EFFECT OF SOS OF ENTEROBACTERIA ON THEIR INTERACTION WITH THE COMPLEMENT SYSTEM

V. V. Tets, S. V. Andreev,
A. M. Ishchenko, and V. A. Pasechnik

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One of the most important systems for adaptation of bacteria to changing external environmental conditions is the SOS repair system. It is responsible for changes in population structure ranging from slowing of multiplication and a change in mutation activity to self-destruction of a proportion of the cells. It has been shown that SOS repair has great universality and plasticity, and has several different levels of activation in response to possible influences leading ultimately to the appearance of single-stranded breaks in the DNA molecule [2, 10]. The functions of some genes of the SOS system have not yet been studied. An SOS system is found in various bacteria (of the genera *Escherichia, Salmonella, Streptococcus,* etc.) and its organization is similar [3, 4, 9].

The main genes controlling the SOS response are the recA and lexA genes. The main activator is RecA-protein, a product of the recA gene.

Adaptation of bacteria in the human and animal body is closely linked with changes in activity of the immune defense systems. An important component of the latter is complement. One of the first defense responses of the host to microbial invasion is considered to be activation of proteins of the complement system via an alternative path, which does not require the participation of antibodies, by direct interaction of component C3 with elements of the bacterial surface [5].

The aim of this investigation was to study the role of the recA gene in interaction between enterobacteria and serum complement, during activation of the alternative path.

EXPERIMENTAL METHOD

Strains of *Escherichia* serovar 0124 were used: the original VT 1240, the S-form, virulent in Sereny's tests and in a pulmonary model with intranasal infection of albino mice; nonvirulent — VT 2240 and VT 3251, obtained by genetic recombination. Strain VT 2240 contains the recA56 gene, transmitted to it from *E. coli* K12 JC 10240, as a result of which it virtually does not synthesize the RecA-protein; strain VT 3251 contains the recA441 (tif 1) gene from *E. coli* K12 JM 12, whose RecA-protein is activated constitutively at 42°C. Strains VT 2240 and VT 3251 has an unchanged antigenic structure compared with the original VT 1240, as shown by data of the agglutination test and crossed exhaustion of sera prepared against it. To verify the specificity of effects arising with a change in the recA gene, an avirulent mutant VT 1241, which is a R-form, was used.

TABLE 1. Viability of *E. coli* 0.24 Cells after Treatment with NHS

Strain	Volume of EGTA serum in incubation sample, μl	Number of cells in incubation sample	Number of living cells, % of control
VT 1240	800	$(1,2\pm0,1)\cdot10^8$	$(5,5\pm0,2)\cdot10^{-3}$
VT 1241	800	$(1,5\pm0,3)\cdot10^8$	$3,8\pm0,3$
VT 2240	800	$(1,4\pm0,2)\cdot10^8$	$(1,2\pm0,2)\cdot10^{-3}$
VT 3251	800	$(1,3\pm0,2)\cdot10^8$	59 ± 1

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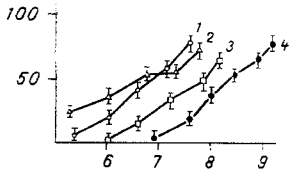

Fig. 1

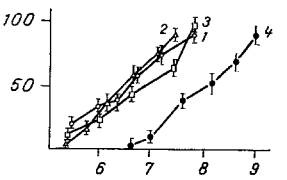

Fig. 2

Fig. 1. Percentage of utilization of alternative path components of complement (AH50) after contact of serum (1 h, 37°C) containing EGTA and bacteria. Abscissa, $\log_{10}$ of cell concentration. 1) VT 1240; 2) VT 2240; 3) VT 3251; 4) VT 1241.

Fig. 2. Percengage utilization of factor B of alternative path of complement (BH50) after contact between serum (1 h, 37°C) containing EGTA and bacteria. Legend as to Fig. 1.

All the bacteria were taken for study in the middle of the exponential growth phase. The cells were treated with complement by mixing the bacterial suspension (from 10^6 to 10^{11} cells) with normal human serum (NHS) and incubating it for 1 h, at 37°C, in the presence of 10 mM EGTA (ethylene-glycol bis-2-aminoethyl ester of N,N'-tetra-acetic acid), 0.1% gelatin, and 1.5 mM $MgCl_2$ in isotonic veronal buffer (pH 7.4). The volume of the samples was constant (1 ml). After incubation the bacteria were sedimented by centrifugation and seeded on nutrient medium to determine the number of surviving cells. Levels of complement (AH50) and factor B (BH50) in the supernatant were determined [1, 8] and the percentage utilized was calculated by the formula:

$$\frac{\text{Initial H50} - \text{H50 after incubation}}{\text{Initial H50}} \times 100\%.$$

The percengage of the component C3 hydrolyzed was determined by measuring the quantity of C3a fragment formed, using the immunoblotting method [6] and monoclonal labeled ^{125}I-anti-C3a-immunoglobulins, specific both for the native C3 molecule and for its small C3a fragment. The C3a was detected by analysis of the autoradiographs on a Ultroscan scanner (LKB, Sweden).

EXPERIMENTAL RESULTS

During activation of the alternative path, complement exhibited marked bacteriocidal activity within the range of ratios of bacteria to serum of $1.5 \cdot 10^8$–$1.9 \cdot 10^8$ cells to 1 ml NHS (Table 1).

It will be clear from Table 1 that the micro-organisms differed in their resistance to complement. Bacteria of strains VT 1240 and VT 2240 were most sensitive, whereas the viability of mutants with an activated SOS system (VT 3251) and of the R-mutants (VT 1241) remained at the levels of 60 and 4%, respectively.

Dependence of the utilization of the total alternative path components (Fig. 1), factor B (Fig. 2), and component C3 (Fig. 3) during interaction of serum complement with the test micro-organisms also was studied. It will be clear from Figs. 1 and 2 that the total complement and factor B of cells of strains VT 1240 and VT 2240, which were most vulnerable to the bacteriocidal action of the serum, were utilized most rapidly. To reduce the content of AH50 and BH50 by 50%, $(3.5\text{-}4.5) \cdot 10^6$ cells were required. To produce the same decrease in these values by bacteria of strains VT 1241 and VT 3251, a much larger number of cells were required (to reduce AH50, $6.4 \cdot 10^7$ and $2.6 \cdot 10^7$, respectively, to reduce BH50 — $1.1 \cdot 10^8$ and $4.3 \cdot 10^6$ cells, respectively).

Thus, the R mutants (VT 1241) were the weakest activators of the alternative path of complement and were intermediate in their resistance to its bacteriocidal activity.

It will be clear from Fig. 3 that interaction with the bacteria led to degradation of C3 (mol. wt. 185 kD), depending on their number, and the formation of a low-molecular-weight C3a fragment (mol. wt. 9.2 kD). Densitometry of the autoradiographs showed that with the same ratio of micro-organisms to serum ($4.0 \cdot 10^8$ cells/ml NHS) the percentage of conversion of C3, induced by bacteria of different strains, differed, namely 14.9 for VT 1240, 8.9 for VT 1241, 4.7 for VT 2240, and 5.8 for VT 3251. Comparison of data on the utilization of com-

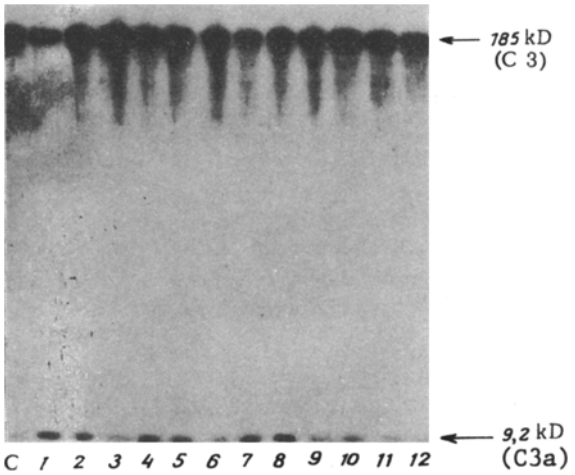

Fig. 3. Autoradiograph of electrophoresis of 1 ml of serum samples after contact with bacteria (after electrophoresis, plate with gel was processed as described in the text). C) Control (native serum); 1-3) serum after contact with $2 \cdot 10^8$, $5 \cdot 10^7$, and 10^7 VT 2240 cells, respectively; 4-6) with $2 \cdot 10^8$, $5 \cdot 10^7$, and 10^7 VT 3251 cells; 7-9) with $2 \cdot 10^8$, $5 \cdot 10^7$, and 10^7 VT1240 cells; 10-12) with $2 \cdot 10^8$, $5 \cdot 10^7$, and 10^7 VT 1241 cells.

plement and its bactericidal activity showed that virtually the same fall in the BH50 level was produced, in the case of the wild-type strain (VT 1240) by 3 times more degradation of C3 than with the recA56 and recA441 mutants. This degree of activation of C3 was sufficient to cause death of bacteria of the first two strains, but it has no bactericidal action on mutants with an activated SOS repair system. The mechanism of defense of recA441 mutants against attack by complement is not clear. However, it can be tentatively suggested that changes in the composition of the bacterial membrane caused by mutation of the recA gene prevents assembly of the membrane-attacking complex. This hypothesis was confirmed by experimental data on the quantity of sialic acids, which was $1.4 \cdot 10^{-2}$ μmole/10^9 cells in the case of strain VT 3251, which is an order of magnitude higher than for R-mutants ($1.7 \cdot 10^{-3}$) and 40-60 times higher than for strains VT 2240 and VT 1240 ($2.2 \cdot 10^{-4}$ and $3.8 \cdot 10^{-4}$, respectively). We know that sialic acids potentiate the action of the inhibitor I of complement as a result of an increase of the affinity of the H cofactor for membrane-bound C3b, which leads to the formation of an inactivated C3b molecule, unable to initiate the formation of the membrane-attacking complex (C3b-9) [7].

Changes in the recA gene thus affects relations between bacteria and the complement system. It has been shown in the case of invasive escherichias of the 0124 serovar and some of its mutants that activation of SOS repair increases the resistance of micro-organisms to the action of serum complement. The mechanism responsible for this resistance is not clear. It can be tentatively suggested that realization of the activity of the membrane-attacking complex is impeded through changes on the surface of the bacterial cell.

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