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CYTOPATHOGENIC ACTION OF STRAINS OF *Escherichia coli* CONTAINING SIMILAR HETEROGENETIC ANTIGENS OF THE AB0 TYPE ON HUMAN CELL CULTURES

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The character of interaction between different strains of Escherichia coli serotype O26 and cells of continuous cultures of human strains HeLa, Tg-33, and RH was studied in vitro. The phenomenon of cytopathogenic action (CPA) of uropathogenic strains of E. coli containing heterogenetic type O(H) and B antigens on human cell strains with the corresponding isoantigens was detected after interaction for 6 h. The number of dead cells in these cultures was 1.5-3 times greater than their number in control cultures to which E. coli cells not containing heterogenetic antigens or containing dissimilar heterogenetic antigens of the human AB0 type were added. It is postulated that this phenomenon plays an important role in the development of chronic forms of colibacillary pyelonephritis.

KEY WORDS: human cell cultures; Escherichia coli; heterogenetic antigens.

In recent years human cell cultures have been used on an increasingly wide scale to determine the virulence of microorganisms. A particularly sensitive model for this purpose is the use of continuous cultures of human cells by means of which the virulence of several enteropathogenic [2, 8, 14] and uropathogenic [11] strains of Escherichia coli has been revealed. These freshly isolated strains, by contrast with reference strains, as a rule had a cytopathogenic action (CPA) and they were often found to be capable of intracellular reproduction. Many features of this interaction still remain unstudied. Nevertheless, the attention of immunologists and microbiologists is increasingly being drawn to the hypothesis that heterogenetic antigens of microorganisms, similar to antigens of human cells, play an essential role in the formation both of their virulence

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TABLE 1. Results of Interaction for 6 h between Cells of Continuous Cultures and E. coli Strains Containing Heterogenetic Antigens of the Human AB0 Type (M ± m)

Series of expts.	Number of expts.	Strain of human cells	Isoantigens of cell cult.	No. of strain of E. coli	Type of hetero-antigen	Mean percentage of dead cells	P
I	4	HeLa K-41	0	26a	0	30,1±4,8	<0,05
II	3		0	15	—	10,0±3,2	<0,5
IV	3		0	—	—	12,9±1,1	—
I	4	HeLa K-1	0	2	0	47,6±3,6	<0,01
II	5		0	28a	—	20,3±2,5	<0,5
IV	4		0	—	—	21,6±2,3	—
I	8	HeLa K-2	0	8	0	45,1±1,1	<0,001
II	8		0	37	—	17,2±2,2	<0,2
III	6		0	206	A	12,9±1,5	<0,5
IV	8		0	—	—	13,2±3,0	—
I	7	RH	0	26a	0	22,1±2,2	<0,01
II	7		0	28a	—	15,0±0,8	<0,2
III	4		0	206	A	16,3±1,2	=0,05
IV	9		0	—	—	13,2±0,8	—
I	3	Tg-33	B	54, 14	B	35,7±5,0	<0,02
II	3		B	28a	—	20,0±0,9	<0,1
IV	3		B	—	—	13,6±0,7	—

Note. Explanation of series of experiments: I) interaction between cell cultures and E. coli containing similar antigen; II) experiments with E. coli not containing heterogenetic antigen of the human AB0 type; III) experiments with E. coli containing dissimilar heterogenetic antigenetic antigen; IV) experiments without addition of E. coli.

and of the low level of the immune response of the host organism [1, 3-6, 12, 13]. Such heterogenetic antigens, similar to human AB0 antigens, have been discovered in many enteropathogenic [4, 13] and uropathogenic [4, 6, 10] strains of E. coli, and accordingly it has been postulated [4, 6] that their presence reflects one manifestation of the "biological mimicry" of the pathogenic agent toward the human body. However, no direct evidence in support of the pathogenic role of these heterogenetic antigens has yet been obtained.

For the reasons given above, in the investigation now to be described a model of interaction between continuous human cell cultures and various strains of E. coli was used to study the possible connection between the cytopathogenic action of those strains and the presence of heterogenetic human type AB0 antigens in them.

EXPERIMENTAL METHOD

Continuous cultures of five strains of human cells were used as the target cells: 3 clones (K-41, K-1, K-2) obtained from strain HeLa (tissue from carcinoma of the cervix uteri), strain Tg-33 (from the tissue of the fallopian tube), and strain RH (from kidney tissue). These cell cultures were grown in Wassermann tubes on medium No. 199 with the addition of 20% bovine serum. To each tube was added $5 \cdot 10^4$ cells in 2 ml medium; on the third day of growth the medium was changed and a suspension of E. coli ($1 \cdot 10^6$ bacterial cells) added. After interaction for 6 h at 37°C the living and dead cells were counted in a Goryaev's chamber, using a 1% solution of trypan blue. Coverslips were placed in some tubes, fixed with Bouin's solution, and then started with Mayer's hematoxylin. The experiments were repeated from 3 to 8 times and the results obtained for the COA were subjected to statistical analysis.

The cell cultures were infected with strains of E. coli* isolated from patients with pyelonephritis. Strains of serotype O26 were selected, in which the specific absorption of antisera method [5] showed the presence or absence of heterogenetic antigens of the AB0 type. The presence of group antigens of the AB0 system was established correspondingly in the cell cultures by methods of mixed agglutination and specific absorption [7].

EXPERIMENTAL RESULTS

In some series of experiments toward the sixth hour of interaction between the strains of E. coli and the cell cultures, a cytopathogenic effect could be seen. Intravital observations and the study of preparations (Fig. 1) revealed marked destruction of the cell monolayer in these series.

Counting the number of dead cells (stained with 1% trypan blue solution), as Table 1 shows, revealed a regular increase (by 1.5-3 times) in mortality of the human cell cultures to which strains of E. coli containing similar heterogenetic antigens had been added. In the series of the experiment in which strains of E. coli containing a dissimilar heterogenetic antigen of the AB0 type or strains not containing such antigens were added, the number of dead cells in the experimental series did not differ significantly from their number in the control cultures, to which no E. coli cells were added.

Clonal cultures of HeLa cells (containing O isoantigen) were particularly sensitive (Table 1, Fig. 1), for after addition of strains of E. coli (Nos. 2, 8, 26a) containing O(H)-like antigen up to 45-47% of dying cells was found (13-21% in control cultures). A somewhat smaller percentage of dead cells (not more than 35) was

*The strains of E. coli were obtained from the No. 1 Central Hospital of the Ministry of Communications.

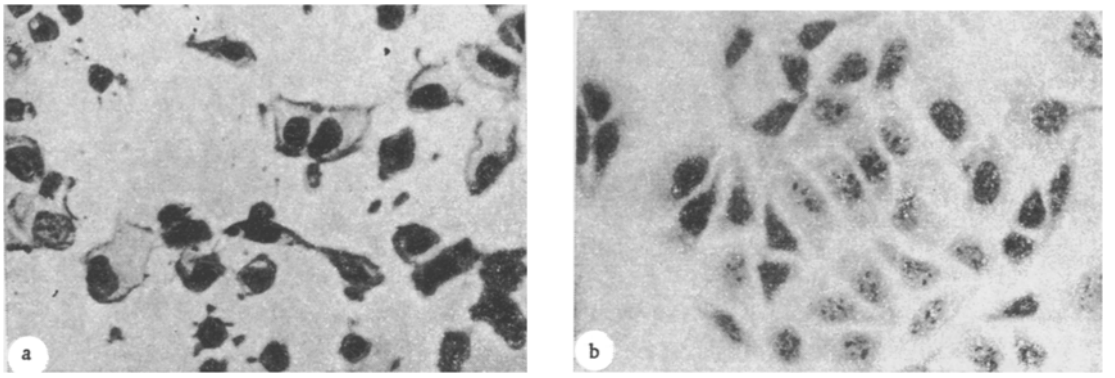

Fig. 1. Human HeLa cells from continuous culture (clone K-1) after interaction for 6 h with E. coli O26: a) cytopathogenic action of E. coli containing similar heterogenetic type O antigen; b) control: action of E. coli strains not containing heterogenetic antigen. Stained with Meyer's hematoxylin, 100 ×.

found in a culture of Tg-33 cells (containing B isoantigen) when infected with E. coli strains (Nos. 14, 54) containing similar type B antigen, but these results also differed significantly from the controls, where not more than 13-20% of cells died. In an RH culture containing O(H) isoantigen the weakest (22%) cytopathogenic effect was observed after addition of an E. coli strain with O(H)-like antigen (No. 26a), but it also was significantly higher than the values observed in the control cultures (13-16%).

The results thus revealed an increase in the CPA of E. coli strains on human cells in continuous culture when the heterogenetic antigen of the microorganism corresponded to the group antigen of the cell culture. The mechanism of this phenomemon remains unexplained. E.coli cells of serotype O26 have been identified in man as frequent causative agents of colitis, enteritis, and pyelonephritis [6, 9], but not all strains of this serotype contain heterogenetic antigens. Meanwhile, our observations show that it is in these cases of correlation between the host's group antigens and the heterogenetic antigens of E. coli that a protracted or chronic course of pyelonephritis is most frequently observed. It can be postulated on the basis of these findings that such a course can be attributed to the more severe local pathogenic action of the agent on the kidney cells coupled with a general weakening of the immune response of the host.

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