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## Protective Effect of Antilipoplysaccharide Monoclonal Antibody in Experimental *Klebsiella* Infection

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**An O-antigen-specific murine monoclonal antibody (MAb) directed against an immunodominant epitope expressed on *Klebsiella* O1, O6, and O8 lipopolysaccharides (LPS) was examined with respect to its binding to nonencapsulated and encapsulated bacterial cells and its ability to protect against lethal murine *Klebsiella* sepsis. While the MAb (clone Ru-O1, mouse immunoglobulin G2b) bound well to nonencapsulated organisms of the O1 serogroup, binding was significantly, but not completely, abolished by the presence of the K2 capsule. In a model of experimental *Klebsiella* peritonitis and sepsis induced by a virulent O1:K2 serogroup strain, higher doses of anti-LPS MAb Ru-O1 than of a previously described anticapsular MAb specific for the K2 capsular polysaccharide were needed to provide protection. However, high-dose (40 µg/g of body weight) pretreatment with anti-LPS MAb Ru-O1 significantly reduced bacterial dissemination to various organs as well as macroscopic and histologic pulmonary alterations. Thus, since the number of *Klebsiella* capsular antigens occurring in clinical material is too large to be completely “covered” by a K-antigen-specific hyperimmunoglobulin preparation, O-antigen-specific antibodies may supplement K-antigen-specific immunoprophylaxis and -therapy of clinical *Klebsiella* infection.**

Organisms of the genus *Klebsiella* are an important cause of hospital-acquired infections (17, 18). In particular, nosocomial pneumonia (3) and septicemia (2) due to *Klebsiella* spp. are a frequent problem in both medical and surgical intensive care units. In spite of the development of new and potent antibiotics, *Klebsiella* infections are still associated with a high mortality rate of up to 40% (2, 3). Therefore, various studies have been performed in order to elucidate the pathogenesis of *Klebsiella* infections and to characterize possible virulence factors. The final goal of these studies is the identification of bacterial surface antigens that might serve as target structures for active vaccination or passive immunotherapy.

The prominent morphologic feature of *Klebsiella* organisms is the large capsule (K antigen), which plays a significant role in pathogenicity (12, 29, 39). It has been shown that K-antigen-specific polyclonal and monoclonal antibodies (MAbs) may enhance phagocytosis of *Klebsiella* organisms and protect against experimental *Klebsiella* infections (9, 15, 23, 34). Recently, a multivalent *Klebsiella* capsular polysaccharide vaccine which has been shown to elicit a capsule-specific serum antibody response in volunteers was developed (7, 11). The vaccine contains 24 different capsular antigens in order to provide broad-spectrum coverage against K serotypes occurring in clinical infections (11). A hyperimmune intravenous (i.v.) immunoglobulin preparation made from the postvaccination plasma of volunteers immunized simultaneously with the *Klebsiella* capsular antigen and *Pseudomonas* vaccines recently underwent a randomized placebo-controlled prophylactic trial in intensive care unit patients (8). The results of this study showed

that the incidence of vaccine-specific *Klebsiella* infections was reduced by >50% in patients receiving the hyperimmune globulin preparation ( $P = 0.05$ ), although this difference was not significant at the preassigned 1% level (10). However, approximately 30% of all *Klebsiella* infections occurring in this study were caused by capsular serotypes not included in the vaccine. Since more than 70 different capsular antigens have been found in clinical material (26), it would be necessary to add many additional K serotypes in order to broaden the antimicrobial spectrum of the currently available vaccine.

An alternative approach may be the development of a composite *Klebsiella* vaccine containing other surface determinants in addition to capsular antigens. The lipopolysaccharide (LPS) (O antigen) is an attractive candidate, since the number of clinically relevant O-antigenic types in *Klebsiella* is relatively small. In a recent study, only four O antigens (O1, O2ab, O2ac, and O3) were shown to account for 72% of clinical isolates and for >80% of O serotypeable isolates (37). The percentage of clinical strains that were typeable by O-antigen-specific sera varied between 79% (37) and 93.6% (14). Thus, few O-antibody specificities may be sufficient to significantly broaden the antimicrobial spectrum of a K-antigen-based vaccine, provided that protection by O-antigen-specific antibodies can be demonstrated in vivo.

However, although it has been shown that O-specific antibodies may penetrate through the *Klebsiella* capsule and bind their target antigen on the bacterial cell surface (24, 32, 33), little is known about the ability of such antibodies to protect against infection with virulent *Klebsiella* strains. In the present study, we therefore prepared an O-specific MAb and examined its functional effect in a model of lethal murine *Klebsiella* infection. Since the O1 antigen appears to play an important role in clinical strains, being detectable in ~25 to 30% of isolates (1, 37), experiments were performed with a MAb generated against this antigen.

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## MATERIALS AND METHODS

**Animals.** Eight- to 10-week-old pathogen-free male BALB/c mice weighing 20 to 25 g each were used throughout the study.

**Bacteria and LPS.** The following *Klebsiella* O serogroup reference strains were obtained from the Statens Seruminstitut, Copenhagen, Denmark: *Klebsiella pneumoniae* Friedländer 201 (O1:K-), 8238 (O1:K37), 7380 (O2ab:K-), 5053 (O2ac:K-), 390 (O3:K11), 1702 (O4:K42), 4425/51 (O5:K57), NCTC 8172 (O6:K64), 264(1) (O7:K67), 889 (O8:K69), 1205 (O9:K72), 337 (O10:K73), 378 (O11:K78), and 708 (O12:K80). As a positive control strain for the *Klebsiella* O1 antigen, we used *Escherichia coli* 8188/41 (O19ab), since the O19b component of this strain is known to be immunologically identical to *Klebsiella* O1 (19, 26). Negative controls for enzyme-linked immunosorbent assay (ELISA) binding studies included *E. coli* Bort (O18:K1:H7) (34) as well as *E. coli* 8858/41 (19), which expresses the O19a antigen only. *K. pneumoniae* 23 (O1:K2) and its isogenic nonencapsulated derivative 23/3 as well as strains 37 (O1:K7), 58 (O1:K7), and 557 (O1:K21) were kindly supplied by R. Podschun, Kiel, Germany. The production of LPS from the O-serogroup reference strains and from *E. coli* Bort was described previously (37, 38). LPS preparations from the other *E. coli* strains were obtained by the same methods. Whole-bacterial-cell ELISA experiments and experimental infections were performed using the highly virulent variant of strain *K. pneumoniae* Caroli (O1:K2), which has been used before by ourselves (15, 34) and by other authors (4). A nonencapsulated mutant of this strain was kindly prepared by R. Podschun.

**MAbs.** The capsule-specific MAb III/5-1, a murine immunoglobulin M (IgM) antibody, was described previously (34). MAb Ru-O1, a mouse IgG2b MAb specific for *Klebsiella* O1 LPS, was obtained in the same way as described previously for the capsule-specific MAb except that mice were immunized twice with *K. pneumoniae* Friedländer 201 (O1:K-). The bacterial suspension used for immunization was prepared as follows. Bacteria were grown in Trypticase soy broth for 3 h, washed three times in physiological saline, and adjusted nephelometrically to a concentration of  $2 \times 10^7$  organisms per ml. The bacteria were inactivated at 60°C for 1 h, and the suspension was stabilized by the addition of 0.5% formalin. A 1-ml volume of this suspension was mixed with an equal volume of complete Freund's adjuvant (Sigma, Deisenhofen, Germany) and injected subcutaneously into two mice. Three weeks later, the animals were boosted with 10 µg of LPS O1 given intraperitoneally (i.p.). Four days later, the animals were sacrificed, and their spleen cells were fused with the murine SP2/O-Ag14 myeloma cell line. Ascites fluid was obtained by injection of cloned hybridoma cells into pristane-primed mice (34). The immunoglobulin class and subclass were determined by ELISA, using typing reagents obtained from Sigma. Control MAbs were commercially available; we used purified mouse IgM (clone TEPC 183) and IgG2b (clone 74-12-4, ATCC HB147, or clone MOPC 141, all from Sigma) myeloma antibodies.

**Antibody purification.** MAb Ru-O1 was purified from ascites fluid by column chromatography over protein G-Sepharose by following the instructions of the supplier (HiTrap Protein-G; Pharmacia, Uppsala, Sweden). Purified IgG was concentrated by means of Centrprep-10 concentrators (Amicon, Beverly, Mass.). The protein concentration was determined by the Coomassie blue dye binding method with bovine serum albumin (BSA) as the standard. Purification of MAb III/5-1 was described previously (34).

**Direct LPS-ELISA.** To study the specificity of MAb Ru-O1, samples of LPS from O-antigen reference strains were dissolved in phosphate-buffered saline (PBS), pH 7.4, at 25 µg/ml. Flat-bottomed 96-well ELISA plates (Greiner, Nürtingen, Germany) were coated with the LPS solutions (100 µl/well) and incubated at 4°C for 18 to 24 h. After removal of antigen, nonspecific binding sites were blocked with 0.5% casein-0.5% BSA in PBS (casein-BSA) as described previously (35). Purified MAb or isotype-matched mouse IgG2b control MAb was added to the plates at 10 µg/ml and incubated for 24 h at 4°C. After being washed with PBS, bound mouse immunoglobulin was traced with appropriately diluted alkaline phosphatase-conjugated goat anti-mouse IgG antibody (Sigma). Plates were washed again, and reactions were developed by adding phosphatase substrate (no. 104; Sigma) dissolved at a concentration of 1 mg/ml in diethanolamine buffer, pH 9.8. The optical densities of the wells at 405 nm (OD<sub>405</sub>) were read in a Titertek Multiscan Plus reader (Flow Laboratories, Helsinki, Finland).

**Whole-bacterial-cell ELISA.** Binding of MAb to whole bacterial cells was examined by using a slight modification of a previously described method (36). In short, approximately  $4 \times 10^8$  organisms were inactivated by a short heat exposure (90°C, 10 min) in a water bath to abolish intrinsic alkaline phosphatase activity. After bacterial cells had been washed, MAbs were added to the bacterial pellets at 10 µg/ml in casein-BSA. After incubation and repeated washings, MAbs bound to the bacterial surface were traced with alkaline phosphatase-conjugated anti-mouse IgG or IgM goat antibodies (Sigma) at appropriate working dilutions. Reactions were developed further as described previously (36).

**Experimental *Klebsiella* infection.** To ensure the virulence of the challenge strain, bacteria were injected i.p. into BALB/c mice and reisolated from liver and spleen homogenates 24 h later. For experimental infections, bacteria grown on blood agar plates for 18 h were suspended in sterile PBS, pH 7.4. The challenge organisms were washed two times in PBS to remove loose slime containing extracellular polysaccharides. Bacterial suspensions were adjusted densitometrically at 365 nm to the desired concentration, which was confirmed by colony

counts on blood agar plates after serial 10-fold dilutions. For determination of the 50% lethal dose (LD<sub>50</sub>), various bacterial concentrations in a volume of 0.2 ml were injected i.p. into BALB/c mice. The animals were observed daily for 7 days, and mortality in different groups was recorded. The LD<sub>50</sub> of *K. pneumoniae* Caroli as calculated by the method of Reed and Muench (28) was 10 organisms per mouse. LD<sub>50</sub> determinations for *Klebsiella* sp. strains NCTC 8172 (O6:K64) and 889 (O8:K69) were performed in the same way.

**Protection studies.** Groups of 10 animals were pretreated with an i.p. injection of purified MAb Ru-O1 at the doses indicated or with PBS. Four hours later, the animals were infected i.p. with an estimated dose of 50 organisms of *K. pneumoniae* Caroli, corresponding to five times the LD<sub>50</sub>. This dose was selected because in the LD<sub>50</sub> determination study, all animals challenged with this dose died within 4 days, with a mortality of approximately 50% after 2 days. Mortality was recorded daily for 2 weeks. Animals surviving beyond this time point were considered healthy, because visible signs of illness were absent and bacteria could not be isolated from their tissues. In experiments comparing the protective effect of MAb III/5-1 and MAb Ru-O1, mice were pretreated i.p. with increasing doses of each of the MAbs and challenged with five times the LD<sub>50</sub> of strain Caroli 4 h later.

For bacterial clearance experiments, groups of five animals were pretreated with anti-LPS MAb Ru-O1 and anticapsular MAb III/5-1 at the doses indicated in the Results section and challenged with 50 organisms of *K. pneumoniae* Caroli, given i.p., 4 h later. After 90 min after infection, 0.2 ml of blood was obtained from the tail of each animal after careful skin disinfection and plated on blood agar. Colonies were counted after incubation at 37°C for 18 h, and the number of organisms per ml of blood was calculated.

To evaluate the influence of different routes of antibody application, we also performed experiments in which MAb Ru-O1 (40 µg/g) was injected i.v. 4 h before i.p. or i.v. challenge with 50 organisms of *K. pneumoniae* Caroli.

**Quantification of viable *K. pneumoniae* in infected organs.** In quantification experiments, mice were sacrificed on the first and second day after infection. The lungs, livers, and spleens were excised aseptically, placed on sterile steel nets, and homogenated by passage into sterile distilled water. Serial 10-fold dilutions of the homogenates were plated in duplicate onto blood agar plates (100 µl per plate). After incubation at 37°C for 24 h, colonies of *K. pneumoniae* were counted, and bacterial counts were expressed as log<sub>10</sub> CFU per organ.

**Histological examination.** Two randomly selected animals from each group were sacrificed on the first and second day after infection, and their lungs, livers, and spleens were excised and placed in 10% formalin. After fixation for 1 day, the tissues were fixed in ethanol and embedded in paraffin. Sections 5 µm thick were placed on glass slides and stained with hematoxylin and eosin.

**Statistical methods.** Bacterial counts in infected organs and blood were compared by using the Mann-Whitney *U* test.

## RESULTS

**Binding of MAb Ru-O1 to *Klebsiella* LPS.** The results of ELISA binding studies with MAb Ru-O1 are given in Table 1. The MAb reacted with LPS prepared from strain Friedländer 201 (O1:K-), which had been used for immunization, but also with other LPS prepared from strains belonging to the O1 serogroup, irrespective of their capsular types. In addition, binding was noted against the O19b antigen of *E. coli*, which has been shown to be immunologically identical to *Klebsiella* O1 (19). Furthermore, positive reactions were obtained also with the O6 and O8 antigens. In this respect, it is of interest that the O6 antigen has been found to be highly related to, if not identical to, the O1 antigen (1, 26). The O8 antigen was found recently to be very closely related to O1, although it differs from the latter serogroup by an O-acetylated polysaccharide side chain (20). In our own experiments, we could not differentiate between the O1 and O8 antigens by conventional serological methods, since homologous LPS-specific antibodies present in rabbit antisera prepared against reference strains Friedländer 201 (O1:K-) and 889 (O8:K69) could be completely removed by absorption with the heterologous strains (data not shown). Thus, both the strain representing the O6 antigen (strain NCTC 8172) and the one representing the O8 antigen (strain 889) may in fact belong to the O1 serogroup. To our knowledge, no strains of serogroups O6 and O8 other than the two mentioned have ever been described.

In addition to the studies summarized in Table 1, we performed ELISA inhibition experiments with all clinical *Klebsiella* strains of known O-antigen serotype that we described in

TABLE 1. Reaction of MAb Ru-O1 with LPS preparations from *Klebsiella* O-serogroup reference strains, additional *Klebsiella* strains, and serologically related *E. coli* strains

Source of LPS	Serogroup	Reaction with MAb Ru-O1 (OD <sub>405</sub> ± 1 SD) <sup>a</sup>
<i>K. pneumoniae</i> Friedländer		
201 <sup>b</sup>	O1:K-	3.10 ± 0.02
7380 <sup>b</sup>	O2ab:K-	0.02 ± 0.02
5053 <sup>b</sup>	O2ac:K-	0.07 ± 0.03
390 <sup>b</sup>	O3:K11	0.01 ± 0.01
1702 <sup>b</sup>	O4:K42	0.02 ± 0.01
4425/51 <sup>b</sup>	O5:K57	0.01 ± 0.01
NCTC 8172 <sup>b</sup>	O6:K64	3.24 ± 0.12
264(1) <sup>b</sup>	O7:K67	0.01 ± 0.01
889 <sup>b</sup>	O8:K69	2.99 ± 0.25
1205 <sup>b</sup>	O9:K72	0.02 ± 0.00
337 <sup>c</sup>	O10:K73	0.01 ± 0.01
378 <sup>b</sup>	O11:K78	0.00 ± 0.00
708 <sup>b</sup>	O12:K80	0.00 ± 0.00
<i>K. pneumoniae</i> Caroli		
37	O1:K2	2.91 ± 0.58
58	O1:K7	3.11 ± 0.08
557	O1:K7	3.32 ± 0.46
8238	O1:K21	3.19 ± 0.09
	O1:K37	3.21 ± 0.02
<i>E. coli</i>		
8188/41	O19ab <sup>d</sup>	2.09 ± 0.55
8858/41	O19a <sup>d</sup>	0.02 ± 0.00

<sup>a</sup> ELISA reaction was read after 30 min of incubation time. MAb Ru-O1 was used at a concentration of 10 µg/ml in casein-BSA. Results are from three independent experiments.

<sup>b</sup> *Klebsiella* O serogroup reference strains.

<sup>c</sup> Strain 337 was removed from the list of *Klebsiella* O-serogroup reference strains because it was found to belong to the genus *Enterobacter*. This LPS was included as a negative control.

<sup>d</sup> Figures refer to *E. coli* O serogroups. *E. coli* serogroup O19b has been found to be immunologically identical to *Klebsiella* O1 (19).

our previous report (37). MAb Ru-O1 reacted with 19 of 19 O1 serogroup isolates (sensitivity, 100%) but with none of 46 isolates belonging to other serogroups and none of 17 isolates that were not O-antigen typeable (specificity, 100%).

The conclusion that MAb Ru-O1 is directed against an immunodominant O-antigenic determinant representing the *Klebsiella* O1 serogroup is thus based on the following observations. (i) The MAb reacts with *E. coli* O19ab but not with a

corresponding *E. coli* strain expressing an O antigen that is the same except for the 19b component (Table 1). Thus, the MAb is specific for the O19b determinant, which is an immunodominant determinant of *E. coli* 8188/41 and is known to be immunologically identical to the major antigenic determinant of *Klebsiella* O1 (19). (ii) The MAb reacts with all clinical *Klebsiella* O1 serogroup isolates studied so far. (iii) The MAb does not react with the shared O-antigenic determinants of *Klebsiella* LPS that have been described. All *Klebsiella* strains listed in Table 1 except strain 264(1) (O7) have a common LPS epitope previously described by our group (36). However, MAb Ru-O1 reacts only with a subset of these strains containing O1, O6, or O8 serogroup LPS. Furthermore, strain 7380 (O2ab, Table 1) has been described as a strain harboring the D-galactan I component of LPS which is present in O1, O2, O6, O8, and O9 serogroup reference strains and may thus be regarded as a shared antigenic determinant of these strains (21). However, MAb Ru-O1 did not react with LPS derived from strains 7380 (O2ab) and 1205 (O9) and was thus not specific for D-galactan I. (iv) Immunoblotting experiments showed that MAb Ru-O1 reacted with high-molecular-weight components present in strains representing the O1, O6, O8, and *E. coli* O19b serogroups. The reaction pattern was identical to that obtained with a rabbit immune serum directed against the *E. coli* O19b determinant (data not shown).

**ELISA binding of MAb to whole bacteria.** The respective experiments are summarized in Table 2. It can be seen that MAb Ru-O1 bound well to the nonencapsulated mutant of strain Caroli, while insignificant binding occurred in the presence of the K2 capsule. In these experiments, the mouse IgG-specific antibody conjugate used as a tracer antibody was diluted at 1:30,000, corresponding to the working dilution suggested by the manufacturer. When the antibody conjugate was used at a 10-fold-higher concentration (1:3,000), mean ODs ± 1 standard deviation (SD) rose to 0.72 ± 0.11 for the encapsulated *K. pneumoniae* Caroli parent strain compared to 0.03 ± 0.01 for the control strain *E. coli* Bort (four experiments; *P* < 0.05). Thus, under very sensitive conditions, some degree of antibody penetration through the capsule could be detected. The findings were reproducible with a second serotype O1:K2 strain (strain 23). As anticipated, MAb III/5-1 bound strongly to the encapsulated K2 strains (Table 2).

**Protection studies.** Preliminary experiments showed no difference in mortality between animals pretreated with PBS or an irrelevant MAb of the IgG2b subclass, since in both groups,

TABLE 2. Binding of MAb Ru-O1 (O-antigen specific) and MAb III/5-1 (K2-antigen specific) to whole bacterial cells

MAb tested	OD <sub>405</sub> ± 1 SD				<i>E. coli</i> Bort (control)
	<i>K. pneumoniae</i> Caroli (O1:K2)		<i>K. pneumoniae</i> 23 (O1:K2)		
	Encapsulated parent strain	Nonencapsulated variant	Encapsulated parent strain	Nonencapsulated variant	
Ru-O1 (IgG2b)					
Normal test sensitivity <sup>a</sup>	0.09 ± 0.03	2.47 ± 0.07	0.13 ± 0.01	2.86 ± 0.32	0.03 ± 0.00
Enhanced sensitivity <sup>a</sup>	0.72 ± 0.11	≥4.00	0.67 ± 0.04	3.36 ± 0.13	0.03 ± 0.00
IgG2b control <sup>b</sup>					
Normal test sensitivity	0.03 ± 0.01	0.15 ± 0.06	0.03 ± 0.01	0.01 ± 0.00	0.03 ± 0.01
Enhanced sensitivity	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.02	0.07 ± 0.02	0.05 ± 0.03
III/5-1 (IgM)					
Normal test sensitivity	3.36 ± 0.14	0.15 ± 0.06	2.87 ± 0.29	0.10 ± 0.03	0.08 ± 0.00
IgM control <sup>b</sup>					
Normal test sensitivity	0.17 ± 0.11	0.08 ± 0.03	0.17 ± 0.04	0.13 ± 0.07	0.07 ± 0.03

<sup>a</sup> For details, see text.

<sup>b</sup> Control MAbs were clones MOPC 141 (IgG2b) and TEPC 183 (IgM).

TABLE 3. Protective effect of MAb III/5-1 and MAb Ru-O1 in animals challenged with *K. pneumoniae* Caroli

Dose of MAb ( $\mu\text{g/g}$ )	No. of animals surviving/no. challenged	
	MAb III/5-1	MAb Ru-O1
0.25	5/5	ND <sup>a</sup>
0.50	5/5	ND
1.00	10/10	0/10
10.0	10/10	0/10
20.0	10/10	1/10
40.0	ND	7/10
200.0	ND	6/10

<sup>a</sup> ND, not done.

all animals died within 4 days after challenge. Therefore, control animals received PBS in further experiments. When animals were pretreated with graded doses of MAb III/5-1 (capsule specific) and MAb Ru-O1 (O-antigen specific), it became apparent that the anticapsular antibody was by far more protective on a weight basis. The dose of antibody protecting 50% of the animals was  $<0.25 \mu\text{g/g}$  for MAb III/5-1 but  $\sim 30 \mu\text{g/g}$  for MAb Ru-O1 ( $P < 0.01$ ) (Table 3).

The influence of MAb pretreatment on the level of bacteremia was examined in groups of five animals 90 min after i.p. challenge with 50 CFU of *K. pneumoniae* Caroli. The numbers of bacteria in the blood, expressed as CFU per milliliter  $\pm 1$  SD, were  $77 \pm 49$  (control without pretreatment),  $0 \pm 0$  (pretreatment with MAb III/5-1 at  $0.25 \mu\text{g/g}$ ;  $P \leq 0.01$  versus control),  $22 \pm 17$  (pretreatment with MAb Ru-O1 at  $0.25 \mu\text{g/g}$ ;  $P \geq 0.05$  versus control), and  $10 \pm 8$  (pretreatment with MAb Ru-O1 at  $40 \mu\text{g/g}$ ;  $P \leq 0.01$  versus control). Thus, the reduction of bacteremia effected by the MAbs was closely correlated to the overall protective efficacy of the MAbs as measured by survival.

In order to determine whether MAb Ru-O1 would protect also when given via the i.v. route, we performed an experiment in which the MAb ( $40 \mu\text{g/g}$ ) was injected i.v. to animals given 50 CFU of *K. pneumoniae* Caroli i.p. or i.v. (five animals per group). None of the control animals without MAb pretreatment survived, while three of five animals given i.v. MAb before i.p. infection and five of five animals given i.v. MAb before i.v. infection survived. Thus, protection obtained by i.v. pretreatment was at least as good as that obtained by i.p. pretreatment with anti-LPS MAb.

In an attempt to show the protective effects of the anti-LPS MAb for strains harboring the O6 and O8 antigens, we used the respective reference strains (strains NCTC 8172 and 889, Table 1) for LD<sub>50</sub> determinations in groups of five mice. However, both strains were weakly pathogenic, with LD<sub>50</sub>s exceeding  $10^8$  organisms per animal (data not shown). At these doses, animals died rapidly as a result of intoxication rather than infection. Therefore, no attempt was made to use these strains for protection experiments. The observation that strains harboring capsular antigens other than K1 and K2 are weakly pathogenic for mice has been described previously by Kauffmann (19) and Mizuta and coworkers (25). Thus, although the O1 antigen appears to be relevant for full expression of pathogenicity (30, 31), the presence of this antigen (and of the related antigens O6 and O8) is by itself not sufficient to cause lethal infection.

**Bacterial counts in infected organs.** In further experiments with MAb Ru-O1, the optimally protective dose of  $40 \mu\text{g/g}$  was used. As shown in Fig. 1, prophylactic administration of MAb Ru-O1 led to a significant reduction of bacterial counts in

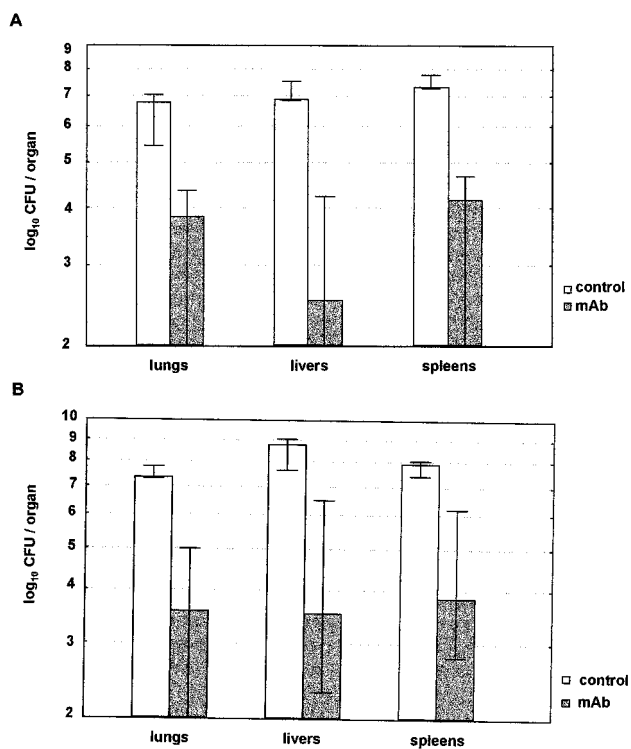


FIG. 1. Bacterial counts in organs of mice infected with *K. pneumoniae* Caroli and previously protected or not protected with MAb Ru-O1 on day 1 (A) or day 2 (B) after infection. Columns represent the median CFU per organ for 10 animals. Error bars represent the range between minimal and maximal CFUs per organ.

lungs, livers, and spleens compared to those in control mice. At macroscopic inspection, lungs of MAb-treated mice were smaller than those of control animals, and multiple visible liver abscesses were found in the control group only (data not shown).

**Histological examination.** Twenty-four hours after infection, lungs of MAb-pretreated animals appeared to be almost normal, with mild peribronchial and septal infiltration by granulocytes and monocytes in less than 5% of the areas examined (Fig. 2A). At the same time, cellular infiltration was much more pronounced and was associated with perivascular edema in the lungs of control animals in more than 30% of the areas investigated (Fig. 2B). Histological changes in the control group were even more pronounced at 48 h after infection, with consolidated areas filled with extravasated granulocytes, monocytes, and erythrocytes, while cellular infiltration in the MAb-pretreated group remained moderate (data not shown).

## DISCUSSION

O-antigen-specific antibodies have been shown to be protective in experimental models of *E. coli* (13, 22) and *Pseudomonas aeruginosa* infection (16, 27). The immunoprophylactic effect of such antibodies has led to the development of O-antigen-based vaccines comprising the *E. coli* and *P. aeruginosa* O antigens most commonly encountered in clinical material (6, 7). Furthermore, an O-antigen-specific hyperimmune globulin preparation made from the plasma of volunteers screened for antibody against a panel of *Pseudomonas* O antigens has been in clinical use in Europe for several years (5).

By contrast, little is known about the immunoprotective ef-

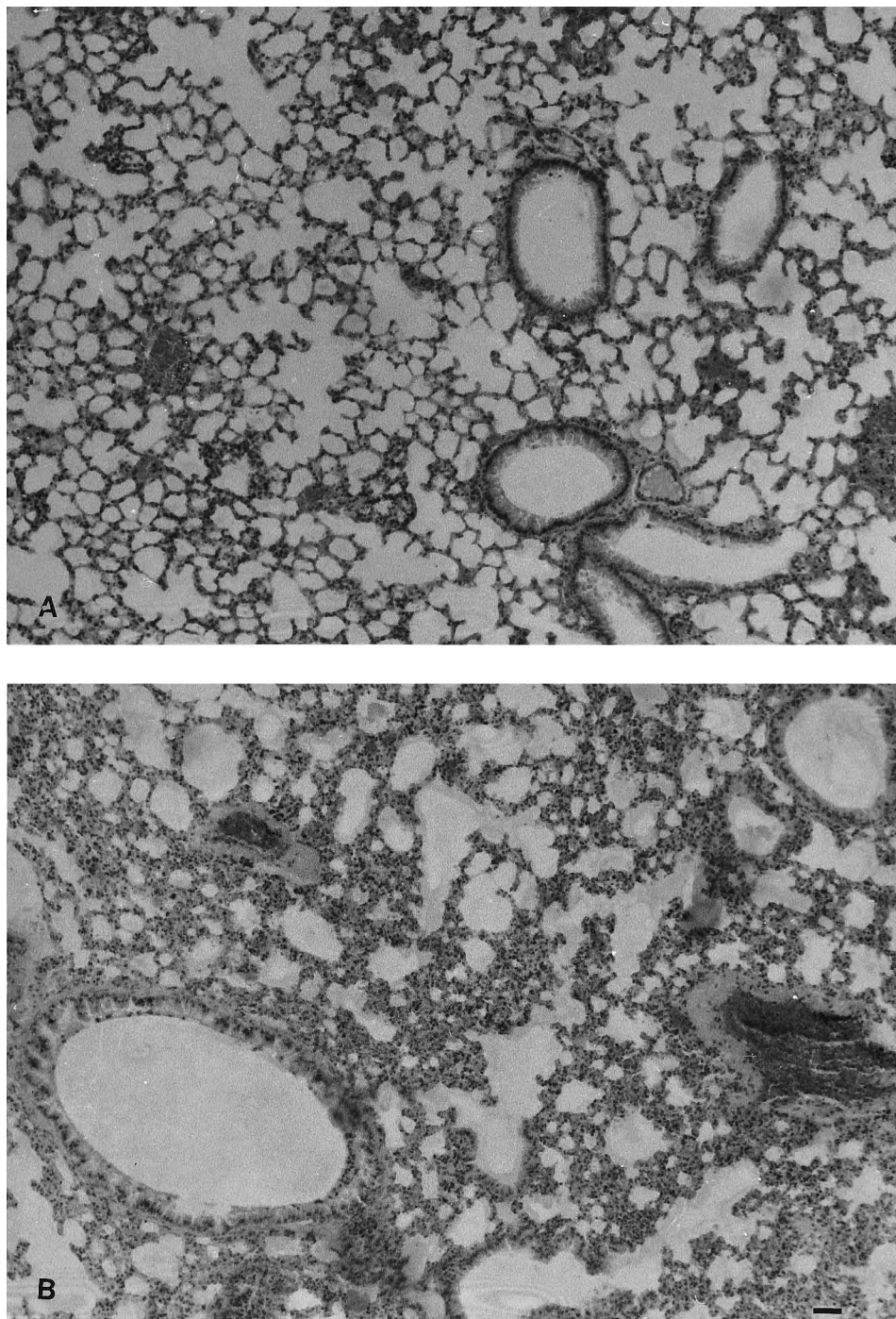


FIG. 2. Photomicrograph of hematoxylin-and-eosin-stained mouse lung 24 h after infection with *K. pneumoniae* Caroli. The mice were pretreated with MAb Ru-O1 (A) or PBS (B). The bar represents 50  $\mu$ m.

fect of O-antigen-specific antibodies in *Klebsiella* infections. Since, compared to the capsular antigens, the number of O serogroups in the genus *Klebsiella* is relatively small (with a total of nine established serogroups), an O-antigen-targeted immunotherapy of *Klebsiella* infection may be feasible, provided that O-antigen-specific antibodies can be shown to afford protection against encapsulated *Klebsiella* strains.

It has been shown previously by other authors that polyclonal antibodies raised against *Klebsiella* LPS may penetrate through the *Klebsiella* capsule and react with their target

epitopes on the bacterial cell surface, as detected by ELISA (32, 33) or immunogold staining (24). However, different *Klebsiella* capsular serotypes appear to have various capacities for "shielding" the bacterial cell surface against O-specific antibodies. Thus, while the K1, K10, and K16 capsules act as complete barriers against the penetration of O-specific antibodies, other serotypes, including K2, allow at least partial binding of O antibodies compared to what nonencapsulated control strains allow (32, 33).

The MAb examined in the present study was therefore char-

acterized not only with respect to its binding to purified LPS but also for its ability to detect cell-bound LPS on the surfaces of both nonencapsulated and encapsulated *Klebsiella* strains. In experiments using purified LPS from various O-antigen prototype strains, we found that MAb Ru-O1 reacted with *Klebsiella* serogroups O1, O6, and O8. The cross-reaction of the O1-specific MAb with strains representing the O6 and O8 serogroups was not surprising, since it was shown previously that both the O6 and the O8 serogroups are highly related to O1 (20, 21, 26). In fact, although the O8 antigen appears to differ chemically from the O1 antigen (20, 21), our own data show that these two antigens cannot be differentiated by conventional serology. We also found that MAb Ru-O1 reacted with *E. coli* 8188/41 (O19ab), which expresses an O antigen found previously to be serologically identical to that of *Klebsiella* O1 (19). Therefore, the LPS binding data show that MAb Ru-O1 recognizes an immunodominant epitope present in the side chain of *Klebsiella* O1 LPS.

For binding studies with whole organisms, we used a serogroup O1:K2 strain, *K. pneumoniae* Caroli, which had been found in previous studies to be highly virulent for mice and rats (4, 15, 34). As shown in Table 2, the MAb bound well to a nonencapsulated mutant of this strain, while its binding to the parent strain was considerably reduced by the presence of the K2 capsule. Similar findings were obtained with a second serotype O1:K2 strain and its isogenic, nonencapsulated variant. The fact that some degree of binding to the encapsulated strains was noted under conditions of enhanced sensitivity may possibly be explained by heterogeneity of the bacterial population, which may include subpopulations exhibiting reduced capsule layers (see below).

We subsequently analyzed the ability of the MAb to protect in a model of experimental *Klebsiella* infection induced by strain Caroli. Compared to a capsule-specific MAb described previously, the amount of MAb needed to provide 50% protection was more than 100-fold higher ( $\sim 30 \mu\text{g/g}$  compared to  $<0.25 \mu\text{g/g}$ ). However, subsequent studies using the optimally protective dose of  $40 \mu\text{g/g}$  showed that the MAb was able to reduce the number of organisms in the blood, to inhibit bacterial dissemination to various organs, and to reduce sepsis-related lung injury (Fig. 1 and 2). The fact that the MAb promoted bacterial clearance in the bloodstream shows that it is opsonic under in vivo conditions.

In contrast to these in vivo findings, initial in vitro phagocytosis experiments with MAb Ru-O1 performed in our laboratory by means of a previously described microplate method (34) showed that the MAb opsonizes only nonencapsulated O1 serogroup strains. Thus, at a 2:1 ratio of bacteria to human granulocytes, a  $5\text{-}\mu\text{g/ml}$  final MAb concentration in the assay system induced a  $94.4\% \pm 3.0\%$  uptake of the nonencapsulated variant of *K. pneumoniae* Caroli. By contrast, the encapsulated parent strain was not phagocytosed under the same assay conditions, while the anticapsular MAb III/5-1 was highly opsonic, with a mean bacterial uptake of  $92.2\% \pm 5.1\%$  ( $P < 0.001$ ) (33a). At first site, these data appear to be at variance with the observation of an in vivo phagocytosis-promoting effect of the anti-LPS MAb. However, during multiplication in vivo, significant subpopulations of encapsulated organisms may have a reduced capsule thickness or even lack the capsule, as suggested by data reported by Frasa et al. for *E. coli* (13). Therefore, more detailed studies are needed to fully elucidate the opsonophagocytic effect of MAb Ru-O1. Furthermore, in addition to promoting phagocytosis, O-antigen-specific MAbs may also exert protection by neutralizing circulating free LPS and thereby reduce activation of proinflammatory cytokines (13, 22). Straus et al. showed that the release of soluble LPS

plays a significant role in the pathogenesis of *Klebsiella*-induced lung injury (30, 31). Further studies, in which we will analyze the contribution of various mechanisms to the overall protective effect exerted by MAb Ru-O1, are therefore under way in our laboratory.

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#### REFERENCES

- Albertí, S., S. Hernández-Allés, J. Gil, et al. 1993. Development of an enzyme-linked immunosorbent assay method for typing and quantitation of *Klebsiella pneumoniae* lipopolysaccharide: application to serotype O1. *J. Clin. Microbiol.* **31**:1379-1381.
- Bryan, C. S., K. L. Reynolds, and E. R. Brenner. 1983. Analysis of 1,186 episodes of gram-negative bacteremia in non-university hospitals: the effects of antimicrobial therapy. *Rev. Infect. Dis.* **5**:629-638.
- Carpenter, J. L. 1990. *Klebsiella* pulmonary infections: occurrence at one medical center and review. *Rev. Infect. Dis.* **12**:672-682.
- Chedd, L., M. Parant, F. Parant, and F. Boyer. 1968. A proposed mechanism for natural immunity to enterobacterial pathogens. *J. Immunol.* **100**:292-301.
- Collins, M. S., and R. E. Roby. 1984. Protective activity of an intravenous immune globulin (human) enriched in antibody against lipopolysaccharide antigens of *Pseudomonas aeruginosa*. *Am. J. Med.* **76**:168-174.
- Cross, A., A. Arstenstein, J. Que, T. Fredeking, E. Furer, J. C. Sadoff, and S. J. Cryz, Jr. 1994. Safety and immunogenicity of a polyvalent *Escherichia coli* vaccine in human volunteers. *J. Infect. Dis.* **170**:834-840.
- Cross, A. S., and S. J. Cryz, Jr. 1990. Vaccines against *Klebsiella* and *Pseudomonas* infections, p. 699-713. *In* G. C. Woodrow and M. M. Levine (ed.), *New generation vaccines*. Marcel Dekker, Inc., New York.
- Cross, A. S. 1995. Intravenous immunoglobulins (IVIGs) to prevent and treat infectious diseases, p. 123-130. *In* M. Z. Atassi and G. S. Bixler (ed.), *Immunobiology of proteins and peptides VIII*. Plenum Press, New York.
- Cryz, S. J., Jr., A. S. Cross, E. Furer, N. Chariatte, J. C. Sadoff, and R. Germanier. 1986. Activity of intravenous immune globulins against *Klebsiella*. *J. Lab. Clin. Med.* **108**:182-189.
- Donta, S. T., P. Peduzzi, A. S. Cross, et al. 1996. Immunoprophylaxis against *Klebsiella* and *Pseudomonas aeruginosa* infections. *J. Infect. Dis.* **174**:537-543.
- Edelman, R., D. N. Taylor, S. S. Wassermann, J. B. McClain, A. S. Cross, J. C. Sadoff, J. U. Que, and S. J. Cryz. 1994. Phase 1 trial of a 24-valent *Klebsiella* capsular polysaccharide vaccine and an eight-valent *Pseudomonas* O-polysaccharide conjugate vaccine administered simultaneously. *Vaccine* **12**:1288-1294.
- Ehrenworth, L., and H. Baer. 1956. The pathogenicity of *Klebsiella pneumoniae* for mice: the relationship to the quantity and rate of production of type-specific capsular polysaccharide. *J. Bacteriol.* **72**:713-717.
- Frasa, H., B. Benaissa-Trouw, L. Tavares, K. van Kessel, M. Poppelier, K. Kraaijeveld, and J. Verhoef. 1996. Enhanced protection by use of a combination of anticapsule and antilipopolysaccharide monoclonal antibodies against lethal *Escherichia coli* O18K5 infection of mice. *Infect. Immun.* **64**:775-781.
- Fujita, S., and F. Matsubara. 1984. Latex agglutination test for O serotyping of *Klebsiella* species. *Microbiol. Immunol.* **28**:731-734.
- Held, T. K., M. Trautmann, M. E. A. Mielke, H. Neudeck, S. J. Cryz, and A. S. Cross. 1992. Monoclonal antibody against *Klebsiella* capsular polysaccharide reduces severity and hematogenic spread of experimental *Klebsiella pneumoniae* pneumonia. *Infect. Immun.* **60**:1771-1778.
- Holder, I. A. 1988. *Pseudomonas* immunotherapy. *Serodiagn. Immunother.* **2**:7-16.
- Horan, T., D. Culver, W. Jarvis, et al. 1988. Pathogens causing nosocomial infections. Preliminary data from the National Nosocomial Infections Surveillance System. *Antimicrob. Newsl.* **5**:65-67.
- Jarvis, V. R., V. P. Munn, A. K. Highsmith, D. H. Culver, and J. M. Hughes. 1985. The epidemiology of nosocomial infection caused by *Klebsiella pneumoniae*. *Infect. Control* **6**:68-74.
- Kauffmann, F. 1949. On the serology of the *Klebsiella* group. *Acta Pathol. Microbiol. Scand.* **26**:381-406.
- Kelly, R. F., W. B. Severn, J. C. Richards, M. B. Perry, L. L. MacLean, J. M. Tomas, S. Merino, and C. Whitfield. 1993. Structural variation in the O specific polysaccharides of *Klebsiella pneumoniae* serotype O1 and O8 lipopolysaccharide: evidence for clonal diversity in rfb genes. *Mol. Microbiol.* **10**:615-625.
- Kelly, R. F., and C. Whitfield. 1996. Clonally diverse *rfb* gene clusters are involved in expression of a family of related D-galactan O antigens in *Klebsiella* species. *J. Bacteriol.* **178**:5205-5214.



22. Kim, K. S., J. H. Kang, A. S. Cross, B. Kaufman, W. Zollinger, and J. Sadoff. 1988. Functional activities of monoclonal antibodies to the O side chain of *Escherichia coli* lipopolysaccharides in vitro and in vivo. *J. Infect. Dis.* **157**:47–53.
23. Lang, A. B., U. Bruderer, G. Senyk, T. L. Pitt, J. W. Larrick, and S. J. Cryz, Jr. 1991. Human monoclonal antibodies specific for capsular polysaccharides of *Klebsiella* recognize clusters of multiple serotypes. *J. Immunol.* **146**:3160–3164.
24. Meno, Y., and K. Amako. 1990. Morphological evidence for penetration of anti-O antibody through the capsule of *Klebsiella pneumoniae*. *Infect. Immun.* **58**:1421–1428.
25. Mizuta, K., M. Ohta, M. Mori, T. Hasegawa, I. Nakashima, and N. Kato. 1983. Virulence for mice of *Klebsiella* strains belonging to the O1 group: relationship to their capsular (K) types. *Infect. Immun.* **40**:56–61.
26. Ørskov, I., and F. Ørskov. 1984. Serotyping of *Klebsiella*. *Methods Microbiol.* **14**:143–164.
27. Pennington, J. E., G. J. Small, M. E. Lostrom, and G. B. Pier. 1986. Polyclonal and monoclonal antibody therapy for experimental *Pseudomonas aeruginosa* pneumonia. *Infect. Immun.* **54**:239–244.
28. Reed, L. J., and H. Muench. 1938. A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.* **27**:493–497.
29. Simoons-Smit, A. M., A. M. Verweij-Van Vught, and D. M. MacLaren. 1986. The role of K antigens as virulence factors in *Klebsiella*. *J. Med. Microbiol.* **21**:133–137.
30. Straus, D. C., D. L. Atkisson, and C. W. Garner. 1985. Importance of a lipopolysaccharide-containing extracellular toxic complex in infections produced by *Klebsiella pneumoniae*. *Infect. Immun.* **50**:787–795.
31. Straus, D. C. 1987. Production of an extracellular toxic complex by various strains of *Klebsiella pneumoniae*. *Infect. Immun.* **55**:44–48.
32. Tomas, J. M., S. Camprubi, and P. Williams. 1988. Surface exposure of the O-antigen in *Klebsiella pneumoniae* O1-K1 serotype strains. *Microb. Pathog.* **5**:141–147.
33. Tomas, J. M., S. Camprubi, S. Merino, M. R. Davey, and P. Williams. 1991. Surface exposure of O1 serotype lipopolysaccharide in *Klebsiella pneumoniae* strains expressing different K antigens. *Infect. Immun.* **59**:2006–2011.
- 33a. Trautmann, M. Unpublished data.
34. Trautmann, M., S. J. Cryz, Jr., J. C. Sadoff, and A. S. Cross. 1988. A murine monoclonal antibody against *Klebsiella* capsular polysaccharide is opsonic in vitro and protects against experimental *Klebsiella pneumoniae* infection. *Microb. Pathog.* **5**:177–187.
35. Trautmann, M., M. Ghandchi, T. Held, S. J. Cryz, and A. S. Cross. 1991. An enzyme-linked immunosorbent assay for the detection of soluble *Klebsiella pneumoniae* capsular polysaccharide. *J. Microbiol. Methods* **13**:305–313.
36. Trautmann, M., K. Vogt, C. Hammack, and A. S. Cross. 1994. A murine monoclonal antibody defines a unique epitope shared by *Klebsiella* lipopolysaccharides. *Infect. Immun.* **62**:1282–1288.
37. Trautmann, M., A. S. Cross, G. Reich, T. K. Held, R. Podschun, and R. Marre. 1996. Evaluation of a competitive ELISA method for the determination of *Klebsiella* O antigens. *J. Med. Microbiol.* **44**:44–51.
38. Westphal, O., and K. Jann. 1965. Bacterial lipopolysaccharides: extraction with phenol-water and further applications of the procedure. *Methods Carbohydr. Chem.* **5**:83–91.
39. Williams, P., P. A. Lambert, M. R. W. Brown, and J. Jones. 1983. The role of the O and K antigens in determining the resistance of *Klebsiella* aerogenes to serum killing and phagocytosis. *J. Gen. Microbiol.* **129**:2181–2191.

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