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Significance of Circulating Capsular Antigen in Klebsiella Infections

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Klebsiella capsular antigen (KCA) was detected in serum by counterimmunoelectrophoresis in 8 of 31 patients with klebsiella bacteremia, in two nonbacteremic patients with pneumonia and meningitis, respectively, and in the cerebrospinal fluid only of 1 of the 31 bacteremic patients. It was also detected in cerebrospinal fluid, urine (two patients each) empyema fluid, and abscess drainage (one patient each). Patients whose bacteremias were associated with a discernible tissue focus (e.g., pneumonia) tended to have detectable serum KCA more often than those with "primary bacteremia." A fatal outcome occurred in six of nine bacteremia patients with detectable serum KCA compared with 4 of 22 without demonstrable antigen ($P < 0.05$). Persistent antigenemia and antigenuria aided in the diagnosis of perinephric abscess in one patient, and increasing levels of serum KCA anticipated treatment failure in another patient with pneumonia. The presence of detectable KCA in the serum of patients infected with klebsiella thus appeared to correlate with severity of infection, with persistence of active foci, and with a poorer prognosis than in those patients who had no detectable antigen. Whether the presence of this antigen itself plays any pathogenic role needs to be further clarified.

Klebsiella infections are an important cause of hospital-associated morbidity and mortality. Recent figures indicate that *Klebsiella pneumoniae* is the most common bacterial agent in hospital-acquired respiratory infections and second only to *Escherichia coli* as a cause of nosocomial bacteremias (Center for Disease Control, National Nosocomial Infections Study data, 1970 to 1974).

Pathogenic klebsiella strains are usually encapsulated with a polysaccharide (K antigen) whose structural variability forms the basis for a typing system which includes more than 72 different immunotypes (9). The physiochemical properties of klebsiella capsular polysaccharides are similar to those of the pneumococcus, meningococcus, and *Haemophilus influenzae* type b. The detection of capsular material from these latter organisms in body fluids of infected patients has proven diagnostically and prognostically useful (1, 3-8, 10, 12-18). It has also added to our understanding of the natural course of these infections and of the development of specific host immunity (3, 4, 13, 18).

We report here the detection of circulating klebsiella capsular antigen (KCA) in patients with klebsiella infections. Data are presented which suggest a correlation between antigenemia and extent, persistence, and outcome of infection. The significance of these findings, as well as the possible diagnostic usefulness of antigen detection in klebsiella infections, are discussed.

MATERIALS AND METHODS

Patients. Thirty-three patients with severe klebsiella infections were studied. Thirty-one were bacteremic and two without bacteremia had culture-positive meningitis and cavitary pneumonia, respectively.

Clinical samples. Initial blood samples were obtained for antigen testing as close to the time of diagnosis of bacteremia as possible. Control blood samples were drawn from six normal medical students and house officers and from seven patients with bacteremias due to *E. coli* (three), pseudomonas (two), enterobacter (one), and pneumococcus (one).

Blood cultures. Venous blood samples were inoculated into a Trypticase soy broth bottle (BBL), which was vented, and a Vacutainer-supplemented peptone broth tube (Becton-Dickinson), which was unvented. These were examined daily for hemolysis and turbidity and subcultured at the first sign of either, or at 2, 5, and 10 days. Isolates were identified as *K. pneumoniae* on the basis of standard biochemical reactions and motility (9).

Klebsiella isolates. Klebsiella strains were grown overnight at 37 C on Worfel-Ferguson agar plates

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(Difco). Organisms were suspended in phosphate-buffered saline and typed by the Quellung or Neufeld reaction (9) using klebsiella typing sera described below.

Klebsiella antisera. Rabbit antisera to all of the known 72 klebsiella capsular types were obtained from the Center for Disease Control, Atlanta, Ga. They were prepared by immunizing rabbits with acetone-killed whole-cell vaccine according to a 2-week intravenous dosage schedule. The lyophilized antisera were reconstituted with distilled water and kept at 4 C.

Counterimmunoelectrophoresis. A counterimmunoelectrophoresis (CIE) kit (Hyland) designed to screen serum samples for hepatitis antigen was used. It consisted of an electrophoresis chamber, agarose plate with prepunched wells, sponge contacts, electrodes, and veronal buffer (pH 8.3 to 8.6). The capsular types of klebsiella isolated from patients were determined beforehand by the Quellung reaction and the appropriate type antiserum was used for antigen testing. Approximately 10 μl of serum or other samples and the same volume of homologous klebsiella antiserum were placed in wells opposite one another. The samples were subjected to a current of 40 mA for 60 min at room temperature. Precipitin lines were read immediately and again after 2 h of refrigeration at 4 C and were then scored on a 0 to 4+ scale. Washing and staining agarose plates with amido black did not increase the resolution of precipitin lines.

Testing antisera against klebsiella culture supernatants by CIE. Supernatants from overnight broth cultures of all 72 klebsiella capsular types were run by CIE against homologous as well as known cross-reacting heterologous klebsiella rabbit antisera, determined by the Quellung reaction (9). Strong precipitin lines were formed, in most cases, between culture supernatants and homologous-type antisera. Cross-reactions occurred but were readily eliminated by absorbing sera with cross-reacting organisms.

Specificity of precipitin reactions obtained by CIE for KCA (K antigen). The specificity of precipitin reactions obtained by CIE for capsular polysaccharide antigens was supported by three lines of evidence. (i) As indicated above, positive CIE tests correlated precisely with capsular typing by the Quellung method, which, in turn, is thought to be specific for capsular antigens (9). (ii) Cross-reactions by CIE among heterologous klebsiella capsular type strains corresponded to known cross-reactions among different Quellung types (9) and could be eliminated by first absorbing antisera with heterologous cross-reacting strains. These cross-reactions are thought to be due to antigenic determinants common to certain heterologous capsular immunotypes (9). (iii) Isolation and purification of capsular polysaccharide from a type 2 klebsiella strain, as outlined below, resulted in an antigen that gave a greatly enhanced precipitin line on CIE when run against type 2 antiserum; conversely, absorption of type 2 antiserum with homologous polysaccharide resulted in loss of precipitin lines when this antiserum was run against either type 2 culture filtrates or CIE-positive serum from patient no. 7 who had a type 2 klebsiella infection.

Quantification of type 2 KCA in clinical samples. Capsular polysaccharide was extracted by the method of Gormus and Wheat (11) from a type 2 klebsiella strain isolated from the blood of patient no. 11. The resulting preparation was weighed and serial twofold dilutions in phosphate-buffered saline and human serum were run by CIE against type 2 antiserum. The "standard curve" thus obtained was used to quantify by serial dilution the amount of capsular polysaccharide present in serum and other body fluids of the same patient (no. 11).

RESULTS

Results of antigen detection by CIE are summarized in Tables 1 and 2. KCA, identified by precipitin lines, was present in the blood of 8 of 31 patients with klebsiella bacteremia and the two nonbacteremic patients. Another bacteremic patient (no. 9) with klebsiella meningitis had detectable cerebrospinal fluid (CSF) antigen not detected in a blood sample taken simultaneously.

Only 2 of 14 patients with "primary bacteremia" had detectable serum KCA, compared with 7 of 17 whose bacteremias were associated with a discernible primary focus (Table 2). Although this difference was suggestive, it was not statistically significant ($0.10 < P < 0.20$) possibly because of the limited number of patients. All four patients with klebsiella pneumonia had antigenemia; large amounts of KCA (precipitin lines scored as 3 or 4+) were present in three. As noted, one of these patients did not have positive blood cultures, and a second developed bacteremia relatively late in the course of his infection, several weeks after antigen first appeared in his blood (Fig. 1). Serum KCA was also detected in patients with meningitis and abdominal and urinary tract infections, but in none of four with septic thrombophlebitis (Table 2).

KCA was detectable in various body fluids in addition to serum. It was demonstrated in the CSF in two patients, in pleural empyema fluid in another patient (both initially when the fluid was culture-positive as well as later when sterile), and in drainage from a surgically incised perinephric abscess. Urine was positive for KCA in two of five patients tested, one with a perinephric abscess and another with pneumonia whose serum was simultaneously positive for KCA. Control sera were negative when run with antisera against all 72 klebsiella types.

Detectable antigen persisted from 3 days to more than 4 months (Table 1). Patient no. 7 with fatal klebsiella pneumonia had detectable antigen in blood and urine for 52 days until the

TABLE 1. *Patients positive for klebsiella antigen*

Patient	Infection	Blood culture	Klebsiella type	Outcome	CIE		
					Sample	Score	Duration of + test
1	Pneumonia	−	1	Survived	Blood	3+	5 days +
2	Meningitis	−	30	Survived	CSF[a]	4+	3 days
					Blood	1+	3 days
3	Primary bacteremia	+	23	Died	Blood	1+	8 days
4	Pyelonephritis	+	1	Died	Blood	1+	?
5	Pneumonia	+	2	Died	Blood	4+	Died 2nd
					CSF	3+	day
6	Primary bacteremia	+	54	Died	Blood	2+	11 days
7	Pneumonia	−→+	1	Died	Blood	4+	52 days
					Urine	2+	
8	Empyema	+	20	Survived	Blood	1+	?
9	Meningitis	+	2	Survived	CSF	1+	?
10	Peritonitis	+	11/21	Died	Blood	1+	13 days
11	Perinephric abscess, empyema	+	2	Survived, not cured	Blood	2+	4.5 months
					Urine	4+	4.5 months
					Empyema	4+	2 months
					Abscess	4+	2 months

[a] CSF, Cerebrospinal fluid.

TABLE 2. *Detection of klebsiella capsular polysaccharide in serum by CIE*

Infection	Capsular polysaccharide		
	Present	Absent	Total
Primary bacteremia	2	12	14
Urinary tract infection	2	5	7
Pneumonia (1 empyema)	4	0	4
Thrombophlebitis	0	4	4
Abdominal infection	1	1	2
Meningitis	2	0	2
All klebsiella infections	11	22	33
Non-klebsiella bacteremia	0	7	7
Normal controls	0	6	6
All controls	0	13	13

time of his death. Patient no. 11 was followed for a total of 20 weeks with small amounts of serum antigen still present at the end of that time; this coincided with gradual resolution of her perinephric abscess and empyema.

There was a close correlation between circulating KCA and the severity of underlying infection. Patients with readily apparent tissue infections, such as those with pneumonia or an abscess, had high and sometimes persistent levels of serum KCA. Further, a positive antigen test was associated with a poor prognosis. Among patients with klebsiella bacteremia, six of nine with detectable serum KCA died, compared to 4 deaths among 22 patients without demonstrable antigen ($P < 0.05$). Of the six patients with positive antigen tests who died, five had clinical evidence that their klebsiella infections had directly contributed to their death, and in these same five patients circulating KCA was detectable immediately before death. On the other hand, among the four patients without detectable antigen who died, death was temporally remote from clinical infection in three and therefore not ostensibly attributable to their klebsiella infections.

KCA in serum and other fluids was semiquantified in all patients by scoring precipitin lines on a 0 to 4+ scale (Table 1). Type 2 KCA extracted from the strain infecting patient no. 11 (perinephric abscess, empyema, sepsis) was used to "titrate" precipitin lines obtained by CIE of the polysaccharide against its homologous antiserum. The results are shown in Table 3. The smallest concentration of polysaccharide detectable was 0.25 μg/ml. Based on this titration, Fig. 2 shows the results of serial determinations of type 2 polysaccharide concentration in the serum, urine, and pleural fluid of patient no. 11 over the course of 20 weeks. Values shown are the lowest possible concentrations represented by precipitin lines of various intensities. The rapid drop-off of antigen in the urine between weeks 4 and 5 corresponds in time to surgical drainage of the patient's perinephric abscess, and the persistence of antigen in the

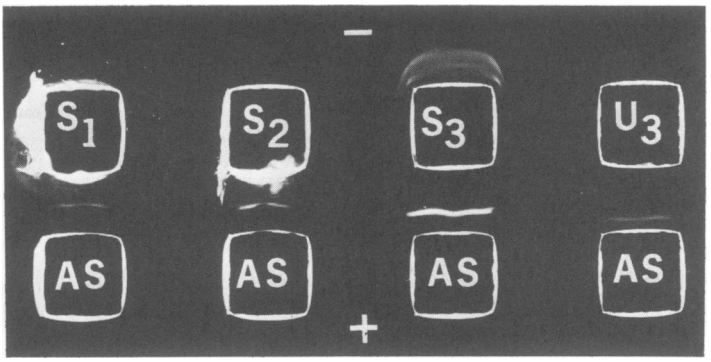

FIG. 1. *CIE test for KCA in patient no. 7 with fatal klebsiella pneumonia. Wells closest to anode (+) contain type 1 klebsiella antiserum (AS). Wells closest to cathode (−) contain serial serum samples obtained at approximately 14-day intervals (S_1, S_2, S_3) and the last well in this row contains unconcentrated urine (U_3) obtained at the same time as serum sample S_3. These latter serum and urine samples (S_3 and U_3) were the last obtained from the patient shortly before death. Note the increasing intensity of precipitin lines obtained in serial serum samples, coinciding with worsening and ultimately fatal pneumonia. Despite frequent blood cultures, bacteremia was detected only once, in the interval between S_2 and S_3.*

TABLE 3. *Quantification of klebsiella type 2 capsular polysaccharide by CIE*

Precipitin line	Capsular polysaccharide concn (μg/ml)
1+	0.25–0.5
2+	1–2
3+	4–8
4+	>15

serum corresponds to the more gradual resolution of her bilateral pleural empyemas and systemic symptoms.

DISCUSSION

KCA was detectable in the blood and other body fluids of some but not all patients with serious klebsiella infections. Our data suggest that a positive test for serum KCA correlated better with the presence of an extravascular focus of infection than with bacteremia. If we consider the levels of circulating antigen as indicated by the intensity of precipitin lines, the correlation between tissue infection and antigenemia was even more pronounced. Likewise, all four patients with pneumonia due to klebsiella who had obvious radiographic and other clinical evidence of extensive tissue infection demonstrated circulating KCA, and among these patients there was little relation between the finding of serum antigen and positive blood cultures. In one there was no detectable bacteremia despite circulating KCA, in another antigenemia preceded positive blood cultures by more than 2 weeks, and in two other patients detectable serum antigen persisted long after bacteremia.

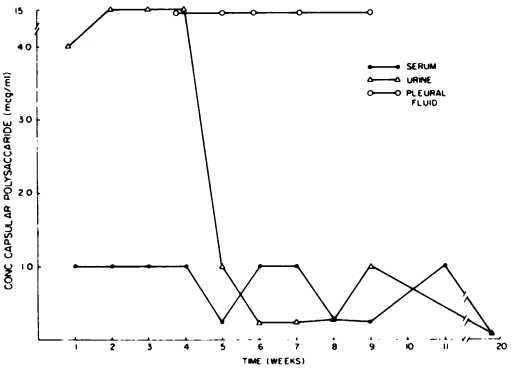

FIG. 2. *Levels of klebsiella type 2 capsular polysaccharide in the serum, urine, and pleural fluid of patient no. 11 (perinephric abscess, empyema, klebsiella sepsis). Concentrations are based on serial dilutions of purified type 2 capsular polysaccharide run by CIE against type-specific rabbit antiserum.*

The persistence or failure to clear KCA also appeared to be significant. Of six patients with detectable circulating KCA who subsequently died, five had demonstrable antigen in their serum immediately before death. One of these patients (no. 7), who had progressive klebsiella pneumonia, demonstrated circulating KCA in increasing amounts for 52 days until his final demise. Another patient (no. 11), who survived, also had prolonged antigenemia; she had multiple and persistent tissue foci which gradually resolved, as, in parallel fashion, did her antigenemia.

Further, our data illustrate the apparent prognostic importance of circulating KCA. The negative antigen tests in patients with nonfatal

"primary bacteremia" and the high mortality rates in patients with positive tests support the close relationship between detectable serum KCA and an unfavorable prognosis.

Our findings in patients with klebsiella infections tend to parallel those in other infections in which antigenemia has been demonstrated. Dochez and Avery (7) detected circulating capsular polysaccharide in patients with pneumococcal pneumonia and showed that the presence and amount of antigen in serum correlated with persistence of disease and prognosis. These findings were later supported by the work of Blake (3), Bukantz et al. (4), and others (5, 15). Similarly, in the case of group C meningococcemia, Hoffman and Edwards (13) found that the amount of circulating group-specific polysaccharide indicated the severity of disease and that clearance of antigen correlated with the formation of bactericidal antibody and with survival. Finally, in central nervous system infections due to *H. influenzae* type b, O'Reilly and his co-workers (18) demonstrated polyribosephosphate, a capsular polysaccharide, in serum and CSF for periods of from 1 to 30 days. They found that patients with prolonged antigenemia had protracted fevers and severe neurological symptoms, frequently with focal complications. Further, the intensity of antibody response was reliably predicted by the efficiency of antigen clearance.

Although antibody to KCA was not measured in our study, we would suspect the same reciprocal relationship between circulating capsular antigen and specific antibody which occurs in infections due to *Streptococcus pneumoniae, H. influenzae* type b, and *Neisseria meningitidis*, group C. Although data do not exist for human klebsiella infections, Batshon et al. (2) showed that large doses of klebsiella type 2 polysaccharide given to mice, unlike smaller immunizing doses, caused a reversible immunological paralysis, resulting in the death of mice after challenge with living organisms. These findings remind us of those of O'Reilly et al. (18), who found the appearance of protective polyribosephosphate-specific antibody delayed in children whose *H. influenzae* infections were associated with large and persistent levels of circulating antigen. The same phenomenon may have occurred in our patients with large amounts of circulating antigen, which, conceivably, may have blocked effective humoral immunity and thus aggravated or perpetuated existing infection. That is to say, circulating KCA may actually play a pathogenic role as well as reflecting the presence and extent of underlying infection. This point needs to be further elucidated.

The possible diagnostic usefulness of CIE for detection of klebsiella antigen is diminished by the multiplicity of klebsiella serotypes represented in human infections as well as by the small amounts of circulating KCA that are apparently present in self-limited bacteremias. Perhaps the major potential application of antigen testing would be in following infections in which the diagnosis was already established, rather than as a means of making an initial or rapid diagnosis. In these instances, as illustrated by several of our cases, serial determinations of circulating antigen may be helpful in following disease activity, raising suspicions concerning hidden foci, assessing the efficacy of treatment, and evaluating prognosis.

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