The presence of different subpopulations of lymphocytes in the tissue of the developing lung was thus found in 8-10-week human fetuses. A significant increase was found in the number of lymphocytes carrying various surface markers at the later stages of development, starting from the 17th-18th week of gestation. As the lung develops, the proliferative capacity of the lymphoid cells decreases.

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ROLE OF HISTAMINE RECEPTORS OF MOUSE AND GUINEA PIG PERITONEAL LEUKOCYTES IN THE PATHOGENETIC ACTION OF *Yersinia pestis* ADENYLATE CYCLASE

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The writers previously showed that β-adrenergic receptors of animal cells are involved in the pathogenetic action of the adenylate cyclase of *Yersinia pestis.* However, the group of histamine H_1 and H_2 receptors found on the surface of mixed lymphocytes and neutrophils remained unstudied. Considering the complex role of histamine in the development of infectious diseases and in the immune response, and its action on the cell through the adenylate cyclase–AMP system [10], we decided to study the possible role of peritoneal leukocyte histamine receptors of animals in the mechanism of the suppressive action of the recently discovered [1, 3] adenylate cyclase of *Yersinia pestis.*

EXPERIMENTAL METHOD

Experiments were carried out on a purified preparation of *Y. pestis* adenylate cyclase with specific activity of 1700 pmoles $\cdot mg^{-1} \cdot min^{-1}$ [1]. Peritoneal leukocytes were obtained from albino mice and guinea pigs by the usual method. The chemiluminescence of the cell suspension was recorded as described previously [1]. Expression of F^- and C_3 receptors (FcR and C_3R) was determined on guinea pig macrophages, by the rosette-formation method (EA-RFC) and chemiluminescence [5]. The kinetics of incorporation of $^{45}Ca^{2+}$ ("Izotop," USSR) was studied by methods in [2, 6]. Activity of Ca^{2+}-calmodulin-dependent protein kinase (CaM-PK) was determined as described in

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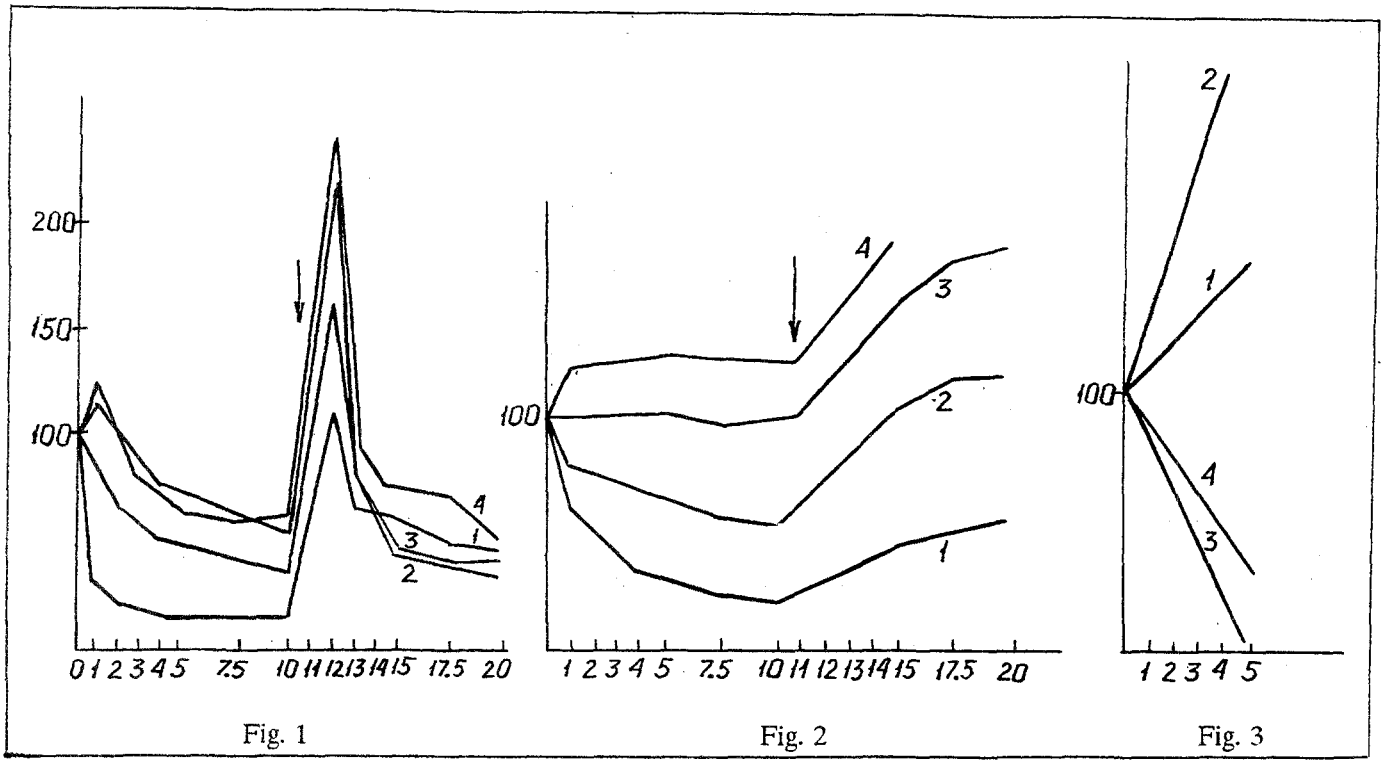

Fig. 1 Fig. 2 Fig. 3

Fig. 1. Effect of different histamine concentrations: 10^{-5} (1), 10^{-6} (2), 10^{-7} (3), and 10^{-8} M (4) on intensity of chemiluminescence of albino mouse leukocytes. Arrow indicates time of addition of *E. coli* QD 5003 (10^7 million cells) in Figs. 1 and 2. Here and in Figs. 2 and 3: abscissa, time (in min); ordinate, intensity of chemiluminescence (in %).

Fig. 2. Effect of different histamine concentrations in doses of: 10^{-5} (1), 10^{-6} (2), 10^{-7} (3), and 10^{-8} M (4) on intensity of chemiluminescence of guinea pig leukocytes.

Fig. 3. Chemiluminescence profiles of C_3- (1, 2) and Fc-receptors (3, 4) after treatment with adenylate cyclase from *Y. pestis* (10^{-6} and 10^{-7} g/ml) of guinea pig peritoneal macrophages.

[4]. Total protein was determined by Lowry's method in the modification [11] and by differential spectrophotometry [8]. Histamine and blockers of H_1- and H_2-receptors (diphenhydramine, chloropyramine, promethacin, cimetidine) of Russian manufacture, calmodulin ("Serva"), and ^{32}P-ATP ("Izotop," USSR) were used.

EXPERIMENTAL RESULTS

Histamine, exerting its action through activation of the adenylate cyclase–cAMP system of the cell [10], had a dose-dependent effect on chemiluminescence (ChL) of albino mouse peritoneal leukocytes (Fig. 1). For instance whereas large doses of histamine (10^{-5}-10^{-6} M) strongly inhibited ChL, in smaller concentrations (10^{-7}-10^{-8} M) during the first few minutes of contact, increased ChL to 115-120%, which was followed by a lytic decline to 60-70%. Stimulation of ChL in this way by low doses (10^{-8} M) of histamine has been observed by other workers also [14]. Addition of a suspension (10^7 cells/ml) of *E. coli* QD 5003 to samples of luekocytes treated with histamine, in order to verify the functional state of the leukocytes, led to an increase in ChL to 115-270%, evidence of the transient character of the suppressive action of histamine.

Preliminary contact of albino mouse leukocytes with promethacin, diphenhydramine, and chloropyramine, inhibitors of histamine H_1 receptors, in a dose of $(2.5\text{-}5)\cdot10^{-6}$ M did not prevent the inhibitory action of adenylate cyclase, whereas cimetidine (an inhibitor of H_2 receptors) neutralized the action of the enzyme regardless of when it

was added. It can be postulated on the basis of these results that the action of *Y. pestis* adenylate cyclase is mediated chiefly through histamine H_2 receptors.

The chemiluminescence profile of guinea pig peritoneal leukocytes (Fig. 2), treated with different doses (10^{-5}-10^{-7} M) of histamine, differed from that of albino mouse leukocytes in its somewhat lower sensitivity to the concentrations of the preparation used. The action of histamine was reversible in character and was abolished by the addition of *E. coli* cells.

Of the antihistamine drugs used, promethacin, a blocker of H_1-receptors, most effectively prevented the inhibitory action of adenylate cyclase, and in a dose of $2.5 \cdot 10^{-6}$ M it actually exceeded the effect of cimetidine. Because of the selectivity of action of the antihistamine blockers, it can be concluded that histamine-specific receptors exist on the surface of animal leukocytes, and through them, the adenylate cyclase of *Y. pestis* is able to realize its pathogenetic potential.

An increase of 1.5 times in the number of C_3 receptors after treatment of guinea pig peritoneal macrophages by adenylate cyclase was demonstrated by rosette formation. This correlated with the increase in chemiluminescence (Fig. 3) of samples of macrophages after fixation of erythrocytes on them. If it is recalled that activation of complement is accompanied by removal from C_3 of a C_{3a} fragment with mol. wt. of 7 kD, endowered with activity of anaphylatoxin and capable of inducing degranulation of leukocytes and macrophages, with release of histamine from them [12], and that some effects of C_{3a} are inhibited by antihistamine preparations which block the H_1-receptor, it can be tentatively suggested that the adenylate cyclase of *Y. pestis,* by activating C_3R, facilitates histamine release.

Receptors for Fc fragments (FcR) of leukophilic IgG, which was present in neutrophils and other cells, were detected on the plasma membrane of the mononuclear phagocytes. The biochemical principles governing changes in the number of Fc receptors have not yet been explained and the role of FcR in activation of intracellular processes has not been studied.

It was found that adenylate cyclase of *Y. pestis* in a concentration of 10^{-7} g/ml significantly inhibits expression of FcR on the surface of guinea pig peritoneal macrophages by comparison with intact macrophages (EA-RFC 55 and 277 respectively). This also applies to the number of EA-RFC binding more than six EA. The number of macrophages which adsorbed 6-8 erythrocytes was an order of magnitude less after incubation with the adenylate cyclase preparation.

The use of the highly sensitive chemiluminescence method to record the number of erythrocytes involved in the formation of FcR-mediated rosettes revealed an even greater difference in the expression of these receptors in control and enzyme-treated guinea pig peritoneal macrophages. For instance, with adenylate cyclase in a dose of 10^{-6} g/ml the ChL level fell to 3% of the control value, but in a dose of 10^{-7} g/ml, it fell to 35% (Fig. 3).

Weakening of FcR expression by *Y. pestis* adenylate cyclase can be explained both by the direct action of the enzyme on the receptor surface of the cells and by the effect of cAMP, formed in the target cell through the action of the *Y. pestis* enzyme [1], a view that is in agreement with the fact that expression of Fc receptors on mature guinea pig macrophages is induced by cGMP but inhibited by cAMP.

Histamine stimulates the increase in concentration of free intracellular Ca^{2+}. Some workers [15] consider that this effect is mediated by histamine H_2-receptors, others that this action is abolished by H_1-receptor antagonists, but not by cAMP [7].

The increase in the intracellular Ca^{2+} concentration under the influence of histamine (10 μM) takes place in two phases: a fast "peak" and subsequent smaller but stable rise. The peak increase in calcium is connected with its release from intracellular binding sites, the second phase with its arrival from the extracellular space. The role of Ca^{2+} in histamine release is known [9].

Our experiments on albino mouse peritoneal leukocytes revealed a considerable decrease in incorporation of labeled Ca^{2+} into cells under the influence of bacterial adenylate cyclase in a dose of 10^{-7} g/ml (control cells 6750 cpm, enzyme-treated cells 2850 cpm). This effect of adenylate cyclase is evidently indirect in character, for the inward flow of Ca^{2+} is regulated by Ca-channels, on the opening of which Ca^{2+} passes into the cells along the concentration gradient formed by secondary messengers, including Fc [13].

Like the adenylate cyclase toxin of *Bordetella pertussis,* the adenylate cyclase of *Y. pestis* proved to be sensitive to calmodulin. Determination of activity of calmodulin-dependent protein kinase (CaM-PK) of the soluble and membrane fractions, and also of whole cells (albino mouse peritoneal leukocytes) showed that *Y. pestis* adenylate cyclase reduced by 1.5 times the CaM-PK activity of soluble fractions compared with the control (6775 and 13645

cpm respectively). On the addition of calmodulin (10^{-6} M) to the sample, however, the inhibitory effect of the enzyme was totally abolished (13405 cpm). Weakening of CaM-PK activity by adenylate cyclase also was observed in the membrane fractions, but exogenous calmodulin had no stimulating effect under these circumstances, as it did with the soluble fraction. As regards whole cells, no change was observed in CaM-PK activity under the influence of adenylate cyclase and calmodulin.

Calcium transport is regulated by two mediators: Ca^{2+} and cAMP. The latest studies of the regulatory properties of CaM-PK showed [16] that the link between Ca^{2+} and cAMP is highly complex, and that under the appropriate conditions, the cell may prefer only one of the regulatory activities.

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