CYTOPATHOGENIC ACTION OF *Escherichia coli* CELLS CONTAINING HETEROLOGOUS HUMAN TYPE O(H) ANTIGEN ON HUMAN CELLS IN CULTURE

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The character of interaction between two enteropathogenic strains of *Escherichia coli* of serotype 055:K59 with human HeLa cells containing O(H) isoantigen was studied. On the addition of strain *E. coli* No. 5789, containing heterologous type O(H) antigen to a culture of HeLa cells, a cytopathogenic action was discovered on the third day of interaction in the presence of doses of bacterial cells of $2 \cdot 10^{10}$, $2 \cdot 10^{5}$, and $2 \cdot 10^{4}$. A dose of $2 \cdot 10^{3}$ bacterial cells of *E. coli* did not give this effect. Strain No. 3827, not containing heterologous antigen of ABO type, had no cytopathogenic action in maximal, average, and small doses of bacterial cells. It is suggested that the cytopathogenic action of strain No. 5789 is connected with the presence of an antigen in this strain which is identical with the group antigen of the human cell culture studied.

KEY WORDS: *cell culture;* Escherichia coli; *heterologous antigens.*

The attention of immunologists and microbiologists is currently being drawn to the hypothesis that the pathogenicity of microorganisms may be due to antigens which they contain that are similar to human group antigens [3-6, 8, 14, 15]. Interaction between microorganisms containing such antigens and human cells must evidently cause weakening of the immune response [4-6, 9] and the development of a chronic infection [6, 10]. A culture of human cells is a sufficiently sensitive model with which to study some aspects of this interaction. Several investigations have revealed the cytopathogenic action of uropathogenic [11] and enteropathogenic [1, 12, 13, 16] strains of *E. coli* on human cells in vitro when these strains contained heterologous antigens.

The object of this investigation was to study the cytopathogenic action of an enteropathogenic strain of *E. coli* containing heterologous antigen of the O(H) type on human cells in culture* and to detect the minimal dose of bacterial cells capable of inducing a cytopathogenic effect.

EXPERIMENTAL METHOD

A culture of HeLa cells was seeded in Wassermann tubes on medium No. 199 with 15% bovine serum in a dose of 200,000 cells per tube. Enteropathogenic strains of *E. coli* No. 3827 and No. 5789 of the 055:K59 serotype were generously provided by the All-Union Escherichia Center. A study of these strains for their content of type ABO antigens by the adsorption of specific isoagglutinins method [2, 8] showed that strain No. 5789 contains heterologous antigen of the O(H) type and that strain No. 3827 contains no antigens of the ABO type.

To discover the minimal dose of bacterial cells capable of exerting a cytopathogenic action on human cells four series of experiments were performed (the number of bacterial cells was $2 \cdot 10^{10}$, $2 \cdot 10^{5}$, $2 \cdot 10^{4}$, and $2 \cdot 10^{3}$ in 1 ml). On the second day of growth of a culture of HeLa cells, bacteria of strains *E. coli* No. 5789 and No. 3827 were added, and in the control

*A culture of HeLa cells containing the O blood group antigen was used.

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TABLE 1. Statistical Significance of Differences (1 − P)* from Control in Number of Living and Dead Cells of HeLa Culture after Interaction with Strains of *E. coli* Serotype 055:K59 Containing or Not Containing Heterologous Human O(H) Antigen

Experiment No.	Dose of bacterial cells	Strain No. 3827		Strain No. 5789	
		living	dead	living	dead
1	$2 \cdot 10^{10}$	0,997	0,302	1,0	1,0
2		0,430	0,856	1,0	0,997
3		0,659	0,077	1,0	0,997
1		0,299	0,731	1,0	1,0
2		0,433	0,368	1,0	1,0
3	$2 \cdot 10^{5}$	0,227	0,846	1,0	1,0
4		0,076	0,644	1,0	1,0
5		0,077	0,649	1,0	1,0
1		0,365	0,947	1,0	0,997
2		0,759	0,644	1,0	1,0
3	$2 \cdot 10^{4}$	0,227	0,358	1,0	1,0
4		0,151	0,868	1,0	1,0
5		0,531	0,290	1,0	0,942
1		0,368	0,796	0,867	0,990
2		0,152	0,878	0,846	0,368
3	$2 \cdot 10^{3}$	—	—	0,430	0,789
4		0,430	0,878	0,076	0,597
5		0,227	0,433	0,815	0,290
6		0,602	0,227	0,789	0,725

*Results obtained in experiment are considered to be significant if $1 - P \geq 0.950$.

the medium was changed. On the fifth day of growth (the third day of interaction), after staining with 1% trypan blue, the number of living and dead cells was counted in a Goryaev chamber and the coefficient of proliferation and the percentage of dead cells were determined; the significance of differences in the number of living and dead cells (1 − P) was determined by the Student–Fisher method as simplified by Moshkovskii [7].

EXPERIMENTAL RESULTS

In the experiments of series I, after addition of the maximal dose of $2 \cdot 10^{10}$ bacterial cells of strain No. 5789, containing heterologous human O(H) antigen, to the cells of the HeLa culture a marked cytopathogenic effect was obtained (79% of dead cells in the experimental and 4% in the control series). A marked decrease (by 67%) in proliferation of the HeLa cells also was observed (the coefficient of proliferation was 1.0 in the experimental and 3.8 in the control series). Statistical analysis of the data showed that differences between the numbers of living and dead cells in all experiments were significant (Table 1).

After addition of the maximal dose of bacterial cells of strain No. 3827, not containing heterologous antigen, no significant increase in the number of dead cells was found. A significant decrease in the number of living cells was observed in one of the three experiments (Table 1). The coefficient of proliferation in the experimental groups was 3.0 and in the control 3.8. The percentage of dead cells in the experimental groups was 4.6 and in the control 4.0.

In the experiments of series II (mean dose of bacterial cells $2 \cdot 10^{5}$ in 1 ml) strain No. 5789 caused a significant decrease in the number of living cells and an increase in the number of dead cells in all the experiments (Table 1). After interaction with bacterial cells of strain No. 3827 no significant difference from the control for the number of living and dead cells was found. Similar results were obtained when the coefficient of proliferation and percentage of dead cells in the test culture were calculated.

In the experiments of series III the minimal dose ($2 \cdot 10^{4}$) of bacterial cells of strain No. 5789 capable of exerting a cytopathogenic action on human cells in culture was identified. On the addition of *E. coli* strain No. 3827 in this dose to HeLa cells the number of living and dead cells was indistinguishable from the control.

Bacterial cells of strain No. 5789 in a dose of $2 \cdot 10^3$ no longer had a cytopathogenic action on the culture of HeLa cells: No difference was observed between the experimental and control groups as regards the number of living cells. A significant increase in the number of dead cells after the action of strain No. 5789 was observed in only one experiment.

The results thus showed that enteropathogenic strain *E. coli* serotype 055:K59 No. 5789, in which the heterologous O(H) antigen corresponds to the group antigen of the culture of HeLa cells, by contrast with strain *E. coli* serotype 055:K59 No. 3827, which does not contain this antigen, had a marked cytopathogenic action on the test culture. A dose of $2 \cdot 10^4$ bacterial cells for strain No. 5789 proved to be the minimal dose at which it manifested its cytopathogenic action.

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