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MANNOSE-SPECIFIC INTERCELLULAR AGGREGATION OF RAT THYMOCYTES AND ESCHERICHIA COLI CELLS TRIGGERED BY TEMPERATURE

D-mannose was shown to induce disaggregation of rat thymocytes and E. coli AB1157 cells at any stage of the process. Mannose-specific aggregation of thymocytes and E. coli was inhibited by heating as opposed to concanavalin A-induced thymocyte aggregation. The process was completely reversible at the rapid temperature changes, indicating its membrane not intracellular mechanism. Taken together, the results demonstrate that mannose-specific intercellular contacts can be affected differently by temperature.

Introduction. Results of recent research emphasize that a notable part of intercellular contacts are based on the protein-carbohydrate interaction [1, 2]. Bacterial cells can effectively interact with animal cells in adhesion and intercellular aggregation reactions [3, 4]. The mechanisms of these processes are being studied intensively since they are the basis of the development of various bacterial infections [5]. It has been shown that *E. coli* cells bearing type 1 fimbrial lectin bind to mannose-containing glycoligands on the surface of macrophages and neutrophils [6]. Recently, we have found that *E. coli* AB1157 cells also induce aggregation of rat thymocytes, while heat-inactivated bacterial cells lose this activity [7]. Earlier, heating was shown to affect lectin-dependent interactions in cellular and model (liposomes) systems [8, 9]. The mannose-specific intercellular aggregation of rat thymocytes and *E. coli* may be used to model lectin-carbohydrate contacts between animal and bacterial cells in suspensions, because, in comparison with phagocytes, thymocytes have little unspecific adhesion to a majority of surfaces.

In the present study we investigated the effect of the temperature provoking structural and morphological changes in the cell plasma membrane on the rat thymocyte aggregation induced by *E. coli* AB1157 and mannose-binding lectin concanavalin A (Con-A). Our results show that in contrast to Con-A-induced aggregation, the mannose-specific intercellular aggregation of rat thymocytes and *E. coli* is triggered by the rapid temperature change and occurs at the another temperature range.

Experimental. D-mannose and Con-A were obtained from «Sigma» (USA).

The thymocytes were isolated from the thymus gland of male Wistar strain rats, 6—8 weeks of age, and suspended in phosphate-buffered saline (PBS), pH 7.3, containing 137 mM NaCl, 2.7 mM KCl, 5 mM Na_2HPO_4 and 1.5 mM KH_2PO_4, as previously described [7].

E. coli AB1157 cells were cultured by the standard method [10]. Briefly, the bacteria were grown overnight at 37 °C in a beef-extract broth and washed two times with PBS before use.

The aggregation of rat thymocytes induced by *E. coli* as well as by Con-A was measured by changes in light transmission at 590 nm of cell suspensions [7], which were kept at a constant temperature and with continuous stirring.

Results and discussion. The rat thymocytes aggregated in the presence of *E. coli* AB1157 at room temperature (fig. 1). The order in which cells were added played no role in the results, the intercellular aggregates were microscopically visible. To test the stability of cell aggregates the gaptenic sugar D-mannose was added at various time points of the aggregation process. Independent from addition time, D-mannose in concentration of 45 mM elicited the complete disaggregation of cells (fig. 1).

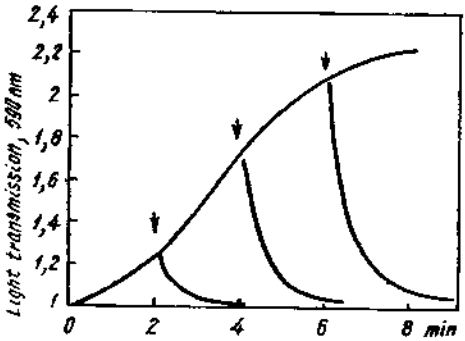

Fig. 1. Aggregation of rat thymocytes by *E. coli*. *E. coli* cells ($1.5 \cdot 10^8$/ml) were added at time zero to thymocyte suspension ($7 \cdot 10^6$/ml) in PBS at 20 °C; D-mannose (45 mM) was added after the bacteria as indicated by the arrows. The traces are representative of at least three experiments

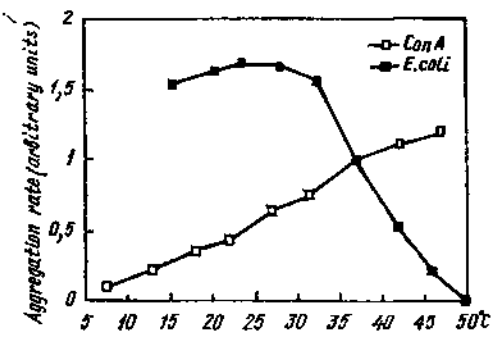

Fig. 2. Effect of temperature on rat thymocyte aggregation induced by *E. coli* and Con-A. *E. coli* cells ($1.3 \cdot 10^8$/ml) or Con-A (50 μg/ml) were added to rat thymocyte suspension ($4.5 \cdot 10^6$/ml) in PBS kept at the indicated temperature. Aggregation rate was calculated as an incline of tangent to bend point of trace. The illustrated data are representative of three experiments

Earlier, other sugars (D-glucose, D-galactose, lactose, D-maltose, L-rhamnose et al.) were found to be inactive to affect the intercellular aggregation [7]. The high carbohydrate specificity of this reaction prompted us to compare it with another mannose-specific aggregation of rat thymocytes induced by Con-A.

Fig. 2 illustrates the effect of temperature on the rat thymocyte aggregation induced by *E. coli* in comparison with those induced by mannose-binding lectin Con-A. It was observed that the rate of thymocyte and *E. coli* intercellular aggregation decreased with the temperature increase. On the contrary, heating raised the rate of Con-A-induced thymocyte aggregation (fig. 2). Thus, mannose-specific contacts of cell surfaces formed by bacterial and plant lectins display different sensitivity to temperature.

Since at 50 °C rat thymocytes and *E. coli* did not interact (fig. 2), we hypothesized that high temperature might destroy intercellular contacts due to the reversible heat-induced structural changes of respective plasma membrane components. This supposition was proven by special experiments, when the temperature of samples was changed rapidly (for 15—25 s) during the process of aggregation. Indeed, the intercellular aggregation of rat thymocytes and *E. coli* proceeding at 16 °C was stopped with the rapid jump in the temperature of the sample to 50 °C, thus the cell aggregation process was replaced by disaggregation (fig. 3). Furthermore, it was reversible with the following rapid decrease in temperature to 16 °C (fig. 3). At the same time, Con-A-induced aggregation of thymocytes could be stopped by rapid fall in temperature without cell disaggregation (not illustrated).

The results of the study reported here show that intercellular contacts between *E. coli* AB1157 and rat thymocytes are based on the mannose-specific interaction of respective surface structures. Apparently, the type 1 fimbrial lectin of bacterial cells takes part in this reaction as well as in process of lectinophagocytosis of the bacteria by phagocytes [6].

Since the lection is expressed by many virulent strains of *E. coli* [11, 12], the possibility of forming intercellular contacts of bacterial and lymphoid cells may be significant for the development of the local immune response.

To the best of our knowledge, the results first demonstrate that mannose-specific intercellular contacts can be affected differently by temperature. Thus, heating stimulates rat thymocyte bridging by exogenous lectin Con-A apparently due to clasterization of mannose-containing plasma membrane glycoligands. However, the mannose-dependent interaction of thymocytes with *E. coli* via bacterial lectin is blocked by heating. In this

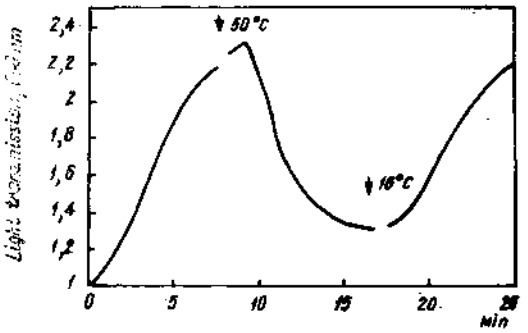

Fig. 3. Aggregation and disaggregation of rat thymocytes induced by *E. coli* at the rapid temperature changes. *E. coli* ($1.3 \cdot 10^8$/ml) were added to rat thymocytes ($5 \cdot 10^6$/ml) in PBS at 16 °C. Where indicated by the arrows, the temperature of sample was rapidly changed and recording was continued. The traces are representative of at least three experiments

case we deal with the direct contacts of cellular surfaces without a connecting intermediate like exogenous Con-A. This phenomenon is an important example of mannose-specific biorecognition triggered by temperature. Remarkably the fact that one can «switch on» and «switch off» this process (fig. 3) indicates membrane involvement but not intracellular mechanisms. High complementary of cell contacting surfaces is supposedly necessary to form the stable intercellular contacts, which are destroyed easily at the temperature change. This is in keeping with the thrombocyte aggregation sensitivity to temperature due to the morphological changes of the cell surface as studied by Samal et al. [13]. A structural state of mannose-specific fimbrial lectin of *E. coli* is also important since it has been shown to affect cells only in immobilized form [14].

Futhermore, hydrophobicity of bacterial cells [15] may also play a role in their adhesion to thymocytes at lower temperature. Further experiments are required to elucidate the mechanisms of the temperature-dependent biorecognition of bacteria and mammalian cells.

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ВПЛИВ ТЕМПЕРАТУРИ
НА МАНОЗОСПЕЦИФІЧНУ АГРЕГАЦІЮ ТИМОЦИТІВ ЩУРІВ
І БАКТЕРІАЛЬНИХ КЛІТИН ESCHERICHIA COLI

Резюме

Встановлено, що D-маноза викликає дезагрегацію тимоцитів щурів і бактеріальних клітин *E. coli* 1157 на будь-якій стадії процесу міжклітинної агрегації. Манозоспецифічна агрегація тимоцитів і *E. coli* пригнічувалася із підвищенням температури на противагу прискоренню агрегації тимоцитів, викликаної конканаваліном А. Процес міжклітинної агрегації тимоцитів і бактеріальних клітин був повністю зворотнім за різкої зміни температури, внаслідок чого нагрівання суспензії до 50 °С призводило до дезагрегації клітин, а подальше зниження температури до 16 °С — до запуску процесу агрегації. Отримані результати свідчать про те, що манозоспецифічні міжклітинні контакти тимоцитів щурів за участю конканаваліну А і бактеріальних клітин виявляють різну чутливість до дії температури.

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