about their capacity to influence the susceptibility of the organism to various toxic stimuli.

The goal of this work was a study of the influence of IM of metalloorganic nature on the susceptibility of mice of different strains to "mouse toxin" (MT) of plague microbes. MT is a plague microbe-derived lethal protein toxin of high activity. Keeping in mind the diurnal variations in the organism's resistance to the toxic effect of various compounds, the experiments were carried out at different times of the day.

MATERIALS AND METHODS

Male mice of the CBA and C57Bl/6 strains and F_1 (CBA×C57Bl/6) were used. The animals were kept in a vivarium under standard conditions of light, heat, and feeding. The acclimatization period before the start of the experiments was not less than 2 weeks. The IM MOP-35 and MOP-79 were administered intravenously at 11:00 and 23:00 h. Control animals received saline.

MT was isolated and purified from the lysate of *Y. pestis* EK 76 cells [7]. The toxin was introduced intraperitoneally in doses of 0.5 and 1 mg per mouse, 5 min after IM infusion. Mortality in the experimental and control groups was recorded. Changes in the susceptibility to the toxic stimulus were estimated as the ratio of mortality in the experimental group to mortality in the control group and expressed in percent.

RESULTS

The results of the experiments examining the effect of MOP-35 and MOP-79 on the susceptibility of mice to MT are presented in Fig. 1 and Fig. 2.

As can be seen in the figures, as early as 5 min after IM intravenous infusion a reliable change of susceptibility to the toxic effect can be seen, depending on the time of day. The effect of IM on the susceptibility

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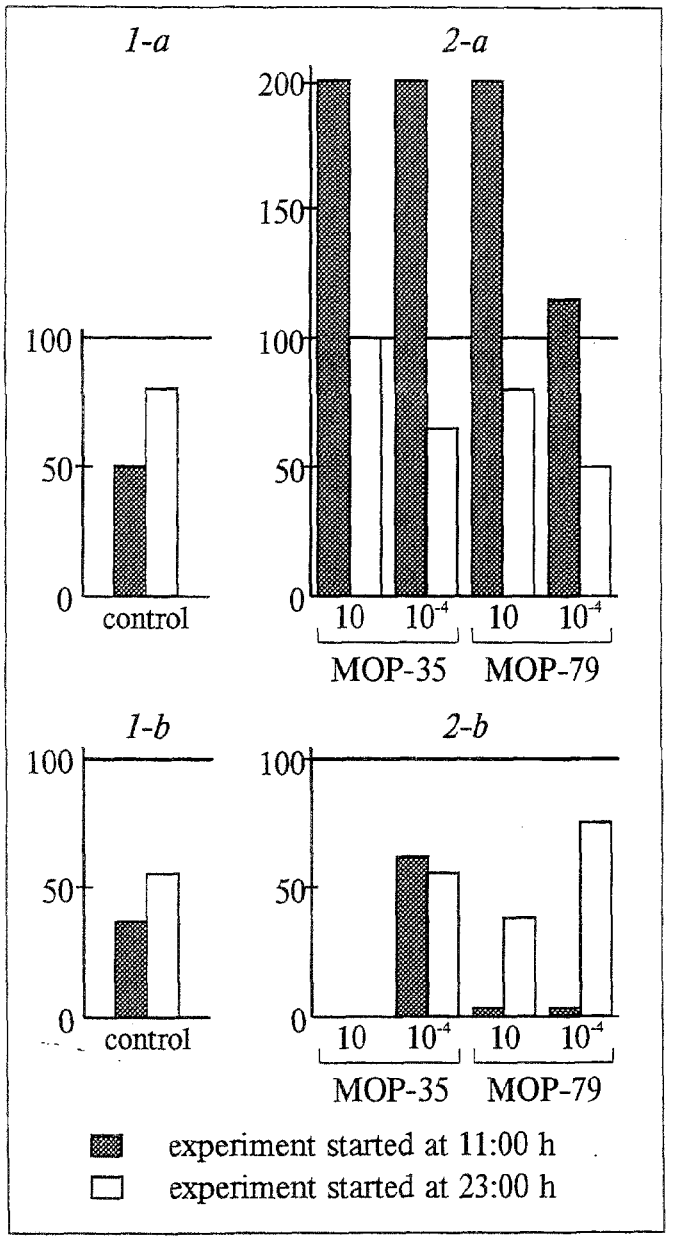

Fig. 1. Immunomodulator – induced change in the sensitivity of CBA mice to "mouse toxin": dependence on the time of day.The toxin was inoculated in a dose of 0.5 μg (*a*) and 1.0 μg (*b*). Abscissa: groups of animals. Ordinate: 1*a*) and 1*b*) mortality of mice in percentage to the total number of mice; 2*a*) and 2*b*) mortality of mice in percentage to the mortality in the control group. *n*: number of animals per group (9 – 12).

to MT was most pronounced in CBA mice (Fig. 1). It should be stressed that the experiments with toxin used in a dose of 1 μg gave results strikingly different from those with a smaller dose of toxin (0.5 μg). The differences were not only of a quantitative, but also of a qualitative nature. For instance, with the dose of 1 μg, administration of IM in the morning led to a rise of MT susceptibility; however, injection of IM in the evening as a rule reduced susceptibility to MT. IM in most cases lowered susceptibility to the lower dose of toxin, irrespective of the time of administration.

In C57Bl/6 mice (Fig. 2) MOP-35 and MOP-79 had a minimal effect on the susceptibility to MT. The time-dependent differences in the IM effect were also weaker.

F_1(CBA×C57Bl/6) hybrids occupied an intermediate position regarding the degree of the effect of IM on the susceptibility to MT when compared to the high-responder CBA strain and low-responder C57Bl/6 strain. Unlike the case with the CBA mice, the morning administration of IM to F_1 animals mostly resulted in a reduction of MT susceptibility; however, in the evening experiments the effect strongly depended on the type and dose of IM.

Thus, in the course of the study it was established that the effect of IM of metalloorganic nature on MT susceptibility depends on such factors as the dose of preparation, the time of injection, the genotype of the animals, and the dose of toxic substance. The last circumstance once again provides evidence that when considering toxicity, one should keep in mind the concept of the interaction between the susceptible organism toward which the action is directed and the damaging factor.

As a rule, the dependence observed by many authors of the effect of the preparation on the time of day is explained by the so-called "rule of initial values" [10]. According to this rule, the more strongly activated any given function is, the more easily it can be suppressed and, conversely, the more effort that is required for its further stimulation. However, we could not register such a tendency in our investigations, i.e., no relationship was found between the initial sensitivity to the toxin in the intact animals and the pattern of its variation following IM administration.

The results obtained do not contradict the earlier established correlation between the susceptibility to MT and the level of ecto-5'-nucleotidase (5'-N) of peritoneal exudate macrophages (PEM) [3,5]. A change in the 5'-N activity in the PEM is known to be one of the manifestations of the immunomodulatory activity of the metalloorganic compounds used in this investigation. A change in this enzyme activity appears as early as 5 min after the injection of the preparation; it depends on the time of injection, the animal's genotype, and the dose of preparation [1,6]. As was demonstrated in our study, the variations in the susceptibility to MT are governed by the same regularities.

The assumption regarding the existence of a certain relationship between susceptibility to MT and the level of PEM 5'-N activity is in good agreement with the current concept of the mechanisms of plague toxin action as well as with published data on the structure and function of 5'-N [2]. It is known that

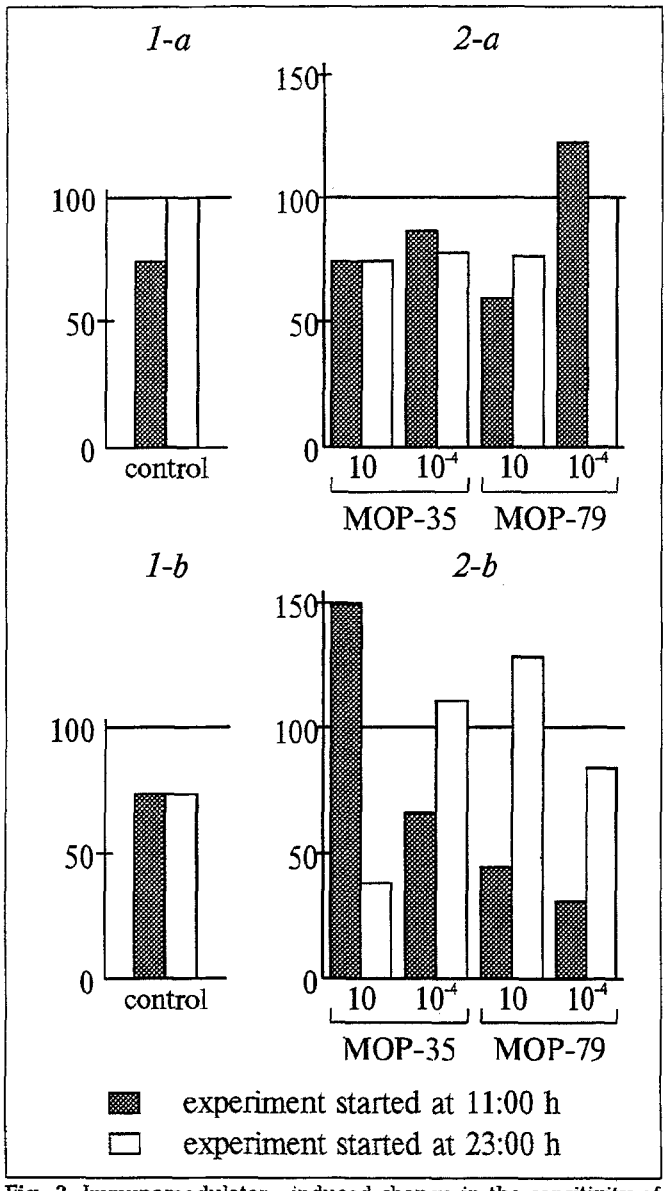

Fig. 2. Immunomodulator–induced change in the sensitivity of C57Bl/6 and F_1(CBA×C57Bl/6) mice to "mouse toxin": dependence on the time of day. *a*) C57Bl/6 mice; *b*) F_1(CBA×C57Bl/6) mice. Axes are the same as in Fig. 1.

MT-induced intoxication resembles the clinical picture of functional adrenalectomy. Similarities of the metabolic changes in plague intoxication and adrenocortical deficiency have been noted. The toxic reaction is considered to be an abrogation of the metabolic response to the adrenal function, while cAMP acts as an antagonist of the lethal effect of MT [9]. Earlier it was shown that 5'-N is a physiologically important regulator of the cAMP level [8]. The level of activity of this enzyme is correlated with the level of glucocorticoids [4].

The results of this investigation, demonstrating: *a*) the effect of IM on MT susceptibility beginning just 5 min following IM administration, and *b*) the dependence of the effect on the time of IM inoculation, present evidence of an apparently weighty contribution of the nonspecific defense mechanisms to the variation of the susceptibility to the toxin; the circadian cycle-related features of hormone production may also be of certain importance.

Thus, in this study it was shown that IM of metalloorganic nature, namely MOP-35 and MOP-79, are potent instruments of emergency change in the susceptibility to plague intoxication.

At present it is impossible to give a complete explanation of the findings. It would be helpful in this regard to perform a multifaceted comparative study of the circadian cycles of variation in the PEM 5'-N activity and related parameters of the neuroendocrine system in mice of different strains.

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