must be admitted that this insertion of an hydrophobic lectin segment in the bilayer could be reversed by addition of a specific inhibitor like N-acetylgalactosamine. Indeed, as demonstrated by Rendi et al.[7] liposome agglutination could be reversed. Alternatively, our results could be explained in terms of ganglioside organization in the lipid matrix. It has been demonstrated that gangliosides are randomly distributed in a fluid lipid matrix[13]. Soybean agglutinin may induce ganglioside clustering and the permeability change may simply result from the formation of hydrophilic pores of GM_1 gangliosides without penetration of the lectin into the lipid bilayer. The lateral mobility of the gangliosides will determine the reversibility of the agglutination process. In model membranes, ganglioside clustering strikingly increases the membrane permeability. Moreover, it has been recently proposed that lentil lectin induces the existence of reversible microclustering of membrane receptors in HeLa cells[14].

Finally, although GM_1 ganglioside appears to be the receptor of cholera toxin[15,16] and of *Ricinus communis* toxin[8], it would be premature to attribute GM_1 as a lectin receptor before demonstration that cells lacking GM_1 could be made agglutinin-responsive after incorporation of GM_1 in their membranes.

1 Reprint requests to J.M.R., Faculté des Sciences-Chimie-Physique des Macromolécules aux Interfaces, Université libre de Bruxelles, Campus Plaine-Accès 5, Blvd. du Triomphe, B-1050 Bruxelles (Belgium).
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Colicin E3 enhances the oxidoreductive activity of guinea-pig leucocytes

J. Lokaj, J. Šmarda[1] and J. Mach

Department of Microbiology and Immunology and Department of Biology, Medical Faculty, J.E. Purkyně University, CS-66243 Brno (Czechoslovakia), and Institute of Sera and Vaccines, CS-10103 Praha (Czechoslovakia), 19 March 1981

Summary. Colicin E3 increased the capacity of peritoneal exudate leucocytes to reduce iodo-nitro-tetrazolium-chloride to formazane. This effect was directly dependent on its concentration within the range 10^3-10^5 lethal units per cell. In control experiments, dectran C exerted no influence on the rate of this acitivity and colicin E3 did not convert INT to formazane.

Colicin E3 evokes distinct biological effects on mammalian cells in vitro. At suitable concentrations, it kills human epithelial cells of the line HeLa and mouse fibroblasts of the line L[2]. It also decreases incorporation of radioactively-labeled precursors into DNA and RNA in murine leukemia cells of the line P388[3]. Furthermore, it profoundly inhibits the concanavalin A-promoted mitogenic activation of mouse T-lymphocytes and interferes with the homing of murine lymph node lymphocytes to the lymph nodes, without altering their migration to the spleen and liver[4]. At a low concentration, colicin E3 stimulates the proliferation of the murine leukemia cells P388[3].

In the present experiments, the action of colicin E3 on phagocytes was checked. We chose these model cells for 2 reasons: 1. they react very sensitively even on subtle changes in their medium, these reactions being detectable by rather simple methods; 2. the influence of colicin E3 on them would stimulate the consideration of a possible role of bacteriocins in inflammatory lesions in vivo.

Materials and methods. Exudate cells were gained by rinsing the peritoneal cavity of guinea-pigs with Hanks solution (Oxoid), following 18 h after i.p. appplication of 20 ml sterile 1% glycogen solution in physiological saline. Cells were washed 3 times in Hanks solution and resuspended in Hanks at 1×10^7 cells per ml. Viability was determined with trypan blue; differential counts were performed in preparations stained with methyl green and pyronine according to Unna-Pappenheim.

Colicin E3, a highly purified substance (product of the Institute of Sera and Vaccines, Praha), was used. Its purity was checked as described previously[2]. The freeze-dried preparation was dissolved and diluted in distilled water (for injections) immediately before each experiment. The activity of stock solution (containing 17.5 mg colicin substance per ml) was 10^5 arbitrary units (i.e. about 2×10^{12} lethal units per ml), using the sensitive strain *Escherichia coli* C6 as indicator.

Tetrazolium-reductase activity was determined by INT-test[5]. To 0.2 ml of 0.1% iodo-nitro-tetrazolium-chloride Lachema (INT) solution in phosphate buffer (pH 7.6), 0.1 ml of colicin solution of given concentration (or solution of a control substance), 0.5 ml of Hanks solution and 0.2 ml of cell suspension were added. The activity of colicin E3 ranged from 1×10^0 lethal units per cell to 1×10^5 lethal units per cell. After 45 min and 90 min incubations at 37 °C (in a water bath, with occasional stirring), the reaction was

stopped by adding 1 ml of 1 M HCl. Each cell suspension was spun down by lowspeed centrifugation (145 × g, 5 min); the pellet was resuspended in 3 ml acetone and extracted 20 min at room temperature. After a 10 min centrifugation at 600 × g, the concentration of extracted formazane was measured by means of the Spekol photocolorimeter at 485 nm. Each sample containing cell suspension from the same animal was tested in 3 parallel testtubes. Experiments according to this design were repeated with cells from other guinea-pigs; representative results of 1 experiment are given as means of A_{485}-values ± SE at $p = 0.05$.

3 sets of controls were run in each experiment: for negative controls, water for injections or dextan C (an electroneutral polysaccharide of mol.wt. about 60,000, equal to that of colicin E3) were added; for positive controls (i.e. for controls of cell stimulability), 1% suspension of rice starch was used. Only siliconized glass, washed for tissue cultures, was used.

Results. Differential cell counts in peritoneal exudates of guinea-pigs, as analyzed 18 h after i.p. application of glycogen, are summarized in table 1.

Control cell suspensions incubated 90 min in a waterbath at 37 °C showed no alteration of viability when tested by trypan blue vital staining.

The influence of colicin E3 on INT-reductase activity of exudate leucocytes is shown in table 2.

None of the colicin concentrations tested was cytotoxic for guinea-pig peritoneal exudate cells (after 45 or 90 min incubation), according to the trypan blue test. However, colicin E3 distinctly increased the capacity of these cells to reduce iodo-nitro-tetrazolium-chloride to colored formazane; this stimulating effect was directly dependent on colicin E3 concentration. Control experiments with dextran C revealed no influence upon the rate of tetrazolium reduction. Colicin alone, without cells, did not convert INT to insoluble formazane.

From 1 guinea-pig, 48 h after peritoneum irritation, exudate was obtained containing 61% of pyroninophilic macrophages, 22% of lymphocytes and 17% of neutrophilic granulocytes only. In experiments with cells of this exudate, the same stimulating effect of colicin E3 - in the same concentrations - on their INT-reduction activity was noticed.

Discussion. Our finding that colicin E3 influences the metabolic activity of mammalian cells from inflammatory exudates is yet another report that the biological activity of colicins is not limited to procaryotic organisms only, but that it is directed against eucaryotic cells as well. In accordance with[4], a direct interaction of colicin E3 with the plasma membrane of mammalian cells may be supposed.

Activation of the phagocyte surface membrane leads to a 'respiratory burst' (increase in oxygen uptake, superoxide production, peroxide production and hexose monophosphate shunt activity) and extracellular release of lysosomal enzymes[6,7]. A metabolic activity analogous to that provoked by a phagocytosed corpuscular material is also induced by a variety of substances which react with surface structures of leukocytes, including surface active agents[8], phospholipase C[9], concanavalin A[10–12], biologically active fragments originating at C-system activation[13,14], just as immune complexes and antibodies against neutrophilic leucocytes[15–17].

From the results of our experiments it is reasonable to suppose that colicin E3 is able to activate the 'respiratory burst' of phagocytes. All stimulating colicin E3 activity degrees used (1×10^3 - 1×10^5 lethal units per cell) fit well with the concentrations stimulating the proliferation of mouse leukemia cells P388 (lower than 1.37×10^5 lethal units per cell[3]).

Further experiments are needed to assess the involvement of colicins in the pathology of inflammatory response.

Table 1. Differential leucocyte type counts in guinea-pig peritoneal exudates

Cell type	Relative frequency
Neutrophilic granulocytes	66–67%
Young pyroninophilic macrophages	27–31%
Lymphocytes	2–7%

Table 2. The effect of colicin E3 on tetrazolium-reductive activity of exudate leucocytes (determined by INT-test)

Agent	Absorbance (± SE) at 485 nm after 45 min	90 min
None (distilled water)	0.076 ± 0.006	0.111 ± 0.013
Dextran C 0.5%	n.t.*	0.095 ± 0.013
Dextran C 5%	n.t.	0.075 ± 0.013
Colicin E3 1×10^0 l.u.**/cell	n.t.	0.105 ± 0.006
Colicin E3 1×10^1 l.u./cell	0.085 ± 0.013	0.106 ± 0.0
Colicin E3 1×10^2 l.u./cell	0.095 ± 0.013	0.105 ± 0.032
Colicin E3 1×10^3 l.u./cell	0.103 ± 0.013	0.136 ± 0.032
Colicin E3 1×10^4 l.u./cell	0.123 ± 0.006	0.195 ± 0.026
Colicin E3 1×10^5 l.u./cell	0.147 ± 0.013	0.228 ± 0.026
Rice starch 1%	0.392 ± 0.045	0.556 ± 0.052

* n.t., not tested. ** l.u., lethal units.

1 Reprint requests to J.Š., Department of Biology, Medical Faculty, Purkyně University, CS-66243 Brno (Czechoslovakia).

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