Identification of the Major Adherence Ligand of *Klebsiella pneumoniae* in the Receptor for Coliphage T7 and Alteration of *Klebsiella* Adherence Properties by Lysogenic Conversion

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We have studied the adherence of both laboratory and wild-type *Klebsiella pneumoniae* strains, isolated from sputum, urine, and stool samples, to human buccal and intestinal and urinary tract epithelial cells. Of 32 unencapsulated strains, 30 adhered to all epithelial cells tested. Four *K. pneumoniae* strains lysogenic for AP3, a phage which causes conversion to resistance to coliphages T3, T7, and ϕ I, were all unable to adhere to epithelial cells. One of these strains was cured from phage infection and became capable of adhering. Spontaneous mutants resistant to coliphage T7, as well as *K. pneumoniae* K59-sensitive cells preadsorbed with inactivated T7 particles, did not adhere to epithelial cells. All strains capable of adhering were able to adsorb coliphage T7 and T3, whereas all nonadhesive strains were not. AP3-like prophages were induced from 7 of 12 nonadhesive *Klebsiella* strains. A laboratory strain which was able to adhere was lysogenized with 2 of these phages. In both cases, the strain lost its ability to adsorb coliphages T3, T7, and ϕ I and to adhere to human epithelial cells. All *K. pneumoniae* adhesive strains agglutinated yeast cells, whereas the nonadhesive strains did not. Competition studies have shown that D-mannose and concanavalin A prevented adherence to human epithelial cells, yeast agglutination, and adsorption of coliphage T7 to *K. pneumoniae* cells. It is concluded that in *K. pneumoniae* adherence to epithelial cells is mediated by the receptor for coliphages T7 (and T3), which in turn recognizes D-mannose in the receptors it binds.

Klebsiella pneumoniae is an important gram-negative pathogen which has the interesting properties of colonizing both the intestine and upper respiratory tract in humans and being responsible for frequent infections of both the urinary and respiratory tracts (4, 7, 10, 16). This suggests that *K. pneumoniae* may show unique adherence properties with respect to other gram-negative strains which colonize either only the lower intestine or respiratory tract. However, adherence of *K. pneumoniae* strains to various human epithelial cells has not been studied (3, 13b), and most of the published work on the pathogenicity of *K. pneumoniae* has dealt with capsules and capsulated strains (4, 7, 10, 16).

In our clinical bacteriology laboratory, we have noticed that a significant number of *K. pneumoniae* strains isolated from both urinary and respiratory tract infections are not capsulated. Furthermore, we have recently found laboratory strains of *K. pneumoniae* sensitive to the coliphages P1, T3, T7, and ϕ I and have described a case of lysogenic conversion in *K. pneumoniae* (32) showing that the *Klebsiella* phages FR2 and AP3 (33) convert *K. pneumo-*

niae K59 and *K. pneumoniae* MirA12 to resistance to phages P1 and T3, and T7 and ϕ I, respectively.

In bacteria, phage receptors can be considered to mediate recognition and interaction between different organisms (11). F and R pili, which serve both as the adsorption sites for male specific bacteriophages and as conjugation bridges (5, 19, 21, 27), can be considered typical examples of bacterial organelles mediating interactions with viruses and other cells. Thus, we thought it possible that other phage receptors could also mediate interactions between bacteria and human epithelial cells.

We studied the adherence of *K. pneumoniae* to human epithelial cells and, in this paper, report the influence of lysogenization by phages AP3 and FR2 and the role of bacteriophage receptors in the adherence of *K. pneumoniae* strains.

It was found that *K. pneumoniae* K59 cells converted to resistance to coliphages T3, T7, and ϕ I by phage AP3 (but not those converted to resistance to coliphage P1 by phage FR2) lost the property of adhering to human epithelial

cells. Furthermore, in *K. pneumoniae* K59, as well as in all other *K. pneumoniae* strains tested, adherence to human epithelial cells was mediated by the T3 and T7 coliphage receptor.

MATERIALS AND METHODS

Strains. The *K. pneumoniae* strains used are listed in Table 1. Absence of capsule, in apparently unencapsulated colonies grown in Worfel Ferguson broth (Difco Laboratories), was confirmed by reacting cells with anti-*Klebsiella* capsule sera and observing the development of a quellung reaction. *Candida albicans* and *Saccharomyces cerevisiae* cells, belonging to the collection of this institute, were also employed.

Klebsiella phages FR2 and AP3 were isolated from *K. pneumoniae* MirM7 (23) and have previously been described (29-33). Bacteriophages V27 and V29 were induced from strains K27 and K29 by mitomycin C treatment (26) and purified by a single plaque isolation on K59 strain; they have the same plaque morphology and host range as FR2 and AP3 (33; E. Debbia et al., Abstr. IV Int. Congr. Virol., p. 386, 1978; G. Satta and R. Fontana, Atti I° Congr. Nazion. Virol., p. 323-335, 1971). Coliphages T3 and T7 were purchased from the American Type Culture Collection. Coliphage ϕ I belongs to our laboratory collection.

Media. Brain heart infusion broth and brain heart infusion agar (Difco) were employed throughout this study. For all adherence experiments, KPB buffer [5.3% K_2HPO_4 , 2.2% KH_2PO_4 , 0.5% $(NH_4)_2SO_4$, and 0.25% sodium citrate (pH 7.2)] was used.

Adherence experiments. Buccal and urinary uroepithelial cells were prepared as described by Ofek et al. (25) and Svanborg Edén and Hansson (37), respectively. The technique of Nagy et al. (24) was employed to prepare intestine epithelial cells from pieces of intestine obtained from humans who had undergone intestinal surgery. Cell adhesion experiments were performed as previously described (25). Adherence inhibition tests with sugars and lectins were performed by first adding to the epithelial cells the substances to be tested (sugars, final concentration of 20 mg/ml; lectins, final concentration of 1 mg/ml), and then adding the bacterial cells. In all cases, inhibition was calculated as the percentage of adherence in the presence of either sugars or lectins, as compared with an untreated control.

For adherence inhibition tests with viral particles, phages killed by a 5-min exposure to a standard 15-W ultraviolet (UV) lamp were added to sensitive bacteria at a multiplicity of 100 particles per cell. After 15 min of incubation, free phages were eliminated by spinning down the cell suspension, and the resulting pellet containing bacteria with adsorbed viruses was resuspended and added to epithelial cells for the adherence test.

Isolation of phage-resistant mutants. To select strains resistant to phage T7, 0.3-ml samples containing ca. 10^8 bacteria were inoculated into 3 ml of soft agar containing a total of 10^{10} T7 phage particles, which was then plated on brain heart infusion agar plates and incubated at 37°C for 48 h. Unencapsulated colonies which appeared after incubation were then purified from contaminating phages by several single-

colony isolations and spot tested for phage resistance. The resistant colonies were tested for inability to adsorb phage T7 and transferred onto slants for further studies.

Isolation of strains unable to adsorb coliphage T7. Two methods were used. In the first method, cells

TABLE 1. *Bacterial strains*

<i>K. pneumoniae</i> strain	Source and characteristics
K59	This institute's collection; sensitive to <i>Klebsiella</i> phages FR2 and AP3 and to coliphages P1, T3, T7, and ϕ I (32, 33)
K59(FR2)	K59 derivative lysogenic for <i>Klebsiella</i> phage FR2; resistant to coliphage P1 (32, 33)
K59(AP3)	K59 derivative lysogenic for <i>Klebsiella</i> phage AP3; resistant to coliphages T3, T7, and ϕ I (32, 33)
KRTT 1 KRTT 2 KRTT 3 KRTT 4	Unencapsulated K59 derivatives selected for resistance to T7 coliphage; resistant also to coliphage T3 (this paper)
MirSa	Laboratory strain; sensitive to both <i>Klebsiella</i> phages FR2 and AP3 and coliphages T3, T7, and P1 (32, 33)
K25, K26	Isolated from urine in the Clinical Bacteriology Laboratory of this institute; unencapsulated and sensitive to <i>Klebsiella</i> phages FR2 and AP3 and to coliphages T3, T7, and P1
K27, K29	Isolated from urine; lysogenic and unencapsulated, unable to adsorb T3 and T7 coliphages
K31, K135	Isolated from urine in the Clinical Bacteriology Laboratory of this institute; unencapsulated, resistant to coliphages T3 and T7 but able to adsorb them
K111, K152	Isolated from sputum in the Clinical Bacteriology Laboratory of this institute; unencapsulated, resistant to coliphages T3 and T7 but able to adsorb them
K120	Isolated from feces in the Clinical Bacteriology Laboratory of this institute; unencapsulated, resistant to coliphages T3 and T7 but able to adsorb them.
K25r, K26r	Derivatives of strains K25 and K26, respectively; selected for resistance to phage T7 (this paper)
K31r K111r K120r K135r K152r	Derivatives of strains K31, K111, K120, K135, and K152, respectively; selected for resistance to coliphage T7 (this paper)
Wild type	Isolated from clinical specimens in the Clinical Bacteriology Laboratory of this institute; 40 were from sputum, 40 from urine, 38 from feces, and 11 from bile; unencapsulated

were mutagenized as described previously (2), grown overnight in brain heart infusion broth, and transferred to fresh medium and infected with coliphage T7 at a multiplicity of 20 viral particles per cell. Under these conditions, the majority (90%) of cells, although unable to support plaque formation by phage T7, were killed. After a 60-min incubation, cells were plated on brain heart infusion agar plates previously inoculated with a total of 1×10^{10} T7 viral particles. Colonies formed after a 36-h incubation period were purified from viral infection as described above. After purification, a total of 60 colonies was tested for coliphage T7 adsorption and plaque formation. Of 110 colonies tested, 3 strains were found (K31, K111r, and K120r) unable to support the growth of and adsorb coliphage T7. These strains were also unable to support the growth of and adsorb coliphage T3.

By the second method, cells mutagenized and grown as described above were plated on brain heart infusion agar. After overnight incubation, colonies were replica plated on identical plates containing 10^{10} T7 particles. Colonies which grew on the master plates, but poorly or not at all on the replicas, were purified and further examined for coliphage T7 growth and plaque formation. Of 120 colonies tested, 2 were found which supported phage growth and plaque formation. From these strains, derivatives resistant to coliphage T7 were selected (K135r, K152r). All resistant derivatives were also unable to adsorb coliphage T7.

Other methods. Experiments for phage adsorption to bacteria, prophage induction, lysogenization, and curing from prophages were performed as previously described (1, 33). Phage growth was assayed in terms of viral plaque-forming ability by using the top-layer agar method (1).

RESULTS

Adherence of *K. pneumoniae* to human epithelial cells. Table 2 shows that the laboratory strain K59 and 30 of 32 tested additional wild-type strains, recently isolated from clinical specimens, adhered to human epithelial cells obtained from the cheek, the intestine, and the urinary tract. The buccal epithelial cells appeared to be those which bound the most bacteria per cell, whereas the intestinal epithelial cells were those which bound the fewest. It is also interesting to note that the apparent affinity of klebsiellae for the three different epithelial cells did not depend on the type of human mucosa they had originally infected before isolation. In fact, although some strains were isolated from sputum, urine, or stool samples, virtually all strains adhered much more effectively to cells of the oral cavity.

Effect of lysogenic conversion by phages FR2 and AP3 on adherence of *K. pneumoniae* K59 to epithelial cells. Table 3 shows that K59 cells lysogenic for *Klebsiella* phage AP3 were essentially unable to adhere to human epithelial cells. On the contrary, no significant differences were observed in the adherence ca-

pability of strains K59 and K59(FR2). Another laboratory strain (MirSa) and two other wild-type *K. pneumoniae* strains (K25, K26) sensitive to phages FR2 and AP3 were then made lysogenic for each of the two phages.

Again, the three strains lysogenic for FR2 exhibited the same degree of adherence to epithelial cells as the parental strains. However, the strains lysogenic for AP3 almost completely lost their adherence capability. Finally, two K59(AP3) strains were cured from the lysogenizing phage. After curing, they reacquired an adherence capability virtually identical to that of the K59 parent strain.

Role of coliphage T7 receptors in adherence of *K. pneumoniae* strains to epithelial cells. Since we have previously shown that lysogenization by phage AP3 causes repression of coliphages T3, T7, and ϕ I receptors, the above findings suggest the possibility that bacterial receptors for these coliphages could also be the bacterial ligands responsible for adherence to human epithelial cells.

TABLE 2. Adherence to various epithelial cells of the laboratory strain *K. pneumoniae* K59 and 32 different wild-type *K. pneumoniae* strains isolated from clinical specimens

Origin and no. of isolates	Range of the no. of bacteria adhering per epithelial cell of ^a :		
	Oral cavity ^b	Intestine	Urinary tract
Laboratory strain K59	180	75	85
Wild-type strain			
Urine 4	100-195	56-80	53-82
Feces 3			
Sputum 2			
Urine 4	100-186	20-49	50-83
Feces 2			
Sputum 4			
Urine 3	52-83	20-47	52-87
Sputum 1			
Urine 3	50-85	22-45	20-49
Feces 2			
Sputum 1			
Bile 1			
Urine 1	5-15	5-18	3-13
Feces 1			

^a The values reported here correspond to the highest and lowest average of bacteria adhering to one epithelial cell in each bacterial group. The average was calculated by counting the bacteria adhering to 20 epithelial cells.

^b Cheek epithelial cells were used.

TABLE 3. Effect of lysogenization by (and curing from) phages FR2 and AP3 on adherence of different *K. pneumoniae* strains to human epithelial cells

Strain	Effect as shown by the mean no. of bacteria adhering to one epithelial cell of ^a :		
	Oral cavity ^b	Intestine	Urinary tract
K59	180 ± 39 ^c	75 ± 18 ^c	75 ± 15 ^c
K59(FR2)	170 ± 11	69 ± 11	70 ± 14
K59(AP3)	15 ± 4	18 ± 5	10 ± 4
K59-1 cured from AP3	179 ± 21	42 ± 11	81 ± 20
K59-2 cured from AP3	181 ± 39	39 ± 5	72 ± 16
MirSa	185 ± 37	51 ± 17	60 ± 12
MirSa(FR2)	171 ± 12	40 ± 6	55 ± 7
MirSa(AP3)	22 ± 12	5 ± 3	5 ± 3
K25	167 ± 27	66 ± 7	59 ± 12
K25(FR2)	169 ± 19	51 ± 11	57 ± 14
K25(AP3)	15 ± 5	10 ± 6	9 ± 5
K26	159 ± 23	60 ± 17	49 ± 10
K26(FR2)	145 ± 17	50 ± 10	61 ± 14
K26(AP3)	21 ± 7	9 ± 5	11 ± 6

^a In all cases the mean was calculated by counting the number of bacteria adhering to 40 epithelial cells.

^b Cheek epithelial cells were used.

^c Mean ± standard deviation.

To test this possibility, we evaluated adherence to human epithelial cells of four K59 spontaneous mutants (KRTT 1 to 4), selected as resistant to infection by coliphages T7 and unable to adsorb it, and of K59 cells previously adsorbed with UV-inactivated T7 particles. It is known that coliphages T3, T7, and ϕ I are closely related and most likely share a common bacterial receptor (22). In our work we have employed primarily one of these coliphages, T7, to test for the presence of the receptor and to study its role in adherence. Table 4 and Fig. 1 show that the K59 spontaneous mutants unable to adsorb coliphage T7, as well as K59 cells preadsorbed with UV-inactivated T7 phage, did not adhere to the different human epithelial cells. It should be noted that K59 cells with adsorbed UV-inactivated particles of FR2, a phage similar to T7 in shape and dimension but binding to bacterial receptors different from those binding T7, did not have altered adherence properties. In contrast, UV-inactivated particles of coliphage T3 almost completely prevented adherence of K59. Since it is known that bacteriophages recognize and bind to specific receptors only, the fact that the K59 derivatives unable to adsorb coliphage T7 were spontaneous mutants most likely differing from the parental strain for an alteration in or lack of a T7 receptor clearly suggests that in *K. pneumoniae* K59 the same bacterial structure

TABLE 4. Adherence to epithelial cells of both K59 derivatives unable to adsorb coliphage T7, and K59 cells preadsorbed with UV-killed coliphages T7 and T3

Strain	Mean no. of bacteria adhering to an epithelial cell of ^a :		
	Oral cavity ^b	Intestine	Urinary tract
KRTT 1 ^c	19 ± 9 ^d	10 ± 7 ^d	12 ± 10 ^d
KRTT 2 ^c	20 ± 16	7 ± 6	10 ± 9
KRTT 3 ^c	20 ± 19	9 ± 8	13 ± 11
KRTT 4 ^c	19 ± 17	5 ± 4	15 ± 5
K59 + T7	15 ± 11	11 ± 10	10 ± 9
K59 + T3	14 ± 13	8 ± 5	9 ± 6
K59 + FR2	178 ± 40	64 ± 15	74 ± 20
K59	184 ± 41	72 ± 17	78 ± 18

^a In all cases, the mean was calculated by counting the number of bacteria adhering to 40 epithelial cells.

^b Cheek epithelial cells were used.

^c KRTT strains were all resistant and unable to adsorb T7 coliphage.

^d Mean ± standard deviation.

mediates both phage adsorption and adherence to human epithelial cells.

To see whether adherence to human epithelial cells in wild-type *K. pneumoniae* could also be mediated by coliphage T7 receptors, we analyzed the sensitivity of the adhesive strains described in Table 2 to this virus. Of 30 strains, 18 were unable to support growth of and allow plaque formation by coliphage T7. Furthermore, only 7 of an additional 98 unencapsulated adhesive *K. pneumoniae* strains isolated from various clinical specimens (including stool, sputum, and urine) supported growth of and allowed plaque formation by coliphage T7. However, all of the wild-type adhesive strains (but none of the nonadhesive ones) isolated in this study adsorbed coliphage T7 (Table 5) at least as efficiently as K59 cells, thus showing that all adhesive strains carried receptors for coliphage T7 in spite of their frequent incapability to support plaque formation. A coliphage receptor role in adherence to epithelial cells in these strains is supported by the fact that three such strains were unable to adhere to epithelial cells after previously adsorbing UV-killed T3 or T7 phages (data not shown). Furthermore, we derived, from five different wild-type *Klebsiella pneumoniae* strains capable of adsorbing T7 but unable to support plaque formation, five mutants unable to adsorb T7 and found that these strains had also become unable to adhere to human epithelial cells (Table 6).

Adherence inability and lysogenization by AP3-like phages among wild-type *K. pneumoniae*. To see whether lysogenic conver-

FIG. 1. Binding of *K. pneumoniae* K59 (a) and KRTT1 (b) to human oral epithelial cells; bar, 15 μ m.

sion could be an important factor in determining adhesive properties of wild-type *K. pneumoniae* we evaluated the frequency of lysogenization of nonadhesive *K. pneumoniae* by inducible

phages. Viruses showing the same host range and plaque morphology as AP3 could be induced in 7 of the 12 strains tested. K59 cells lysogenized by the viruses induced from two strains lost the

TABLE 5. Adherence to oral epithelial cells and capability of supporting growth and adsorbing coliphage T7 of 140 *K. pneumoniae* wild-type strains

No. of strains from:	Presence (+) or absence (-) of:		
	Cell adherence ^a	T7 growth support ^b	T7 adsorption ^c
Urine			
34	+	-	+
6	-	-	-
10	+	+	+
Sputum			
33	+	-	+
3	-	-	-
7	+	+	+
Feces			
2	+	+	+
31	+	-	+
2	-	-	-
Bile			
11	+	-	+
1	-	-	-

^a Capability to adhere to epithelial cells from oral cavity and urinary tract was evaluated for each strain. In the case of adherence to cheek cells, + means at least 40 bacteria adhering per epithelial cell (average from 20 epithelial cells), whereas in the case of adherence to urinary tract cells, + means at least 20 bacteria adhering per epithelial cell (average from 20 epithelial cells).

^b All strains which were able to support T7 growth were also able to support T3 growth.

^c All strains which adsorbed phage T7 adsorbed also T3.

ability to adsorb coliphages T3, T7, and ϕ I and to adhere to human epithelial cells (Table 7). Furthermore, two lysogenic strains cured from prophages were found both sensitive to coliphages T3, T7, and ϕ I and able to adhere to human epithelial cells exactly like the other *K. pneumoniae* strains (Table 7). These findings clearly indicate that inability to adhere to human epithelial cells among wild-type *K. pneumoniae* may often be dependent on lysogenic conversion phenomena.

Identification of the substrate recognized by the coliphage T7 receptor of *K. pneumoniae*. To identify the compound recognized by the *Klebsiella* adherence receptor in human epithelial cells, we performed competition studies with different sugars and lectins. Of the sugars and lectins listed in Table 8, only D-mannose and concanavalin A (ConA) prevented adhesion of K59; of the other seven strains tested, two were sensitive to T3 and T7, and five were apparently resistant to them (Table 8). ConA and D-mannose prevented, approximately

with the same efficiency, adherence to all cells tested (buccal, uroepithelial, and intestinal). This suggested that the compound recognized by *Klebsiella* receptors for adherence could be mannose. To confirm such a possibility, we studied agglutination by *K. pneumoniae* of *C. albicans* and *S. cerevisiae* cells, which are known to contain mannan on their surface (36). Table 9 shows that these cells were agglutinated by all

TABLE 6. Effect of loss of capability of adsorbing coliphage T7 on adherence of wild-type *K. pneumoniae* to epithelial cells

Strain	Presence (+) or absence (-) of:		Mean no. of bacteria adhering to an epithelial cell of ^b :		
	T7 growth	T7 adsorption ^a	Oral cavity ^c	Intestine	Urinary tract
Parental					
K31	-	+	94 $\pm$ 31 ^d	59 $\pm$ 18 ^d	61 $\pm$ 14 ^d
K111	-	+	85 $\pm$ 20	61 $\pm$ 19	74 $\pm$ 26
K120	-	+	80 $\pm$ 15	58 $\pm$ 20	83 $\pm$ 22
K135	-	+	88 $\pm$ 17	47 $\pm$ 11	60 $\pm$ 17
K152	-	+	92 $\pm$ 19	44 $\pm$ 8	55 $\pm$ 13
Mutant					
K31r	-	-	14 $\pm$ 6	12 $\pm$ 5	13 $\pm$ 8
K111r	-	-	10 $\pm$ 8	14 $\pm$ 6	8 $\pm$ 6
K120r	-	-	14 $\pm$ 11	8 $\pm$ 5	10 $\pm$ 4
K135r	-	-	18 $\pm$ 15	10 $\pm$ 5	15 $\pm$ 4
K152r	-	-	15 $\pm$ 5	7 $\pm$ 6	5 $\pm$ 5

^a Strains able to adsorb T7 were also able to adsorb coliphage T3.

^b The mean was calculated by counting the number of bacteria adhering to 20 epithelial cells.

^c Cheek epithelial cells were used.

^d Mean $\pm$ standard deviation.

TABLE 7. Effect of lysogenization by and curing from *Klebsiella* phages V27 and V29 on capability to adsorb coliphages T3, T7, and ϕ I and capability to adhere to human epithelial cells

Strain	Presence (+) or absence (-) of T3, T7, and ϕ I adsorption	Mean no. of bacteria adhering to an epithelial cell of ^b :		
		Oral cavity ^a	Intestine	Urinary tract
K27	-	10 $\pm$ 6 ^c	11 $\pm$ 9 ^c	11 $\pm$ 8 ^c
K29	-	8 $\pm$ 7	9 $\pm$ 6	18 $\pm$ 10
K27 cured from V27 phage	+	180 $\pm$ 31	69 $\pm$ 25	66 $\pm$ 19
K29 cured from V29 phage	+	182 $\pm$ 39	55 $\pm$ 13	50 $\pm$ 18
K59(V27)	-	11 $\pm$ 10	11 $\pm$ 10	9 $\pm$ 8
K59(V29)	-	13 $\pm$ 11	10 $\pm$ 7	13 $\pm$ 10

^a In all cases, the mean was calculated by counting the number of bacteria adhering to 40 epithelial cells.

^b Cheek epithelial cells were used.

^c Mean $\pm$ standard deviation.

TABLE 8. Effect of D-mannose and ConA on adherence of *Klebsiella* to human epithelial cells

Strain	Presence (+) or absence (-) of:		Adherence to cheek epithelial cells ^a in the presence of ^b :	
	T7 growth	T7 adsorption	ConA ^c	D-Man-nose ^c
K59	+	+	58 ± 8 ^d	60 ± 7 ^d
K31	-	+	56 ± 8	57 ± 8
K111	-	+	55 ± 8	56 ± 8
K120	-	+	54 ± 8	54 ± 8
K135	-	+	56 ± 7	60 ± 7
K152	-	+	58 ± 8	52 ± 8
K25	+	+	53 ± 7	51 ± 7
K26	+	+	55 ± 8	59 ± 8

^a Competition experiments using uroepithelial and intestinal cells gave substantially the same results.

^b The inhibition of adherence to oral cells was approximately the same as to intestine and urinary tract epithelial cells.

^c The following sugars and lectins showed no effect on adherence of *klebsiellae* to buccal, uroepithelial, or intestinal cells: D-glucose, L-fucose, D-fucose, D-galactose, D-trehalose, D-arabinose, D-xylose, D-raffinose, D-galactosamine, D-glucosamine, lectin from *Limulus polyphemus*, lectins from *Phaseolus*, lectin from *Phytolacca americana*, lectin from *Ricinus communis*, lectin from *Sophora japonica*, lectin from soybean, lectin from *Triticum vulgare*, lectin from *Ulex europaeus*, fucose-binding protein, peanut agglutinin, and wheat germ agglutinin.

^d Data given as percent ± confidence limits.

K. pneumoniae strains which were capable of adsorbing coliphage T7, whether able to support phage growth or not. Seven strains unable to adsorb coliphage T7 (2 derived from sensitive parents and five from apparently resistant parents) and K59 derivatives resistant to T3 and T7 (KRTT 1 to 4) did not cause agglutination of *Candida* or *Saccharomyces* cells. Also, K59 strain lysogenic for AP3 did not agglutinate any of the yeasts. Finally, competition studies showed that mannose, but none of several other sugars tested, prevented agglutination of *Candida* and *Saccharomyces* cells. Moreover, K59 and other *K. pneumoniae* strains did not agglutinate the yeasts if preadsorbed with UV-inactivated T3 or T7 particles.

These findings raised the possibility that recognition between *Klebsiella* cells and coliphage T7 could also occur through mannose. To test this possibility, we evaluated the effect of D-mannose and ConA on both efficiency of plating and adsorption to *K. pneumoniae* K59 of T3 and T7. Table 10 and Fig. 2 show that both D-mannose and ConA inhibited adsorption of coliphages to sensitive cells and reduced their efficiency of plating on K59 cells. These last findings, by showing that phage adsorption is me-

TABLE 9. Agglutination of *C. albicans* and *S. cerevisiae* cells by various strains of *Klebsiella* and effect of D-mannose, ConA, and preadsorption with UV-inactivated T3 and T7 particles on agglutination

Strain	Agglutination of ^a :		Agglutination inhibition by ^a :	
	<i>C. albicans</i>	<i>S. cerevisiae</i>	D-Man-nose	ConA
<i>K. pneumoniae</i> able to adsorb phage T7				
K59	+	+	+	+
K31				
K111				
K120				
K135				
K152				
K25				
K26				
Derivatives unable to adsorb phage T7				
KRTT 1-KRTT 4	-	-	NT ^b	NT
K59(AP3)				
K31r				
K111r				
K120r				
K135r				
K152r				
K25r				
K26r				
Pretreated with T3 and T7				
K59	-	-	NT	NT
K31				
K25				

^a Presence (+) or absence (-) of the property tested.

^b NT, Not tested.

TABLE 10. Effect of D-mannose and ConA on both adsorption and efficiency of plating of T3 and T7 coliphages on K59

Inhibitor used	% of adsorbed phages after 10 min of infection		Efficiency of plating on K59	
	T3	T7	T3	T7
D-Mannose	28	22	1×10^{-3}	5×10^{-3}
ConA	34	35	1×10^{-2}	3×10^{-3}
Control (no inhibitor)	74	73	1	1

diated by the same sugar involved in adherence to epithelial cells, further support the idea that, in *K. pneumoniae*, coliphage T7 receptors are the ligands responsible for adherence to epithelial cells.

DISCUSSION

Some findings of this study (which appears to be the first extended study on adherence of *K.*

FIG. 2. Effect of D-mannose and ConA on T3 (----) and T7 (—) adsorption to K59 cells. Symbols: ●, no substance added; ▲, D-mannose added (20 mg/ml); and ■, ConA added (1 mg/ml). Experiment was performed as previously described (11, 32).

pneumoniae to human epithelial cells) provide an experimental basis for explaining the peculiarities of the colonization capability of this microorganism. Buccal epithelial cells were found to have a particularly high affinity for *K. pneumoniae* strains. Such cells were in fact those to which all strains tested bound most efficiently, no matter which mucosa they had been isolated from. This fact appears surprising because of the relatively rare *K. pneumoniae* colonization of the oral and upper respiratory tract mucosa compared to colonization of the intestine. It is possible that organism defenses are more effective in the oral cavity and upper respiratory tract than in other body locations. A more likely explanation is that oral secretions can inhibit adherence of gram-negative bacteria to buccal cells. In fact, it has been shown recently that saliva completely inhibits adherence of *Bacteroides melaninogenicus* to buccal epithelial cells (34). A particularly high affinity for epithelial cells could then be necessary for enterobacteria to be able to colonize and infect the upper respiratory tract. Enterobacteria other than klebsiellae which, although capable of adhering in vitro to oral epithelial cells (25, 38), are not known to colonize the oral and upper respiratory tract, may have an adherence capability too low to overcome factors hampering colonization.

Although lysogenic conversion phenomena in-

terfering with several different bacterial functions were described previously (6), this paper contains the first description of a case of lysogenic conversion that causes alterations in the adhesive properties of converted bacteria. The analysis performed has also indicated that this phenomenon must be widely spread among wild-type *K. pneumoniae*. Since it appears clear that adherence capability is a prerequisite for bacterial colonization (12, 14, 15, 20, 28, 38) (a condition in turn necessary to give rise to pathogenic processes), these findings show an additional and very important means by which prophages may interfere with the potential pathogenicity of bacteria. In addition, it has been shown elsewhere that the T3-T7 receptors raise by over 100- to 150-fold the pathogenicity of *K. pneumoniae* for mice (C. Pruzzo, G. Satta, and C. A. Romanzi, Abstr. XII Int. Congr. Microbiol., p. 181, 1978). Several other lysogenic-conversion phenomena causing alterations in surface antigens have been previously described in different bacterial species (6, 18-35). In no case were surface antigen changes studied in relation to cell adherence properties. It appears likely that this relationship may exist in other bacterial species. Investigation of this possibility would lead to a more complete understanding of both genetic determination of mammalian host colonizing and infecting capability of bacteria and influences of stably integrated proviruses on bacterial cell physiology. The changes in adhesive properties of the host cells caused by prophages have, in fact, very important consequences on bacterial host physiology. Adherence properties determine the environment in which bacterial cells will grow. In this specific case, *K. pneumoniae* cells converted by phage AP3 lost the property of colonizing an environment which is nutritionally rich but exposed to the defenses of the human body.

The studies (mainly competition) identifying the substrate recognized and bound by the T3-T7 receptor do not establish whether *Klebsiella* cells contain a ligand recognizing D-mannose in the organisms or bear, on the contrary, a D-mannose-containing receptor which is recognized by ligands in human epithelial cells, yeasts, and phages. Studies on infection of cells by bacteriophages in other systems should suggest that it is the bacteriophage which recognizes and binds a specific sugar on the external surface of bacteria (11). However, a major ligand mediating adherence to epithelial cells and yeast agglutination has recently been identified and purified from *Escherichia coli* cells. This ligand has been purified and shown to be a lectin which recognizes and binds mannose (13). Studies performed in our laboratory (unpublished data) clearly in-

dicates that also in *E. coli* K-12, the receptor for bacteriophages T3 and T7 is the major ligand mediating adherence to epithelial cells and yeast agglutination. It appears likely that in both *K. pneumoniae* and *E. coli* cells one single structure recognizes and binds mannose residues contained in a variety of organisms including certain bacteriophages. Whether this way of interacting between bacterial cells and bacteriophages is peculiar to the system described here or common to other systems cannot be said at the moment.

In the course of our experiments we have also made the additional observation that *K. pneumoniae* strains capable of adsorbing coliphages T3 and T7 constantly agglutinate. When the same cells lose the capability of adsorbing these coliphages, by either lysogenic conversion or spontaneous mutation, they do not agglutinate any longer. The T3-T7 receptor of *K. pneumoniae* appears, therefore, also capable of recognizing and binding bacterial cells. Many phage receptors have been shown to also be receptors mediating transport of various substances such as vitamin B₁₂ (8), iron (9), maltose (39), and nucleosides (17). It therefore appears quite plausible that bacteriophage receptors have additional functions. However, this is the first description of a phage receptor which is the main ligand mediating recognition and binding of other organisms such as human epithelial cells, yeasts, and bacteria. It also seems likely that in other bacterial species phage receptors may be the main bacterial organs for recognition and binding of other organisms.

We suggest that in *K. pneumoniae* the coliphage T3-T7 receptor is a recognition organ mediating interactions between bacteria and other organisms of widely different types. Such receptors have been maintained in most *K. pneumoniae* strains resistant to phage infection. Binding both viruses and mammalian cells, they must bind the latter with an extremely simple mechanism. In addition, the substrate recognized by the T3-T7 receptor must represent a primordial structure still preserved in highly evolved organisms. The recognition mechanisms mediated by these structures appear likely to be the most primitive ones. The coliphages T3-T7 receptor of the *K. pneumoniae* bacteriophage T3-T7 system appears to be a useful model for studying the molecular basis of recognition binding and interaction between microorganisms.

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