analog, it suppressed spontaneous CL in variant 3 (but not in the presence of DMSO).

Hence, some of the immunodominant fragments of the surface HIV glycoproteins were found to disturb the normal CL kinetics of neutrophils, which is known to reflect their production of active oxygen derivatives - significant factors of the organism's nonspecific protection. The data obtained attest to the need for a careful selection of peptides suitable for the creation of vaccine preparations with the aim of preventing possible side effects on neutrophilic function, including the side effects which may occur during DMSO therapy. Furthermore, the effects observed with some peptides suggest that the detected disturbances of CL response and superoxide production [3,4] in neutrophils of AIDS patients may be the result of a direct influence of HIV glycoproteins on phagocytosing cells.

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EXPERIMENTAL GENETICS

Peculiarities of Expression of the Plasmid pAP42 Genetic Region Determining "Sex" Pili Synthesis and the Surface Exclusion System in Different Cells of *Escherichia coli*

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The functional specificity of "sex" pili synthesized by bacteria under the genetic control of plasmids, and of the surface exclusion systems (Sfx systems) determines the efficiency of conjugational transfer of F-like plasmids between cells of different bacterial populations [3,4,6,9,10]. Meanwhile, the patterns of expression of the genetic region represented in the genomes of different plasmids and allowing for the formation of a distinct functional type of pili and group affiliation of the Sfx system in different bacterial cells, e.g., serologically nontyped and typed variants, require scrutinized study.

The goal of this study was an analysis of the expression of this region as a constituent of the F-

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TABLE 1. Relative Inoculation Efficiency (RIE) of F−Pili−Specific Phages in Serologically Typed *E. coli* Cells Carrying Transposon−Containing pAP42 Plasmid Variants

Host strain	Plasmid	RIE of phages,%		
		f_1	f_2	Q_β
C600	pAP42::Tn5	100	100	100
(K−12)	pAP42::Tn9	100	100	100
AP15−2	pAP42::Tn5	124±4	96±4	113±7
(0106)	pAP42::Tn9	84±6	56±7	83±4
AP58−2	pAP42::Tn5	67±4	31±3	(13±0.9) × 10^{-1}
(0147)	pAP42::Tn9	37±2	18±2	(14±1) × 10^{-1}
AP70−3	pAP42::Tn5	8±0.6	(13±0.6) × 10^{-1}	(18±3) × 10^{-1}
(0128)	pAP42::Tn9	8±0.9	(14±2) × 10^{-1}	(27±2) × 10^{-1}

like plasmid (factor of genetic transfer) pAP42, belonging to group Sfx V [5], in the cells of serologically nontyped and typed *E. coli* strains, and possible ways of inducing mutations.

MATERIALS AND METHODS

C600 Str and AP132 Nal variants were derived from the serologically nontyped *E. coli* strain K-12. Serologically typed *E. coli* strains AP15-2 (Str), AP15-3 (Nal), AP58-2 (Str), AP58-3 (Nal), AP70 (Str), and AP70-3 (Nal) expressing, respectively, 0106, 0147, and 0128 antigens, were selected by us earlier. Transposon-containing variants of transfer-derepressed factor pAP42 (pAP42::Tn5 and pAP42::Tn9) served as plasmids. The capacity of plasmid-containing cells for forming "sex" pili was judged by their sensitivity to three different F-pili-specific phages (f_1, f_2 and Q_β) assayed by the method of agar layers [2]. The relative efficiency of phage inoculation (RIE) was defined as the percent ratio of the mean number of infection foci on the monolayer of serotyped strains to the mean number of foci developed on the monolayer of the cells of the K-12 strains. The results of repeated experiments were statistically analyzed. Conjugation-mediated plasmid transfer was performed via standard conjugations of plasmid donors with corresponding recipient cells [2]. The surface exclusion index (SEI) was calculated as the ratio of the number of plasmid transconjugates revealed using a plasmid-free recipient strain to the number of conjugates obtained by crossing with an isogenous, plasmid-bearing strain.

TABLE 2. Surface Exclusion Index (SEI) of Transposon−Containing pAP42 Plasmid Variants in Serologically Nontyped (K−12) and Typed *E. coli* Cells

Donor strain	Donor cell plasmid (transferred)	Recipient strain	Recipient plasmid (resident)	SEI
C600(K−12)	pAP42::Tn5	AP132(K−12)	pAP42::Tn9	(0.8−1.1)x10^2
C600(K−12)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5	(0.8−4.5)x10^2
AP132(K−12)	pAP42::Tn5	C600(K−12)	pAP42::Tn9	(0.7−1.0)×10^2
AP132(K−12)	pAP42::Tn9	C600(K−12)	pAP42::Tn5	(1.4−9.5)×10^2
C600(K−12)	pAP42::Tn9	AP15−3(0106)	pAP42::Tn5	(1.5−2.3)×10^1
AP132(K−12)	pAP42::Tn9	AP15−2(0106)	pAP42::Tn5	(1.7−1.8)×10^1
AP15−2(0106)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5	(1.9−6.6)×10^2
AP15−2(0106)	pAP42::Tn9	AP15−3(0106)	pAP42::Tn5	(1.0−3.0)×10^1
C600(K−12)	pAP42::Tn5	AP58−3(0147)	pAP42::Tn9	6.1−6.5
C600(K−12)	pAP42::Tn9	AP58−3(0147)	pAP42::Tn5	5.9−6.3
AP132(K−12)	pAP42::Tn5	AP58−2(0147)	pAP42::Tn9	3.5−6.2
AP132(K−12)	pAP42::Tn9	AP56−2(0147)	pAP42::Tn5	0.8−2.2
AP58−2(0147)	pAP42::Tn5	AP132(K−12)	pAP42::Tn9	(6.0−9.3)×10^2
AP58−2(0147)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5	(4.7−6.1)×10^3
AP58−2(0147)	pAP42::Tn5	AP58−3(0147)	pAP42::Tn9	2.7−6.3
AP58−2(0147)	pAP42::Tn9	AP58−3(0147)	pAP42::Tn5	5.5−6.2
C600(K−12)	pAP42::Tn5	AP70−3(0128)	pAP42::Tn9	(2.7−4.9)×10^2
C600(K−12)	pAP42::Tn9	AP70−3(0128)	pAP42::Tn5	(0.8−1.3)×10^3
AP70−2(0128)	pAP42::Tn5	AP132(K−12)	pAP42::Tn9	(6.6−7.6)×10^1
AP70−2(0128)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5	(2.3−2.9)×10^2
AP70−2(0128)	pAP42::Tn5	AP70−3(0128)	pAP42::Tn9	(7.5−7.7)×10^1
AP70−2(0128)	pAP42::Tn9	AP70−3(0128)	pAP42::Tn5	(5.9−6.1)×10^1

TABLE 3. Surface Exclusion Index (SEI) of pAP42::Tn9 and Mutant pAP42::Tn5 Plasmids in *E. coli* Cells of Different Strains

Donor strain	Donor cell plasmid (transferred)	Recipient strain	Recipient plasmid (resident)	SEI
AP132(K−12)	pAP42::Tn5−16	C600(K−12)	pAP42::Tn9	1.1−3.1
C600(K−12)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5−16	0.1−0.2
AP132(K−12)	pAP42::Tn5−40	C600(K−12)	pAP42::Tn9	(1.8−5.0) × 10^1
C600(K−12)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5−40	0.3−1.1
AP132(K−12)	pAP42::Tn5−61	C600(K−12)	pAP42::Tn9	.7−8.0
C600(K−12)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5−61	0.6−3.5
AP132(K−12)	pAP42::Tn5−64	C600(K−12)	pAP42::Tn9	(0.4−1.1) × 10^2
C600(K−12)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5−64	0.1−0.7
AP132(K−12)	pAP42::Tn5−66	C600(K−12)	pAP42::Tn9	1.7−10.0
C600(K−12)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5−66	0.1−3.1
AP132(K−12)	pAP42::Tn5−88	C600(K−12)	pAP42::Tn9	(1.9−7.0) × 10^1
C600(K−12)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5−88	0.1−2.7
AP132(K−12)	pAP42::Tn5−99	C600(K−12)	pAP42::Tn9	8.0−11.0
C600(K−12)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5−99	0.1−3.7
AP132(K−12)	pAP42::Tn5−117	C600(K−12)	pAP42::Tn9	(2.0−8.0) × 10^1
C600(K−12)	pAP42::Tn9	AP132(K−12)	pAP42::Tn5−117	(1.1−2.3) × 10^1

The bacteria were treated with a chemical mutagen (nitrosoguanidine) by the routine method [1]. Plasmid DNA separation and restriction analysis were carried out using conventional procedures [7,8].

RESULTS

Keeping in mind that "sex" pili synthesized in bacterial cells under the genetic control of various F-like plasmids can be functionally divided into subtypes on the basis of their similarity (dissimilarity) in the degree of cell sensitivity to F-pili-specific phages f_1, f_2 and Q_β [10], we undertook a study of serologically nontyped (K-12) and typed *E. coli* strains vis-a-vis their sensitivity to the mentioned phages. The results are listed in Table 1. It can be seen that the expression of the plasmid region determining the functional specificity of "sex" pili is strongly influenced by the properties of the host cell. For instance, cells of strain AP15-2 (serogroup 0106), containing F-like derepressed plasmids pAP42::Tn5 and pAP42::Tn9, are characterized by pili which ensure almost equal levels of sensitivity to all three pili-specific phages as compared to the sensitivity of corresponding cells of the serologically untyped strain C600 (K-12). On the other hand, strain AP58-2 (0147) cells support significantly reduced RIE of Q_β phage, and AP70-3 (0128) cells produce decreased RIE of all three phages, especially f_2 and Q_β. This reduction is probably a result of alteration of the type of functional specificity of the pili.

For a study of the mutual surface exclusion of pAP42::Tn5 and pAP42::Tn9, experiments on direct and back crossing were performed, so that each plasmid was alternately in the resident or transferred position. As can be seen in Table 2, the values of SEI vary for different cell types. It is plausible to assume that the given plasmids lose the mutual exclusion capacity in the cells of strains AP58-2 and

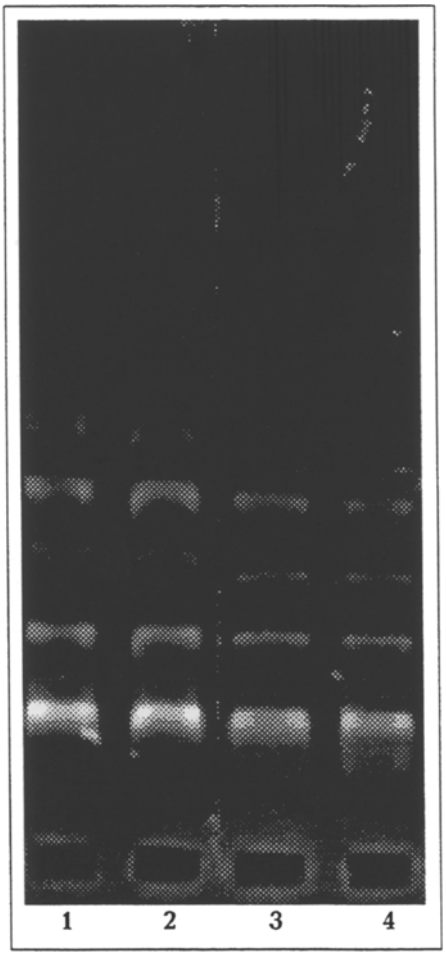

Fig. 1. Electrophoregram of pAP42::Tn5 plasmid DNA and mutation variants after restriction with EcoRI and HindIII.1) pAP42::Tn5, EcoRI; 2) pAP42::Tn5−16, EcoRI; 3) pAP42::Tn5, HindIII; 4) pAP42::Tn5−40, HindIII.

AP58-3 (serogroup 0147), however, this capacity is retained in the cells of other serotyped strains, although sometimes it may be quantitatively expressed more weakly than in the nontyped K-12 strain cells.

In the final experiments we attempted to obtain pAP42 plasmid mutants with altered functional specificity of the genetic region responsible for the specificity of the synthesized pili and Sfx system. For this purpose, *E. coli* AP132 cells containing pAP42::Tn5 plasmid were treated with nitrosoguanidine, and the established clonalcultures were tested with regard to the sensitivity to the three mentioned pili-specific phages and with regard to the mutual exclusion of cell plasmids. Four clones with altered phage sensitivity were selected. The cells of three clones expressed reduced sensitivity to all phages, 20-100 times less than cells of the isogenous strain containing the unaltered pAP42::Tn5 plasmid. The fourth clone was characterized by a reduced sensitivity to Q_β phage and resistance to the other two phages. Thus, the cells contained the mutant plasmid variants.

As was expected, mutant plasmids were less frequently transferred to recipients of different *E. coli* strains during the process of conjugation than was the "wild-type" (unchanged) pAP42::Tn5 plasmid. All transconjugate clones retained the altered pattern of phage sensitivity.

Surface exclusion testing also revealed mutant forms of pAP42::Tn5 plasmid with altered Sfx parameter. The corresponding data are presented in Table 3. All the mutants can be divided into three groups. The first group (pAP42::Tn5-16, pAP42::Tn5-61, pAP42::Tn5-66, and pAP42::Tn5-99 plasmids) is characterized by total loss of the capacity for the specific surface exclusion, the second group (pAP42::Tn5-40, pAP42::Tn5-64, and pAP42::Tn5-88 plasmids) manifests partial preservation of the Sfx feature, in one transfer direction, and the third group (pAP42::Tn5-117) partially retains this feature in both transfer directions. The described pattern of mutant plasmid behavior did not change following the transfer of the plasmids to the cells of other K-12 strains.

Since parallel experiments with initial (pAP42::Tn5) and mutant plasmids, including DNA separation and electrophoretic investigation of EcoRI and HindIII restriction fragments, failed to reveal any difference between the plasmids (Fig. 1), it may be assumed that the formation of the given mutants is a result not of structural rearrangements of the plasmid genome, but rather of gene mutation in the corresponding region of pAP42::Tn5 plasmid.

Summarizing the data, we can conclude that the functional specificity of F-like plasmid "sex" pili and the surface exclusion system can change both under the influence of the host cell genome and as a result of mutations in the plasmid genome. Such changes probably serve as one of the main factors affecting the efficiency of various plasmids distributed in natural bacterial populations.

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