# Influence of the Protein-Polysaccharide Complex and Purified Protein of *Y. Pestis* Fraction 1 on Metabolic Indexes of Macrophages

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The development of antiplague resistance is mainly conditioned by the activation of the cellular component of the immune system. The realization of the immune response on the cellular level is inseparably linked with the function of the system of mononuclear phagocytes (SMP). The course of vaccinal and infectious processes is determined by the interaction between *Y. pestis* and SMP cells. *Y. pestis* capsular antigen - Baker's fraction 1 - is known to play an important role in protecting the microbe against phagocytosis. Fraction 1 may be synthesized by cells in the form of two main serologically identical components, viz., the protein-polysaccharide complex (F1A) and purified protein (F1B). Despite prolonged studies of the role of capsular antigen in protecting the microbe against phagocytosis, only desultory information is available on the mechanism underlying the effect of this virulence determinant, especially of its polysaccharide component on the functional activity of SMP cells.

We present here the results of a comparative study of the influence of the protein-polysaccharide complex and purified protein of fraction 1 on the metabolic indexes of macrophages of peritoneal exudate (MPE) characterizing the functional activity of the cells, namely the chemoluminescent (CL) response and the activity of ecto-5-nucleotidase (5-n) (E.C.-31.35).

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## MATERIALS AND METHODS

Experiments were carried out on (CBA×C57Bl/6)$F_1$ mouse hybrids (males) weighing 16-18 g. Animals were injected hypodermically with the protein-polysaccharide complex (F1A) and purified protein (F1B) of fraction 1 at doses ranging from 2 to 100 μg. The activity of 5-n in MPE was determined 1 and 7 days after the procedure in accordance with a described method [7,8]. Macrophages were obtained according to [2]. Animals given isotonic NaCl solution served as controls. From the experimental data the point and interval estimates of the enzyme activity were found. F1A was obtained by fractionation with ammonium sulfate of the supernatant of a biphase culture of *Y. pestis* strain. F1B was isolated from broth culture filtrate by one-stage column gel-filtration on Ultragel AcA-22 [4].The degree of activity of the oxygen-dependent bactericidal systems of phagocytes was evaluated by measuring the CL level [5].

## RESULTS

The experimental data on the effect of fraction 1 on MPE are presented in Fig.1. The dynamics of all

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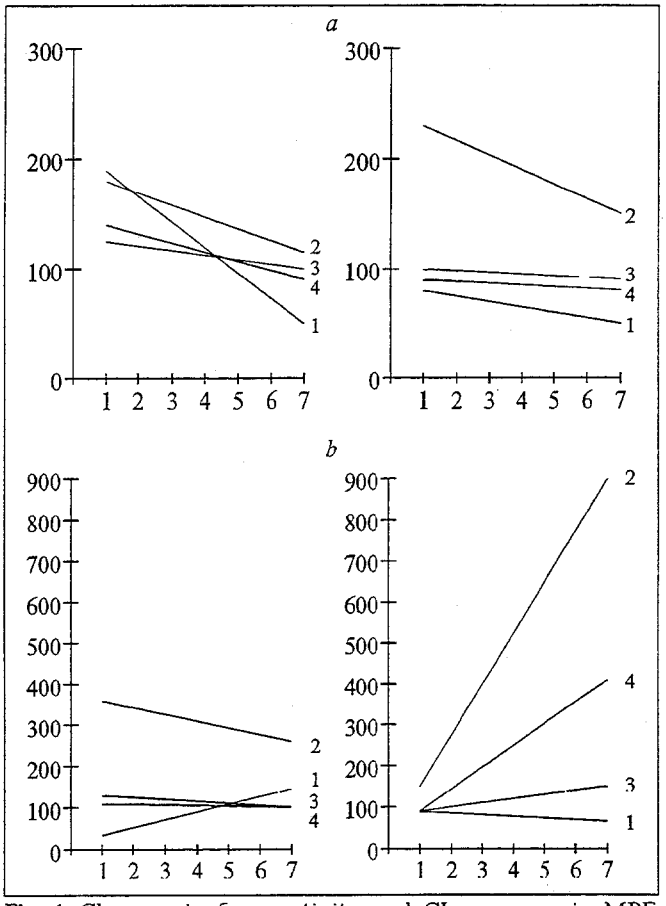


**Fig. 1.** Changes in 5−n activity and CL response in MPE induced by injection of *Y. pestis* fraction 1 (F1A and F1B). Abscissa: time of examination, days; ordinate: activity in percent of control (C). 1) 5−n activity in MPE, % of C; 2) CL induced by *St. aureus*; 3) spontaneous CL; 4) CL induced by *Y. pestis*.

indexes examined is shown, including the level of 5-n activity, spontaneous CL and CL induced by the contact with nonopsonized strains of staphylococcus and plague bacillus (*St. aureus* Wood- 46, *Y. pestis* EV) (Kazan Research Institute of Epidemiology and Hygiene. As shown in Fig.1, the alterations in both the spontaneous CL and *Y. pestis*-induced CL upon F1B injection at a dose of 90 μg became apparent, in the main, only on the 7th day and were not pronounced. Therefore, only the changes in 5-n activity and CL induced by staphylococcus will be described and analyzed. The dependence of these indexes on the dose used on days 1 and 7 is presented in Fig. 2. No discernible changes in 5-n activity were found on the 1st day after F1B injection. After 7 days a decrease of enzyme activity was detected, which was a little more expressed in the case of the lower dose.

The alterations in the CL response were more significant. F1B in both doses examined caused an increase of the induced CL throughout the experiment. The sharp rise of the CL response on day 7 after the F1B injection in a dose of 90 μg should be noted.

Increasing the dose of the preparation led to a certain decrease of the CL level on day 1. On day 7,on the contrary, increasing the dose resulted in a sharp rise of the CL response (up to 90 percent with respect to the control level).

A comparison of the dynamics of the indexes examined (Fig. 1) induced by F1B injection in various doses shows the uniformity of the changes in 5-n activity in both cases, i.e., the absence of any change in enzyme activity on the 1st day, followed by its decrease on the 7th day. On the other hand, the CL response depended to a considerable extent on the dose of the preparation used. F1B in a dose of 1 μg led to a maximum CL increase on the 1st day, whereas a 90 μg dose caused a negligible increase of the CL response on the 1st day, followed by its sharp rise on the 7th day. In other words, the changes in the CL response, in contrast to those in 5-n activity, were found to be dose-dependent.

A comparison of the two metabolic indexes, i.e., 5-n activity and staphyloccocus-induced CL, showed that F1B in a dose of 2 μg affected them in the same manner, whereas a 90 μg dose of the preparation had the opposite effect on these two indexes. All of the above suggests that in the case of F1B use no correlation is found between the two indexes examined which, according to published data, characterize the functional activity of MPE.

In contrast to the situation with F1B, the changes in 5-n activity (Fig. 2) under the influence of F1A were more pronounced on the 1st rather than the 7th day. A strongly expressed dose dependence of this metabolic index should be noted. Thus, an F1A dose of 2 μg (Fig. 1) led to an increase in 5-n activity on the 1st day followed by its decrease on the 7th day. F1A in a dose of 90 μg had the opposite effect on 5-n activity: a decrease on day 1 was followed by a negligible increase on day 7.

The changes in the CL response under the influence of the carbohydrate-containing fraction were found to be dose-dependent to a considerable extent and more pronounced on day 1 rather than 7 (Fig.2). Both on the 1st and 7th day increasing the dose led to an increase in the CL response. This dependence was most pronounced on day 1 .

A comparison of the dynamics of the CL response induced by F1A in various doses (Fig. 1) shows the same direction of the changes in both cases from a higher level on the 1st day to a lower one on the 7th day, the amplitude of the CL response being more significant in the case of the higher dose.

A comparative analysis of the dynamics of the two indexes, 5-n activity and CL response, reveals additional differences in the effect on MPE between various doses of the carbohydrate fraction. Thus, F1A

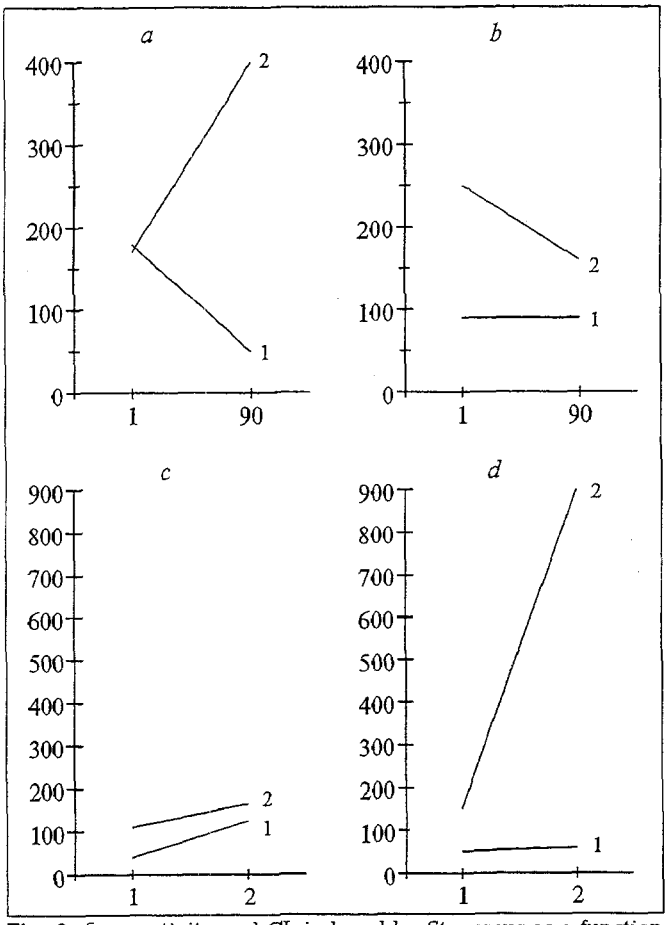


Fig. 2. 5-n activity and CL induced by *St. aureus* as a function of dose of preparation on days 1 and 7. Abscissa: dose, μg; other notation as for Fig. 1.

in a dose of 2 μg caused unidirectional changes of these indexes, whereas for injection of F1A in a dose of 90 μg the changes were differently directed.

Therefore, the analysis of the findings suggests that the preparation of *Y. pestis* capsular antigen causes considerable alterations of the metabolic indexes of MPE, the character and the direction of the changes being different in the cases of F1A and F1B.

Only fragmentary information is available on the effect of fraction 1 on the functional activity and CL response of phagocytes [1,5,6].

The present study testifies to the different effect of the protein- polysaccharide component and the purified protein of fraction 1 on the CL response of macrophages. This metabolic index is found to depend on both the dose and the duration of the experiment. The contradictions found in published data on this problem seem to be due to the nonuniformity of the experimental conditions, including the degree of purity and the dose of preparation used, as well as the time after the influence.

The variation in 5-n activity is known to be one of the factors of the nonspecific resistance of the organism to infection [3].

The observed change in 5-n activity in MPE influenced by *Y. pestis* capsular antigen suggests that the preparation obtained affects the nonspecific resistance to infection, i.e., it possesses immunomodulatory properties. This assumption is in accordance with the modern concept concerning the role of macrophages as the most significant factor in the nonspecific resistance of the organism.

In addition, the fact that the carbohydrate-containing fraction exhibits the most pronounced influence on 5-n activity is assumed to be evidence of its more expressed nonspecific properties.

The correlation between specific and nonspecific factors in the protective system is the focus of attention of workers in the area of theoretical and clinical immunology .

The higher immunological activity of preparations of fraction 1 that contain a polysaccharide component possessing a nonspecific effect on the organism provides further evidence of the important role played by nonspecific in addition to specific factors in the development of antiplague immunity.

## REFERENCES

1. G. I. Vasil'eva, E. P. Dorotenko, and A. K. Kisileva, in: *The Current Status of Theoretical Problems in Epidemiology and Infectious Immunology* [in Russian], Nal'chik (1988), p.113.
2. L. G. Zaitseva and Yu. N. Favorskaya, *Byull. Eksp. Biol.*, **90**, № 11, 576 (1980).
3. G. B. Kirillicheva, V. A. Bekhalo, M. S. Solov'eva, *et al.*, in: *Immunomodulators in Infectious Pathology* [in Russian], Moscow (1988), pp. 105-108.
4. L. N. Serdobintsev, T. M. Taranenko, M. S. Varenkov, and A. V. Naumov, in: *Problems of Prophylaxis of Natural Foci of Infection* [in Russian], Saratov (1983), pp. 37-41.
5. N. Yu. Stukova, *Alterations in the Functional Activity of Oxygen-Dependent Bactericidal Systems of Phagocytes upon Interaction with Y. Pestis and Its Antibodies*, Candidate of Medical Sciences Dissertation [in Russian], Saratov (1991).
6. T. M. Taranenko, A. F. Filippov, V. E. Zhemchugov, and I. P. Andreeva,in: *Current Topics in Laboratory Diagnostics and Biochemistry of Plague and Cholera Pathogens* [in Russian], Saratov (1984), pp. 47-53.
7. M. A. Tumanyan and G. B. Kirillicheva, *Immunologiya*, № 1, 4-5 (1984).
8. M. A. Tumanyan and G. B. Kirillicheva, *Otkrytiya*, № 6, 621 (1986).