CHOLEROGEN AND NEURAMINIDASE PRODUCTION BY CHOLERA VIBRIOS in vitro

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UDC 576.851.315.098.31

A high degree of rank correlation between the synthesis of cholera exotoxin and neuraminidase by cholera vibrios (Vibrio cholerae 569 B) was demonstrated by experiments in vitro, and the appearance of these biologically active substances was shown to depend on the phase of development of the microbial population. It is suggested that they act cooperatively during the development of cholera.

KEY WORDS: cholera exotoxin; neuraminidase of cholera vibrios.

The view is held that the loss of fluid and electrolytes in cholera is due to the action of cholera exotoxin, or cholerogen (CG) [9]. Besides exotoxin, cholera vibrios also produce considerable amounts of neutraminidase (NA) [4], an enzyme which plays an important role in the development of the disease [1].

The dynamics of CG and NA synthesis was investigated during cultivation of Vibrio cholerae 569 B in a liquid nutrient medium.

EXPERIMENTAL METHOD

A suspension of a 24-h culture of V. cholerae 569 B was added to two 3-liter flasks each containing 300 ml of 5% peptone water so that the final concentration of vibrios was $1 \cdot 10^9$ (flask A) and $1 \cdot 10^7$ (flask B) bacterial cells/ml respectively. The original number of living bacterial cells was determined by the serial dilution method and the concentrations of CG and NA were determined in supernatants of samples [4] obtained from each flask. The contents of the flasks were grown with constant shaking for 9 h at 28°C and every 1.5 h 10-ml samples were taken for determination of the number of living bacterial cells and of their content of CG and NA.

Biological activity of CG was determined in albino mice [10] and expressed as active units of action (AA units [2]), assuming that a twofold change in the CG concentration is accompanied by a change of 50 mg in the quantity of edema fluid. Neuraminidase activity was determined from the ability of the supernatant to prevent agglutination of guinea pig red cells by influenza virus [5] and expressed in RD units (reciprocals of dilutions) or by Warren's method [15], using ovomucin as the substrate. Both methods of determination of NA activity gave almost identical results.

Production of GC and NA was estimated from the value of Kendall's general and special rank correlation coefficients [8], accepting the fact, established previously, that CG and NA are different substances [11]. The number of generations of the microbial population was calculated by the usual method [3].

EXPERIMENTAL RESULTS

The dynamics of changes in the concentration of bacterial cells and of CG and NA is shown in Fig. 1, from which it will be seen that with an increase in the time of cultivation all the corresponding indices rose. This is confirmed by the high rank correlation (Table 1). It must, however, be emphasized that statistically significant special rank correlation was found only for NA and CG activity, i.e., correlation calculated with the

Volgograd Plague Institute. Research Laboratory of Experimental Immunobiology, Academy of Medical Sciences of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 5, pp. 566-568, May, 1976. Original article submitted March 12, 1975.

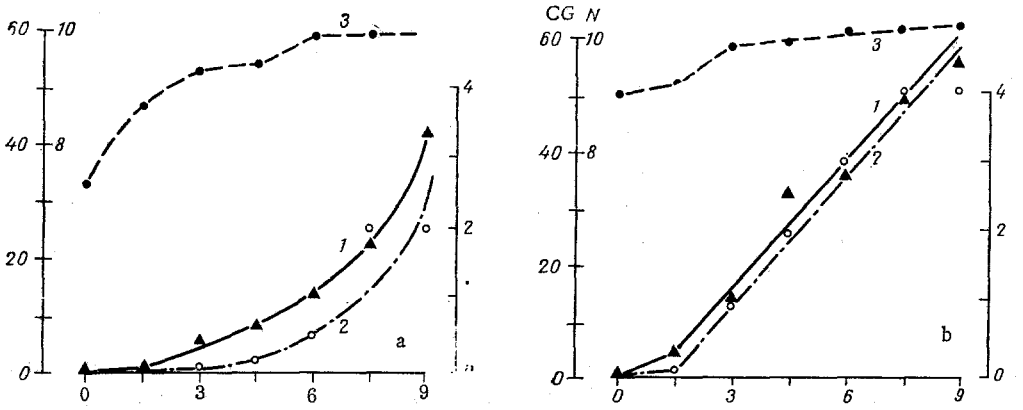

Fig. 1. Dynamics of cholerogenic (1) and neuraminidase (2) activity of supernatants obtained after cultivating V. cholerae 569 B (3) with a seeding dose of $(0.2 \pm 0.04) \cdot 10^8$ (a) and $(12 \pm 1.5) \cdot 10^8$ (b) microbial cells. Ordinate, left: CG) cholerogen activity (in AA units); N) log of number of microbial cells in 1 ml; right, neuraminidase activity (in RD units · 1000); abscissa, time of testing samples (in h).

TABLE 1. Kendall's General and Special Rank Correlation Coefficient (τ) between Biological (CG) and Neuraminidase (NA) Activity of Supernatants and Concentrations of Living Bacterial Cells (N) in Them

Characteristics of supernatants compared	Rank correlation coefficient (M ± m)	
	general	special
CG–NA	+0,96±0,21	+0,68±0,21
CG–N	+0,93±0,21	+0,36±0,21
NA–N	+0,94±0,21	+0,46±0,21

exclusion of the third factor, the concentration of microbial cells. In fact, although cholerogenic activity precedes neuraminidase, excluding the two or three pairs of values that are rather wide of the mark, thereafter there was an almost functional relationship which could be expressed in the statement that on average 80 RD units for flask A and 30 RD units for flask B are equivalent to 1 AA unit.

Correlation between cholerogenic activity and growth of the microbial population was somewhat different. For instance, with a larger seeding dose, after 1.5 h (the first sampling time) the supernatant contained a large quantity of CG: The weight of edema fluid in the limbs of the albino mice (P) was 186 mg (CG was virtually absent from the supernatant obtained immediately after seeding the nutrient medium, P = 14 mg). The concentration of the microbial population rose during this time from $1.2 \cdot 10^9$ to $1.9 \cdot 10^9$ vibrios (0.7 generation). Meanwhile, with a smaller seeding dose (flask B) the concentration of CG remained unchanged during the same time interval (P = 6 and 11 mg), although the concentration of cholera vibrios rose from $2.0 \cdot 10^7$ to $49 \cdot 10^7$ (4.6 generations). Over the next 1.5 h the microbial population rose only to $2 \cdot 10^9$ (2 generations), whereas the CG concentration increased sharply (P = 197 mg). These observations are in agreement with data in the literature [14] indicating the "explosive" appearance of CG in the culture medium once a certain minimal concentration of cholera vibrios was reached (in this experiment $2 \cdot 10^9$).

It can be concluded from these experiments that cholera vibrios (V. cholerae strain 569 B), when cultivated in vitro, once they reach a certain concentration begin to secrete CG and NA particularly intensively into the surrounding medium. Considering that by this time reproduction of the cholera vibrios has slowed down considerably, this feature of CG production must be regarded as a "self-acquired" character [7], by means of which, and through hypersecretion in the small intestine, they can create conditions for the virtually unlimited growth of the microbial population in vivo. Neuraminidase, which destroys the protective mucin of the mucous membrane of the small intestine and increases the content of monosialogangliosides in cell membranes of the mucous membrane, which are receptors for CG fixation [12], considerably increases the chances of direct contact between CG and the brush border of the mucous membrane of the small intestine. Such contact is the first stage of action of CG [13].

These hypotheses are in agreement with the results of an investigation [6] in which the cholerogenic action of nontoxigenic strains of V. cholerae was demonstrated on infantile rabbits as models in which the vibrios were injected simultaneously with NA, which by itself had no pathogenic action on the intestine of the young rabbits.

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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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UDC 576.851.48.06.097.2

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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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 5, pp. 568-570, May, 1976. Original article submitted June 25, 1975.