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Laboratory and Wild-Type *Klebsiella pneumoniae* Strains Carrying Mannose-Inhibitable Adhesins and Receptors for Coliphages T3 and T7 Are More Pathogenic for Mice Than Are Strains Without Such Receptors

CARLA PRUZZO,[1]* SEBASTIANO VALISENA,[1] AND GIUSEPPE SATTA[2]

Istituto di Microbiologia dell'Università di Genova, Genova,[1] and Istituto di Microbiologia e Virologia dell'Università di Cagliari, Cagliari,[2] Italy

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We have shown previously that *Klebsiella pneumoniae* receptors for coliphages T3 and T7 also mediate mannose-inhibitable adherence to human epithelial cells and protect bacteria from phagocytosis and intracellular killing by human polymorphonuclear cells. In this paper we analyze the possible role of such mannose-inhibitable adhesins and T3-T7 receptors (MIAT) in *K. pneumoniae* intraperitoneal pathogenicity for mice. We showed that intraperitoneal pathogenicity for mice of four different *Klebsiella* strains (one laboratory and three wild-type) that carry the MIAT was approximately 60-fold higher than that of four derivative strains that lost such receptors by spontaneous mutation. The MIAT could be repressed by *Klebsiella* phage AP3 lysogenic conversion. Two laboratory and two wild-type strains converted by phage AP3 were also approximately 60-fold less pathogenic for mice than parental strains and showed a pathogenicity level equal to that of the MIAT-negative mutants. Studies of protection in mice with anti-whole cell antisera showed that passive immunization against MIAT-positive cells was more protective than immunization against MIAT-negative cells. Studies of protection in mice by both active and passive immunization with lipopolysaccharide and purified outer membrane proteins have shown that the proteins are the most protective outer membrane components. Since it has been shown previously that the *Klebsiella* receptors for T3-T7 have a proteic component and that an outer membrane protein is missing in the strains resistant to T3-T7 (C. Pruzzo et al., *in* R. C. Berkely (ed.), *Microbial Adhesion to Surfaces*, 1980); the latter finding further supports the role of MIAT in the pathogenicity of *Klebsiella* for mice.

The ability to adhere to epithelial cells is thought to be a prerequisite for the colonization of vertebrate hosts by bacterial strains (1, 4, 10, 24). Ability to colonize, although not sufficient by itself, is a very important and often necessary step in the pathogenicity of microorganisms (8, 9, 11, 14, 25).

Various ligands mediating adherence to epithelial cells have been described for several pathogenic, opportunistic, or saprophytic bacteria (4, 11, 24, 26, 28, 43, 45).

A bacterial ligand mediating adherence to the vertebrate host epithelia also exposes bacteria to the harmful action of the colonized host defenses. Consequently, a powerful selective pressure is present to induce bacteria to lose adherence structures, unless such structures are at the same time capable of protecting bacteria from host defenses, thus playing a more direct role in bacterial pathogenicity. To date, a possible interference of bacterial adhesins in the protection of bacteria from recognition by host immune or cellular defense mechanism has been proposed for gonococci by some authors, but not confirmed and disputed by others (7, 25, 31, 33, 44). On the contrary, several well-documented cases have been reported indicating that bacterial adhesins facilitate phagocytosis and killing of bacteria by both human polymorphonuclear cells (PMNs) and mouse peritoneal phagocytes (3, 19, 41, 42). Recently an intermediate situation in uropathogenic *Escherichia coli* bearing mannose-resistant pili has been shown (5). In fact, mannose-resistant pili mediate adherence to epithelial cells but do not mediate adherence to rat peritoneal macrophages or human PMNs (5).

A bacterial adherence structure which appears to have peculiar properties with respect to those previously described has recently been identified in unencapsulated strains of *Klebsiella pneumoniae* (28, 29). These adhesins (named MIAT) function as both mannose-inhibitable ad-

TABLE 1. Origin and other characteristics of *K. pneumoniae* strains used in this study

Strain	Origin and characteristics
K59	This Institute's collection; unencapsulated, sensitive to *Klebsiella* phages FR2 and AP3 and to coliphages P1, T3, T7, and φ1 (28, 38, 39), MIAT-positive
K59(FR2)	K59 derivative; lysogenic for FR2, resistant to P1 (28), MIAT-positive
K59(AP3)1, K59(AP3)2	K59 derivatives; lysogenic for AP3, resistant to T3, T7 and φ1 (28), MIAT-negative
KRTT1, KRTT2	Unencapsulated K59 derivatives; selected for resistance to T7, resistant also to phage T3 (28), MIAT-negative
K59 rough	K59 derivative; selected as resistant to a wild-type *Klebsiella* phage (2, 35), sensitive to Mu-1 phage and 0.5% sodium desoxycholate, capable of adhering to epithelial cells, MIAT-positive
KRTT1 rough, KRTT2 rough	Derived from strain K59 rough; selected for resistance to T7 as described (28), resistant also to T3, unable to adhere to epithelial cells, MIAT-negative
K25, K26, K31	Unencapsulated wild-type strains isolated from clinical specimens; sensitive to infection by phages T3 and T7 (28), MIAT-positive
K25R, K26R, K31R	Unencapsulated derivative of strains K25, K26, and K31; selected for resistance to T7 (28), resistant also to T3, MIAT-negative
K25 rough	K25 derivative; selected as resistant to a wild-type *Klebsiella* phage (2, 35), sensitive to Mu-1 phage and 0.5% sodium desoxycholate, MIAT-positive
K25R rough	Derived from strain K25 rough; selected for resistance to T7 as described (28), resistant also to T3, does not adhere to human epithelial cells, is sensitive to phagocytosis by human PMN, MIAT-negative

hesins to human epithelial cells and coliphage T3-T7 receptors (28). They also protect bacteria from phagocytosis and intracellular killing by human PMNs (29). Since phagocytosis plays a major role in the aspecific defenses of the vertebrate hosts, such findings suggested the possibility that the MIAT are the major pathogenicity determinants in *K. pneumoniae*.

We showed that both laboratory and wild-type MIAT-positive *Klebsiella* strains show a greater persistence in the mouse peritoneum and are over 60-fold more pathogenic for mice than MIAT-negative strains.

MATERIALS AND METHODS

Strains. The *Klebsiella* strains used in this study 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. *Klebsiella* phages FR2 and AP3 were isolated from *K. pneumoniae* MirM7 (36, 37) and have been described previously (38, 39). Coliphages T3 and T7 were purchased from the American Type Culture Collection.

Media and buffers. Brain heart infusion broth (Difco) and brain heart infusion agar (Difco) were employed throughout this study. Phosphate-buffered saline (0.1 M Na_2HPO_4, 0.1 M KH_2PO_4, 0.15 M NaCl; pH 7.2 to 7.4) and Hanks balanced salt solution were also employed.

Animals. Male Swiss albino mice weighing about 20 g and 3-kg white New Zealand rabbits were employed.

OMPs and LPS preparation. Outer membrane proteins (OMPs) and pure lipopolysaccharide (LPS) were prepared as described by Lindberg and Holme (18).

Preparation of immune sera. Antisera against whole bacteria were raised in rabbits. Exponentially growing bacteria were washed with phosphate-buffered saline, killed by 4% Formalin treatment, and kept at 37°C for 10 h. Rabbits were immunized by six intravenous 1-ml injections of the suspensions given at 2- or 3-day intervals. The immune sera were collected 4 days after the final injections. Antisera against OMPs and LPS were obtained in the same way except that 5 mg of OMPs and 200 μg of LPS in Freund complete adjuvant were inoculated.

Preparation of adsorbed antisera. Two milliliters of antisera against whole bacteria was added to a centrifuged sediment of the desired *Klebsiella* strain containing approximately 10^{10} cells. After 18 h at 4°C, the bacteria were removed by centrifugation, and the supernatant was used in further experiments as adsorbed sera.

Challenge and determination of LD_{50} values. The 50% lethal dose (LD_{50}) values were calculated accord-

TABLE 2. LD_{50} in mice of MIAT-positive and MIAT-negative *Klebsiella* strains

Challenge strain	MIAT[a]	LD_{50} (no. of cells)	Fold difference[b]
K59	+	3.3×10^5	
KRTT1	−	1.9×10^7	57.6
KRTT2	−	2.5×10^7	75.7
K25	+	2.7×10^5	
K25R	−	1.1×10^7	40.7
K26	+	2.2×10^5	
K26R	−	1.3×10^7	59.1
K31	+	3.8×10^5	
K31R	−	2.3×10^7	60.5

[a] +, Presence of MIAT; −, absence of MIAT.
[b] Fold differences were calculated by comparing the LD_{50} values of MIAT-negative strains with those of MIAT-positive parents.

ing to Reed and Muench (32). Four different doses of overnight broth cultures were injected intraperitoneally to a group of five mice for each dose. The calculations were based on the numbers of survivors on day 15. The significance of the difference between the LD_{50} values of the immunized mice and those of the nonimmunized mice was estimated as previously described (46). Results were considered significant for $P < 0.01$.

Vaccination of mice. Mice were inoculated twice intraperitoneally with either LPS (5 μg) or OMPs (50 μg) at 2-week intervals. To evaluate protection, vaccinated mice were injected intraperitoneally with the tested strain 10 days after the last injection.

Passive protection studies in mice. Mice were injected in the central tail vein with 0.2 ml of inactivated (56°C, 30 min) adsorbed or unadsorbed sera 2 or 3 h before and 1 h after intraperitoneal inoculation of the tested strain.

***Klebsiella* intraperitoneal fate in mice.** The intraperitoneal fate of *Klebsiella* strains in mice was studied as described by Medearis et al. (20).

Sensitivity to serum. The serum sensitivity of the *Klebsiella* strains was tested by incubating 5×10^6 bacteria in 1 ml of 40% pooled human or mouse sera diluted in Hanks balanced salt solution. At 15, 30, 45, and 60 min, samples were diluted and plated on brain heart infusion agar to determine the percentage of survivors.

RESULTS

Intraperitoneal pathogenicity for mice of MIAT-positive and MIAT-negative strains of *K. pneumoniae*. To study the possible role of the T3-T7 receptors in the pathogenicity of *K. pneumoniae*, we evaluated the LD_{50} in mice of one laboratory MIAT-positive *Klebsiella* strain and three wild-type MIAT-positive *Klebsiella* strains and of spontaneous T7-resistant mutants derived from them. The T7-resistant derivatives were also all unadhesive and resistant to phagocytosis by human PMNs (MIAT-negative) (28, 29). Table 2 shows that both the laboratory and the wild-type MIAT-positive klebsiellae were 40- to 60-fold more intraperitoneally pathogenic for mice than the respective MIAT-negative spontaneous mutants. It has been shown previously that both the laboratory strain K59 and wild-type strains of *K. pneumoniae* converted to resistance to coliphages T3-T7 by the temperate phage AP3 (or another AP3-like phage) were unable to adhere to human epithelial cells (28) and were sensitive to phagocytosis by human PMNs. To determine whether loss of the MIAT by a mechanism other than spontaneous mutation to coliphage T7 resistance was still associated with a reduction of intraperitoneal pathogenicity, we evaluated the pathogenicity of strain K59 derivatives lysogenic for AP3. Table 3 shows that two K59(AP3) strains were approximately 60-fold less pathogenic for mice than the K59 parent. On the contrary, a K59 derivative lysogenic for phage FR2 which causes resistance to coliphage P1, but does not influence sensitivity to T3-T7 showed approximately the same pathogenicity level as the parent. After curing from phage infection, the K59(AP3) strains recovered the same pathogenicity level as the K59 parent.

Effect of passive immunization against MIAT-positive and MIAT-negative cells on *K. pneumoniae* pathogenicity for mice. Table 4 shows that although antisera were only slightly protective, mice injected with anti-K59 sera both unadsorbed and adsorbed with KRTT1 cells were more resistant to strain K59. Unadsorbed anti-KRTT1 serum protected to a slight (if any) extent against the MIAT-negative strain and gave virtually no protection against the MIAT-positive strain. Anti-K59 sera adsorbed with this strain as well as anti-KRTT1 sera adsorbed either with KRTT1 or K59 cells did not show any protective effect against either strain.

Sensitivity to serum bactericidal effect and persistence in mouse peritoneum of MIAT-positive

TABLE 3. Effect of lysogenic conversion by phages AP3 and FR2 on the LD_{50} for mice of *Klebsiella* strains

Challenge strain	Sensitivity to:[a]			LD_{50} (no. of cells)	Fold difference[b]
	T3	T7	P1		
K59	+	+	+	3.7×10^5	
K59(AP3)1	−	−	+	2.1×10^7	56.7
K59(AP3)2	−	−	+	2.4×10^7	64.8
K59(FR2)	+	+	−	4.1×10^5	1.1
K59(AP3)1 cured	+	+	+	4.8×10^5	1.2
K59(AP3)2 cured	+	+	+	3.9×10^5	1.0

[a] +, Sensitive; −, not sensitive.
[b] Fold differences were calculated by comparing the LD_{50} values of K59 derivatives with that of K59.

TABLE 4. Effect of passive immunization with anti MIAT-positive and MIAT-negative sera on LD_{50} for mice of *K. pneumoniae* strains K59 and KRTT1[a]

Pretreatment	K59			KRTT1		
	LD_{50} (no. of cells)	Fold difference	*P*	LD_{50} (no. of cells)	Fold difference	*P*
Saline	4.2×10^5			2.6×10^7		
Preimmune serum	4.4×10^5	1.0		2.9×10^7	1.1	
Anti-K59 serum						
nonadsorbed	2.6×10^6	6.1	<0.01	4.9×10^7	1.9	>0.01
adsorbed with K59	4.4×10^5	1.0	>0.01	3.1×10^7	1.2	>0.01
adsorbed with KRTT1	2.3×10^6	5.5	<0.01	2.8×10^7	1.1	>0.01
Anti-KRTT1 serum						
nonadsorbed	5.8×10^5	1.4	>0.01	5.3×10^7	2.0	>0.01
adsorbed with K59	3.7×10^5	0.9	>0.01	3.1×10^7	1.2	>0.01
adsorbed with KRTT1	4.4×10^5	1.0	>0.01	2.2×10^7	0.8	>0.01

[a] Fold differences and *P* values were calculated comparing serum-treated mice with saline-treated mice.

and MIAT-negative *Klebsiella* strains. It is known that changes in envelope properties can be associated with alterations in sensitivity to the bactericidal effect of sera. To study whether the reduced pathogenicity of the MIAT-negative strains was dependent on either a higher sensitivity to the bactericidal effect of the mouse blood or the higher sensitivity to phagocytosis by PMNs, we analyzed both sensitivity to the bactericidal effect of sera and the ability of MIAT-positive and MIAT-negative strains to persist within the mouse peritoneum. It was shown that the sensitivities of strains K59, KRTT1, and KRTT2 to the bactericidal effect of both mouse and human sera are virtually equal (about 15% survivors at 60 min, data not shown). No significant differences were observed among strain K59 and K59 derivatives lysogenic for phages AP3 or FR2, which repress receptors for coliphages T3-T7 and P1, respectively. The effect of rough mutation on serum sensitivity was also tested. As expected, the rough mutants were about eight-fold more sensitive than the parental strains. However, the sensitivities of the rough MIAT-positive and MIAT-negative strains were equal (data not shown). As opposed to sensitivity to the serum bactericidal effect, the MIAT receptors significantly influenced the ability of bacteria to persist in the mouse peritoneal cavity. Fifty minutes after injection, the number of MIAT-negative bacteria still present in the peritoneum was approximately half that of the MIAT-positive bacteria (Table 5). It should also be noted that the rough mutation did not influence the persistence of MIAT-positive or MIAT-negative klebsiellae in the mouse peritoneal cavity.

Role of LPS and OMPs in the pathogenicity of MIAT-positive *Klebsiella* strains. The receptors for coliphages T3-T7 are thought to reside in the LPS (17). On the other hand, LPS is known to play a major role in the pathogenicity of certain gram-negative bacteria. To evaluate the possible role of LPS changes in the different pathogenicities of MIAT-positive and MIAT-negative *Klebsiella* strains, we isolated rough mutants from two of the MIAT-positive and two of the MIAT-

TABLE 5. Persistence in mouse peritoneum of MIAT-positive and MIAT-negative *Klebsiella* strains

Strain	Expt	Inoculum (no. of cells $\times 10^4$)[a]	Extracellular bacteria at 50 min (% inoculum) ± SE
K59	A	42	85 ± 11.3
	B	35	60 ± 15.5
	C	26	79 ± 12.8
KRTT1	A	55	41 ± 15.5
	B	66	18 ± 12.1
	C	34	36 ± 15.1
KRTT2	A	21	36 ± 15.1
	B	17	31 ± 14.6
	C	75	24 ± 13.5
K59 rough	A	61	74 ± 13.8
	B	54	94 ± 7.5
	C	98	63 ± 15.2
KRTT1 rough	A	43	20 ± 12.6
	B	54	39 ± 15.4
	C	14	17 ± 11.8
KRTT2 rough	A	67	36 ± 15.1
	B	87	43 ± 15.6
	C	54	21 ± 12.8

[a] Each dose was given to 10 mice.

TABLE 6. Effect of active immunization with LPS and OMPs on LD_{50} for mice of *K. pneumoniae* strains K59 and KRTT1[a]

Vaccine	K59			KRTT1		
	LD_{50} (no. of cells)	Fold difference	*P*	LD_{50} (no. of cells)	Fold difference	*P*
None	2.2×10^5			1.5×10^7		
OMPs						
K59	5.5×10^6	25	<0.01	1.1×10^8	7.3	<0.01
KRTT1	3.9×10^5	1.8	>0.01	9.0×10^7	6	<0.01
LPS						
K59	7.2×10^5	3.3	<0.01	4.2×10^7	2.8	<0.01
KRTT1	4.9×10^5	2.2	<0.01	3.6×10^7	2.4	<0.01

[a] Fold differences and *P* values were calculated by comparing vaccinated mice with nonvaccinated mice.

negative *Klebsiella* strains described in Table 2 and compared intraperitoneal pathogenicities for mice of these mutants with those of the corresponding smooth parents. The pathogenicity of the rough derivatives was not much different from the pathogenicity of the smooth parents both in the MIAT-positive and the MIAT-negative klebsiellae.

We then evaluated the protecting effect of vaccination with the pure LPS from a MIAT-positive strain and from its MIAT-negative derivative. Table 6 shows that vaccination with LPS from MIAT-positive or MIAT-negative *Klebsiella* give little protection against the respective strain. It was also evident that the protection given by the LPS from strain K59 was only slightly higher than that given by the LPS from strain KRTT1. Furthermore, mice vaccinated with the LPS from K59 were almost equally protected against K59 and KRTT1 and vice versa.

The protecting effect of active immunization with OMPs was studied next. Mice vaccinated with OMPs were much more protected than those vaccinated with LPS (Table 6). Vaccination with the K59 OMPs rendered mice almost 25-fold more resistant to this strain, but raised resistance against strain KRTT1 by only 7-fold. Vaccination with the KRTT1 OMPs gave virtually no protection against strain K59, while protecting against strain KRTT1 to approximately the same extent as vaccination with K59 OMPs.

Finally, we evaluated the protective effect of anti-OMPs and anti-LPS sera. Table 7 shows that anti-OMPs sera had a strong protective effect. The strongest protection was given by anti-K59 OMPs sera, which rendered mice 16-fold more resistant to intraperitoneally injected MIAT-positive cells. The antiserum also protected against the MIAT-negative strain, but protection was over fivefold lower. Anti-KRTT1 OMPs sera gave an evident protection against the MIAT-negative strain, but protected very little against K59, causing only a twofold reduction in mice sensitivity. Anti-LPS sera were much less protective. Antisera against LPS from the MIAT-negative cells was almost equally protective against the MIAT-positive and MIAT-negative strains and vice versa.

DISCUSSION

The data presented in this paper clearly show that the MIAT in *K. pneumoniae* are the main determinant of pathogenicity. The MIAT have previously been proved to be receptors for coli-

TABLE 7. Effect of passive immunization against LPS and OMPs on the LD_{50} for mice of *K. pneumoniae* strains K59 and KRTT1

Pretreatment	K59			KRTT1		
	LD_{50} (no. of cells)	Fold difference	*P*	LD_{50} (no. of cells)	Fold difference	*P*
Saline	1.4×10^6			8.2×10^8		
Preimmune serum	1.6×10^6	1.1		8.3×10^8	1.0	
Anti-K59 OMPs serum	2.2×10^7	15.7	<0.01	2.7×10^9	3.2	<0.01
Anti-KRTT1 OMPs serum	2.5×10^6	1.8	>0.01	5.3×10^9	6.4	<0.01
Anti-K59 LPS serum	3.2×10^6	2.3	>0.01	1.8×10^9	2.1	>0.01
Anti-KRTT1 LPS serum	2.9×10^6	2.1	>0.01	1.5×10^9	1.8	>0.01

[a] Fold differences and *P* values were calculated by comparing serum-treated mice with saline-treated mice.

phages T3-T7, to be responsible for adherence to human epithelial cells (28), and to protect against phagocytosis (29). To date, no other bacterial component has been reported to have both epithelial cell adhesive and antiphagocytic properties. As mentioned above, it has been shown recently that *E. coli* strains with mannose-resistant pili do not adhere to phagocytes (5). This situation is different from the one observed with MIAT. In fact, whereas mannose-resistant pili of uropathogenic *E. coli* do not allow binding to phagocytes and do not interfere with phagocytosis, MIAT do not interfere with phagocytes, drastically reducing both uptake and intracellular killing.

The determination that the MIAT of *K. pneumoniae* are pathogenic determinants in mice was concluded from the finding that their loss by two different means, spontaneous mutation and lysogenic conversion, was consistently associated with a dramatic reduction of the pathogenicity level and from the results of active and (although to a lower extent) passive immunization experiments. Four different strains (one laboratory and three wild-type) carrying the MIAT and seven strains (three laboratory and four wild-type) not carrying them were tested for intraperitoneal pathogenicity in mice. All of the MIAT-positive strains were at least 60-fold more pathogenic than all of the MIAT-negative ones. Passive immunization against strain K59 whole cells was only moderately protective; however, it seemed to protect more against K59 than against KRTT1. Immunization against KRTT1 was slightly protective against KRTT1 and had no effect against K59.

Other examples are known of adhesins clearly involved in bacterial pathogenicity from which vaccines are prepared (13, 22, 34, 40). Animals immunized with these vaccines develop humoral antibodies and, in most cases, are protected against infections by the tested strains.

It is generally thought that in *E. coli* the T3-T7 receptors reside in the LPS. LPS was also thought to be the receptor for other coliphages like T4 (47). It has been shown recently that the receptor for T4 phage also involves an OMP (23). It has been shown (30) that the MIAT is proteic in nature and that the *K. pneumoniae* receptor for T3-T7 also involves an OMP. We showed that the different pathogenicity of the MIAT-positive and MIAT-negative strains is not bound to differences in the LPS but to differences in the protein fraction of the outer membrane. In fact, the rough mutation that involves loss of sugar or amino sugars from the variable portion of the LPS did not significantly influence the pathogenicity of either MIAT-positive or MIAT-negative strains, and active and passive immunization with the LPS from K59 did not protect any more than immunization with the LPS from KRTT1. On the other hand, active immunization with K59 OMPs was clearly protective against K59, whereas immunization with KRTT1 OMPs did not protect against the MIAT-positive strain. It is also quite significant that the protection given by passive immunization against K59 OMPs was much higher than that given by immunization against K59 whole cells. It is likely that the OMP components which raise the virulence of *Klebsiella* are present in a small amount in the bacterial envelope and that in the number of cells generally used for active immunization, it is not contained in a sufficient amount for good antigenic stimulation. This may also explain the finding that vaccination with whole cells is often less effective than vaccination with the isolated main pathogenicity determinants (mostly exotoxins) and raises the possibility of preparing vaccines, once the bacterial pathogenicity determinants have been isolated, either by the extraction and purification or by increasing the expression through genetic engineering of these determinants.

The above findings, aside from further supporting the role of MIAT in pathogenicity, show that the major pathogenicity determinants in these *K. pneumoniae* strains reside in the proteic component of the outer membrane. A strong protective effect of active immunization with OMPs has been already shown with *Salmonella typhimurium* (16). Other findings of our laboratory indicate that the T3-T7 receptors of *E. coli* have properties similar to those of *K. pneumoniae*. These findings raise the possibility that OMPs in most *Enterobacteriaceae* (and possibly gram-negative strains) include major pathogenicity determinants. The OMPs of *S. typhimurium* that play a role in pathogenicity have been identified in the so-called "porins" (16). In the *Klebsiella* strains described here, the OMP(s) that raises the pathogenicity has not yet been identified. However, although the mechanism by which the *S. typhimurium* porins raise pathogenicity is not known, the data described above demonstrate that OMPs are major pathogenicity determinants which most likely act by allowing bacterial colonization of mucosa and protecting bacteria against phagocytosis. Although studies on phagocytosis were performed with human PMNs (29) and pathogenicity was studied in mice, we showed that MIAT-positive cells persist more than MIAT-negative ones in mouse peritoneum, thus indicating that the former strains are more resistant than the latter to phagocytosis by mouse phagocytic cells.

This is the first report of a phage receptor that appears to be a major pathogenicity determinant. Since several bacterial cell surface components probably work as receptors for some

phage, phages might be exploited as tools for identifying surface components with roles in bacterial pathogenicity. The same may hold for bacteriocins, which have been shown to often share the same receptors with phages (15). It is then possible that additional determinants of bacterial pathogenicity may be detected by screening changes from sensitivity to resistance to phage or bacteriocin infection. It should also be mentioned that phage or bacteriocin receptor properties of these determinants could greatly facilitate genetic or biochemical studies.

Phage and bacteriocin typing of strains have been and are being used for epidemiological purposes (12, 21, 27). It has been observed in several species that strains belonging to specific phage types were more pathogenic or most often associated with a particular illness (27). The present work may provide an explanation for those findings. In addition the bacterial phage types known to be most often associated with specific illness appear to be good candidates for carrying a pathogenicity determinant in their respective phage receptor.

This paper also demonstrates a bacterial cell surface component prone to repression by lysogenic conversion that plays a major role in pathogenicity. The importance of this finding comes from the fact that the significance of lysogenic conversion in bacterial physiology is poorly understood and that the influence on bacterial physiology of converting phenomena that cause changes in the surface antigens is completely unknown. In this case, the converting phage, aside from causing loss of adhesion to human epithelia, also heavily interferes in resistance to phagocytosis by human PMNs. The fact that the conversion by phage AP3 renders unencapsulated *K. pneumoniae* strains incapable of colonizing human epithelia, more prone to phagocytosis, and less pathogenic could be important in the evolution of the species by causing their spreading into habitats different from the vertebrate hosts. The dramatic increase in sensitivity to phagocytosis caused by the loss of MIAT represents, for instance, a strong selective pressure toward the loss of the prophage or toward mutations that allow expression of the MIAT receptors in the presence of the prophage. A clinically isolated wild-type *Klebsiella* lysogenic for phage AP3 that carries a unique mutation preventing expression of both immunity to superinfection and phage AP3 lysogenic conversion without causing prophage induction has been described previously (38, 39).

Several other cases of bacteriophage receptors that perform other functions have been already described (6, 15), but in no case were the functions associated with the phage receptor of the type described here. Although in a large number of strains tested sensitivity to T3 and T7 was consistently associated with all of the properties mentioned above and the absence of one of these properties was always accompanied by loss of all the others, it cannot be excluded that the functions apparently associated with coliphage T3-T7 receptors are not all coded by the same gene.

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LITERATURE CITED

1. **Aronson, M., O. Medalia, L. Schori, D. Mirelman, N. Sharon, and I. Ofek.** 1979. Prevention of colonization of the urinary tract of mice with *Escherichia coli* by blocking bacterial adherence with methyl-α-D-mannopyranoside. J. Infect. Dis. **139**:329–332.
2. **Bachhuber, M., W. J. Brill, and M. M. Howe.** 1976. Use of bacteriophage Mu to isolate deletions in the *his-nif* region of *Klebsiella pneumoniae*. J. Bacteriol. **128**:749–753.
3. **Bar-Shavit, F., I. Ofek, R. Goldman, D. Mirelman, and N. Sharon.** 1977. Mannose residues on phagocytes as receptors for the attachment of *Escherichia coli* and *Salmonella typhi*. Biochem. Biophys. Res. Commun. **78**:455–460.
4. **Beachey, E. H.** 1981. Bacterial adherence: adhesin-receptor interactions mediating the attachment of bacteria to mucosal surfaces. J. Infect. Dis. **143**:325–345.
5. **Blumenstock, E., and K. Jann.** 1982. Adhesion of piliated *Escherichia coli* strains to phagocytes: differences between bacteria with mannose-sensitive pili and those with mannose-resistant pili. Infect. Immun. **35**:264–269.
6. **Braun, V., and K. Hantke.** 1977. Bacterial receptor for phages and colicins as constituent of specific transport system, p. 99–137. *In* J. L. Reissig (ed.), Microbial interactions. Chapman and Hall, London.
7. **Dilworth, J. A., J. O. Hendley, and G. L. Mandell.** 1975. Attachment and ingestion of gonococci by human neutrophils. Infect. Immun. **11**:512–516.
8. **Duguid, J. P., and R. R. Gillies.** 1957. Fimbriae and adhesive properties in dysentery bacilli. J. Pathol. Bacteriol. **74**:397–411.
9. **Ellen, R. P., and R. J. Gibbons.** 1972. M protein-associated adherence of *Streptococcus pyogenes* to epithelial surfaces: a prerequisite for virulence. Infect. Immun. **5**:826–830.
10. **Fader, R. C., and C. P. Davis.** 1980. Effect of piliation on *Klebsiella pneumoniae* infection in rat bladders. Infect. Immun. **30**:554–561.
11. **Gibbons, R. J.** 1977. Adherence of bacteria to host tissue. Position paper, p. 395–406. *In* D. Schlessinger (ed.), Microbiology—1977. American Society for Microbiology, Washington, D.C.
12. **Gillies, R. R.** 1978. Bacteriocin in typing of *enterobacteriaceae*, p. 79–86. *In* T. Bergan and J. R. Norris (ed.), Methods in microbiology, vol. 11. Academic Press Inc., London.
13. **Isaacson, R. E., E. A. Dean, R. L. Morgan, and H. W. Moon.** 1980. Immunization of suckling pigs against enterotoxigenic *Escherichia coli*-induced diarrheal disease by vaccinating dams with purified K99 or 987P pili: antibody production in response to vaccination. Infect. Immun. **29**:824–826.
14. **Jones, G. W., and R. Freter.** 1976. Adhesive properties of *Vibrio cholerae*: nature of the interactions with isolated rabbit brush border membranes and human erythrocytes. Infect. Immun. **14**:240–245.

15. **Konisky, J.** 1979. Specific transport systems and receptors for colicins and phages, p. 319–359. *In* M. Inuye (ed.), Bacterial outer membranes, biogenesis and functions. Wiley Intersciences Publ., New York.
16. **Kuusi, N., M. Nurminen, H. Saxen, M. Valtonen, and P. H. Makela.** 1978. Immunization with major outer membrane proteins in experimental salmonellosis of mice. Infect. Immun. **25**:857–862.
17. **Lindberg, A. A.** 1973. Bacteriophage receptors. Annu. Rev. Microbiol. **27**:205–241.
18. **Lindberg, A. A., and T. Holrne.** 1972. Evaluation of some extraction methods for the preparation of bacterial lipopolysaccharides for structural analysis. Acta Pathol. Microbiol. Scand. Sect. B **80**:751–759.
19. **Mangan, D. F., and I. S. Snyder.** 1979. Mannose-sensitive interaction of *Escherichia coli* with human peripheral leukocytes in vitro. Infect. Immun. **26**:520–527.
20. **Medearis, D. N., B. M. Camitta, and E. C. Makela.** 1968. Cell wall composition and virulence in *Escherichia coli*. J. Exp. Med. **128**:399–414.
21. **Milch, H.** 1979. Phage typing of *Escherichia coli*, p. 87–155. *In* T. Bergam and J. R. Norris (ed.), Methods in microbiology. Academic Press Inc., London.
22. **Morgan, R. L., R. E. Isaacson, H. W. Moon, C. C. Brinton, and C.-C. To.** 1978. Immunization of suckling pigs against enterotoxigenic *Escherichia coli*-induced diarrheal disease by vaccinating dams with purified 987 or K99 pili: protection correlates with pilus homology of vaccine and challenge. Infect. Immun. **22**:771–777.
23. **Mutoh, N., H. Furukawa, and S. Mizushima.** 1978. Role of lipopolysaccharide and outer membrane proteins of *Escherichia coli* K-12 in the receptor activity for bacteriophage T4. J. Bacteriol. **136**:693–699.
24. **Ofek, I., and E. H. Beachey.** 1980. Bacterial adherence. Adv. Intern. Med. **25**:503–532.
25. **Ofek, I., E. H. Beachey, and A. L. Bisno.** 1974. Resistance of *Neisseria gonorrhoeae* to phagocytosis: relationship to colonial morphology and surface pili. J. Infect. Dis. **129**:310–316.
26. **Ofek, I., D. Mirelman, and N. Sharon.** 1977. Adherence of *Escherichia coli* to human mucosal cells mediated by mannose receptors. Nature (London) **265**:623–625.
27. **Parker, M. T.** 1972. Phage typing of *Staphylococcus aureus*, p. 1–28. *In* J. R. Norris and D. W. Robbins (ed.), Methods in microbiology, vol. 7B Academic Press Inc., London.
28. **Pruzzo, C., E. A. Debbia, and G. Satta.** 1980. Identification of the major adherence ligand of *Klebsiella pneumoniae* in the receptor for coliphage T7 and alteration of *Klebsiella* adherence properties by lysogenic conversion. Infect. Immun. **30**:562–571.
29. **Pruzzo, C., E. Debbia, and G. Satta.** 1982. Mannose-inhibitable adhesins and T3-T7 receptors of *Klebsiella pneumoniae* inhibit phagocytosis and intracellular killing by human polymorphonuclear leukocytes. Infect. Immun. **36**:949–957.
30. **Pruzzo, C., S. Valisena, E. Debbia, and G. Satta.** 1980. Characterization of the *Klebsiella pneumoniae* ligand which mediates adherence to human epithelial cells as the site for attachment for coliphages T3 and T7. Relationship to pathogenicity of unencapsulated strains, p. 509–511. *In* R. C. Berkely (ed.), Microbial adhesion to surfaces. Ellis Horwood Publishers, London.
31. **Punsalung, A. P., Jr., and W. D. Sawyer.** 1973. Role of pili in the virulence of *Neisseria gonorrhoeae*. Infect. Immun. **8**:255–263.
32. **Reed, L. J., and H. Muench.** 1938. A simple method for estimating fifty percent endpoint. Am. J. Hyg. **27**:493–499.
33. **Rosental, R. S., R. S. Fulbright, M. E. Eads, and W. D. Sawyer.** 1977. Ethylenediaminetetraacetic acid-sensitive antiphagocytic activity of *Neisseria gonorrhoeae*. Infect. Immun. **15**:817–827.
34. **Rutter, J. M., and G. W. Jones.** 1973. Protection against enteric disease caused by *Escherichia coli*—a model for vaccination with a virulence determinant. Nature (London) **242**:531–532.
35. **Sanderson, K. E., and P. E. Hartman.** 1978. Linkage map of *Salmonella typhimurium*, edition V. Microbiol. Rev. **42**:471–519.
36. **Satta, G., and R. Fontana.** 1974. Characterization of a conditional mutant with altered envelope showing pH-dependent morphology and temperature-dependent division. J. Gen. Microbiol. **80**:51–63.
37. **Satta, G., R. Fontana, P. Canepari, and G. Botta.** 1979. Peptidoglycan synthesis in cocci and rods of a pH-dependent, morphologically conditional mutant of *Klebsiella pneumoniae*. J. Bacteriol. **137**:727–734.
38. **Satta, G., C. Pruzzo, E. Debbia, and L. Calegari.** 1978. Lysogenic conversion in *Klebsiella pneumoniae*: system which requires active immunity regulation for expression of the conversion phenomenon. J. Virol. **28**:786–794.
39. **Satta, G., C. Pruzzo, E. Debbia, and R. Fontana.** 1978. Close association between shape alteration and loss of immunity to superinfection in a wild-type *Klebsiella pneumoniae* stable lysogen which can be both immune and nonimmune to superinfection. J. Virol. **28**:772–785.
40. **Silverblatt, F. J., and L. S. Cohen.** 1979. Antipili antibody affords protection against experimental ascending pyelonephritis. J. Clin. Invest. **64**:333–336.
41. **Silverblatt, F. J., J. S. Creyer, and S. Schauer.** 1979. Effect of pili on susceptibility of *Escherichia coli* to phagocytosis. Infect. Immun. **24**:218–223.
42. **Silverblatt, F. J., and I. Ofek.** 1978. Influence of pili on virulence of *Proteus mirabilis* in the experimental hematogenous pyelonephritis. J. Infect. Dis. **138**:664–667.
43. **Svanborg-Eden, C., and U. Jodal.** 1979. Attachment of *Escherichia coli* to urinary sediment epithelial cells from urinary tract infection-prone and healthy children. Infect. Immun. **26**:837–840.
44. **Swanson, J., E. Sparks, D. Young, and G. King.** 1975. Studies of gonococcus infection. X. Pili and leukocyte association factor as mediators of interactions between gonococci and eukaryotic cells in vitro. Infect. Immun. **11**:1352–1361.
45. **Vaisanen, V., J. Elo, L. G. Tallgren, A. Siitonen, P. H. Makela, C. Svanborg-Eden, G. Kallenius, S. B. Svenson, H. Hultberg, and T. Korhonen.** 1981. Mannose-resistant haemoagglutination and P antigen recognition are characteristic of *Escherichia coli* causing primary pyelonephritis. Lancet **ii**:1366–1369.
46. **Valtonen, V. V.** 1970. Mouse virulence of *Salmonella* strains: the effect of different smooth-type O side-chains. J. Gen. Microbiol. **64**:255–268.
47. **Wilson, J. H., R. B. Luftig, and W. B. Wood.** 1970. The interaction of bacteriophage T4 tail components with a lipopolysaccharide fraction from *Escherichia coli*. J. Mol. Biol. **51**:423–434.