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BIOCHEMICAL AND IMMUNOCHEMICAL STUDY OF A PROTEIN PREPARATION FROM THE CULTURE LIQUID OF *Yersinia pseudotuberculosis*

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Using ultrafiltration methods of isolation, a culture liquid of Y. pseudotuberculosis *has yielded a protein fraction with a molecular mass of not less than 80-100 kDa which possesses hemagglutinating activity and the properties of an antireceptor. It has been shown for the first time by the methods of affinity chromatography, indirect hemagglutination, and indirect IEA that this protein preparation from a* Y. pseudotuberculosis *culture interacts specifically with the Fab_2 fragment of normal human IgG.*

As established previously [1], fragments of cell receptors formed in catabolic transformations of these proteins have been detected in human blood serum. It has been shown that they are capable of spontaneously forming complexes with IgG — so-called agglutinators (homoreactants). In the norm, fragments of receptor proteins are present at low concentrations in all healthy persons, but in many infectious and autoimmune diseases their level rises substantially, correlating with the severity of the pathological process [2], which has permitted the suggestion of the participation of these compounds in processes of tissue destruction arising in response to the action of the most diverse factors of the environment.

In a recent communication [3] we have shown that in the process of vital activity a number of the strains of the pathogenic bacterium *Y. pseudotuberculosis* secrete into the culture medium products consisting of fragments of receptor proteins analogous to those found in human blood serum.

Results obtained in the present work have shown some functional affinity of the products of the vital activity of the bacteria under investigation with human receptor proteins.

The culture liquid obtained after the cultivation of a number of strains of the pathogenic microorganism *Y. pseudotuberculosis* possessed a capacity for agglutinating rhesus-positive human erythrocytes of the O(I) group [3]. After its fractionation by ultrafiltration on Amicon filters (USA), five fractions containing water-soluble proteins with different molecular weights were obtained (Table 1). A high-molecular-weight protein fraction (HMPF) retained by a Diaflo xm 100 filter (Amicon) and, therefore, having a molecular mass of not less than 80-100 kDa, possessed agglutinating activity. The other fractions either did not possess this property or led to insignificant hemagglutination.

In the hemagglutination reaction with human rhesus-positive erythrocytes of group O(I) previously treated with trypsin, the hemagglutinating activity of the HMPF disappeared, which suggests the protein nature of the active centers of the component forming this fraction.

To reveal the functional significance of the protein preparation obtained from yersinias and its role in the vital activity of the organism under investigation, we have carried out an immunochemical study of it in comparison with a homoreactant from human blood serum.

It had been shown previously that a characteristic feature of "homoreactants" is that they react with the Fab′ fragment of the molecule of the homologous autologous IgG but do not interact with unseparated IgG [4].

The Fab_2 fragment obtained with the aid of pepsin from normal human IgG [5] was immobilized on (cyanogen bromide)-Sepharose and bacterial preparation (HMPF) was passed through a column containing this immunosorbent. The unbound

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TABLE 1. Fractionation of the Culture Liquid after the Cultivation of *Y. pseudotuberculosis* sp. 2781.

Filter	Molecular mass of the protein in the HMPF, kDa	Protein content	
		mg/ml	% of the amount of protein in the native liquid
	Native culture liquid	2.3	100
XM 100	More than 100	0.540	23.5
PM 30	From 30 to 100	0.168	7.3
PM 10	From 10 to 30	0.084	3.5
IM 2	From 1 to 10	0.476	20.7
	Less than 1	1.032	44.8

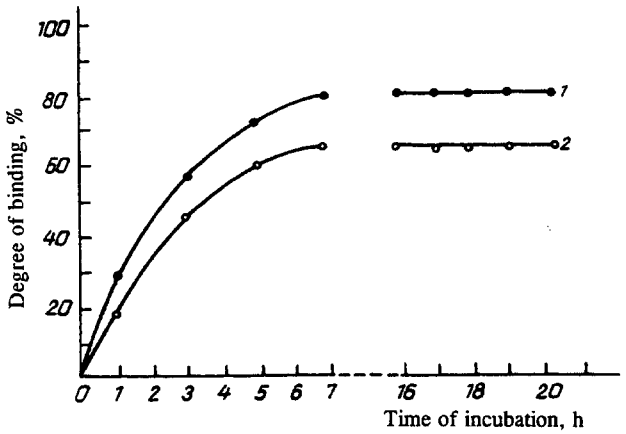

Fig. 1. Kinetics of the affinity binding of the HMPF from *Y. pseudotuberculosis* (*2*) and a homogenate from human blood serum (*1*) on cyanogen bromide-Sepharose 4B with the immobilized Fab_2 fragment of human IgG.

proteins were eliminated by washing the protein with physiological solution (pH 7.8), after which the protein fixed to the immunosorbent was eluted with 2 M potassium iodide [6].

The amount of protein bound with the immunosorbent was about 60% of the total amount in the fraction under investigation (Fig. 1).

The affinity of the HMPF under investigation for the Fab_2 fragment was also determined in the indirect hemagglutination reaction (IHAR) using rhesus-positive erythrocytes of the O(I) group sensitized by the Fab_2 fragment, obtained with the aid of pepsin from normal IgG, and by indirect IEA with the Fab_2 fragment immobilized on polystyrene as ligand. The preparation under investigation reacted with the Fab_2 fragment in the IHAR in a titer of 1/8-1/16 (for the homoreactant, in a titer of 1/16-1/32 [7]).

In the IEA, the preparation under the investigation reacted with the Fab_2 fraction immobilized on a solid phase in concentrations somewhat lower than the homoreactant from human blood serum (Fig. 2).

Investigations on protein preparations from *Y. pseudotuberculosis* and "homoreactant" from human serum cross reacted with Fab_2 fragment from normal human IgG.

It is known [8] that Fab-fragment—homoreactant complexes may be responsible for the appearance in the blood of a patient of a number of biologically active factors thanks to the action of which pathophysiological reactions arise. These complexes participate in the development of general and local inflammatory reactions. The interaction of receptor proteins and their complexes from the blood serum of microorganisms with the proteins of pathogenic microorganisms has not hitherto been investigated. The results that we have obtained therefore open up new possibilities for analyzing the role of the receptor proteins from macro- and microorganisms in the infectious process and the further elucidation of the mechanisms of pathogenesis.

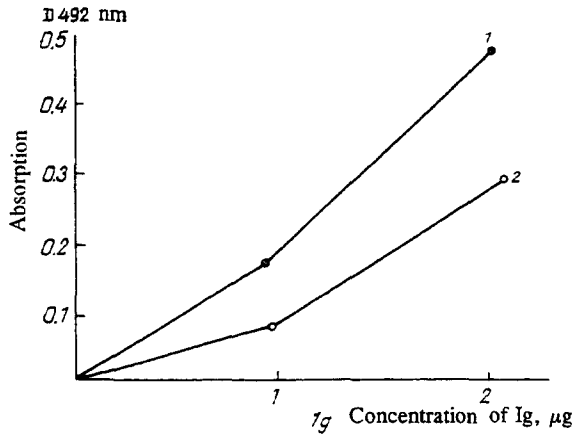

Fig. 2. Binding of the HMPF of *Y. pseudotuberculosis* (*2*) and of the homoreactant from human blood serum (*1*) with a Fab_2 fragment from normal human IgG according to indirect IEA.

EXPERIMENTAL

The microorganism *Y. pseudotuberculosis*, strain 2781, was cultivated at 6-8°C in a nutrient medium containing (g/liter): peptone — 20; $(NH_4)_2SO_4$ — 4; $MgSO_4$ — 0.4; $CaCl_2$ — 0.02; NaCl — 10; $FeCl_3$ — 1; NaH_2PO_4 — 16; distilled water — 1 liter; pH — 7.3. The medium contained 0.1% of glucose. After four days the suspension of microorganisms was separated by centrifugation at 6000 rpm for 5 min. The culture liquid obtained was dialyzed against 0.06 M phosphate buffer (pH 7.4) until ammonium ions had been completely eliminated. The dialysate was centrifuged at 25,000 rpm for 30 min. The precipitate was filtered off with suction. The culture liquid purified in this way was used for further investigation.

Enzymatic Treatment of Erythrocytes. A 10% suspension of erythrocytes in physiological solution (10 ml) was treated with trypsin (2 mg), at 37°C for 30 min. Then the erythrocytes were washed three times with physiological solution (pH 7.8) and used in the hemagglutination reaction.

The hemagglutination reaction of the preparations under investigation was conducted in the usual way [9] with rhesus-positive human erythrocytes of group O(I).

The fractionation according to molecular mass of the protein preparation from the yersinia culture liquid and their concentration to the necessary volume was carried out by ultrafiltration on "Diaflo" xm 100, pm 30, pm 10, UM 2 molecular filters from Amicon (USA).

The affinity of the HMPF from the culture liquid of *Y. pseudotuberculosis* for the Fab fragment was determined by the method of indirect hemagglutination with rhesus-positive human erythrocytes of group O(I) sensitized by the Fab_2 fragment of human IgG, and also with the aid of indirect IEA and by affinity chromatography using the Fab_2 fragment of normal human IgG immobilized on polystyrene and (cyanogen bromide)-Sepharose (Farmacia), respectively [7, 10].

The homoreactant used in this work was obtained from a pool of healthy donor sera as described in [11].

Protein contents were determined by Lowry's method [12].

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