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Common Enterobacterial Antigen

III. Initial Titers and Antibody Response in Bacteremia Caused by Gram-Negative Bacilli

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Antibody titers to common enterobacterial antigen (CA) were determined in 141 controls and in acute serum specimens from 206 patients with bacteremia caused by gram-negative organisms. Levels of antibody to CA ranged from 1:160 to 1:2,560 in 95% of control subjects. These levels did not differ significantly from those in acute serum specimens from bacteremic patients with "nonfatal underlying diseases." Patients with more severe underlying diseases, "ultimately fatal underlying diseases," tended to have lower titers of antibody to CA. Human antibody to CA was predominantly of the 19S variety. A fourfold change in antibody titer to CA was observed in convalescent serum obtained after bacteremia in 32% of 108 patients studied. Correlation of titers of antibody to CA in acute serum specimens with the frequency of occurrence of shock or death failed to demonstrate any protective activity of antibody to CA. These complications occurred equally as often in patients with high titers of antibody to CA as in those with low titers.

The demonstration of identical or very similar chemical composition of the core regions of the cell wall lipopolysaccharides of *Enterobacteriaceae* (7) has led to the recognition that shared, cross-reactive antigens are present in most gram-negative bacilli. The common enterobacterial antigen (CA), described originally by Kunin, is the most extensively studied of these shared antigens (1, 4–8, 12). Most of these studies have been concerned with attempts at purification, delineation of the chemical composition, and definition of the biological activity of CA (4–6). In contrast, relatively little information is available concerning antibody to CA in adults. Although titers to CA have been measured in pyelonephritis and gastroenteritis caused by *Shigella*, *Salmonella*, and enteropathogenic *Escherichia coli*, these studies have been limited almost exclusively to the pediatric age group (1, 4, 12). An antibody response to CA was infrequent in any of these infections except those caused by *Shigella*. Similarly, little is known concerning the distribution of antibodies to CA in normal adult populations, the class of immunoglobulins involved, or their biological activity in man.

Studies of the role of type-specific and cross-reacting antibodies in bacteremia caused by gram-negative bacilli provided the opportunity to delineate the distribution of CA antibodies in adults, to partially characterize it, and to evaluate antibody response in adults in severe systemic infections. In addition, this report provides more detailed information on the relation of antibody to CA to protection against bacteremia than an earlier, more cursory report (11).

MATERIALS AND METHODS

Clinical material. Serum specimens were obtained from 91 consecutive patients admitted to University Hospital for a variety of indications and from 50 blood donors to provide control values of CA antibody titers. Acute serum specimens were obtained from 206 patients with bacteremia caused by gram-negative organisms when bacteremia was suspected clinically or immediately after bacterial growth was observed in blood cultures. Gram-negative bacilli isolated from blood cultures were identified by colonial morphology and standard biochemical techniques. These isolates were maintained by serial transfer on brain heart infusion agar for further use. Follow-up specimens of serum were obtained when possible to measure antibody response to CA as a result of bacteremia.

Patients with bacteremia were carefully observed during hospitalization and their records were subsequently reviewed. Earlier investigations have demonstrated that the severity of the patient's underlying disease is the most important determinant of the outcome of bacteremia caused

by gram-negative organisms. For this reason, patients were classified into the three categories of severity of underlying disease as originally described by McCabe and Jackson (10). Since only seven patients fell into the category of "rapidly fatal underlying diseases," these were tabulated together with patients with "ultimately fatal underlying diseases." The occurrence of shock and the outcome of bacteremia were determined for each patient by using criteria delineated in an earlier publication (11). The frequency of occurrence of shock and a fatal outcome was correlated with CA antibody titers in acute serum specimens by point biserial correlation on the assumption that shock and death reflected more severe bacteremia. Point biserial correlation is a statistical technique which allows identification of trends throughout the entire range of antibody titers and obviates any inherent tendency to demonstrate protection by grouping antibody titers (2). Although it would have been preferable to relate antibody titers to the frequency of development of bacteremia, the importance of surgery and manipulative procedures made selection of an appropriate group of controls impossible.

Serological studies. Titrations of antibody to CA were performed by the indirect hemagglutination technique by using erythrocytes coated with extracts of a bacillus previously shown to contain large quantities of CA, *Proteus rettgeri* 6572, as described in other publications (M. A. Johns et al., J. Immunol., in press; and references 8a and 11). Concomitant hemagglutination inhibition studies were carried out by using extracts of *Enterobacter aerogenes* or *E. coli* 0:14 by previously described methods to insure that antibody to CA, rather than O-specific antibody to *P. rettgeri*, were actually being measured (Johns et al., in press; reference 8a). Antibody titers to CA were reported as the titer of antibody to erythrocytes coated with extracts of *P. rettgeri* which was blocked by extracts of *E. aerogenes* or *E. coli* 0:14. Further confirmation that antibody titers to *P. rettgeri* coated erythrocytes that were blocked by *E. aerogenes* extracts represented antibody to CA was also obtained by the demonstration of blocking with purified CA (Johns et al., in press).

Concomitant titrations of antibody to CA before and after 2-mercaptoethanol treatment of serum, to distinguish 19S from 7S antibody, were also carried out. Serial dilutions of serum were treated with 0.05 ml of 1 M 2-mercaptoethanol for 1 h at 37 C. Sensitized erythrocytes were then added, and the mixture was incubated at 37 C for an additional hour, refrigerated overnight, and read.

RESULTS

Titers of CA antibody in controls and patients with bacteremia. Titers of antibody to CA in serum specimens obtained from 91 consecutive control patients admitted to University Hospital for a variety of indications and 50 blood donors ranged from <1:20 to 1:5,120. The frequency distribution of antibody titers to CA in acute serum specimens from 206 patients with bacteremia caused by gram-negative organisms and the 91 control patients and 50 blood donors are compared in Fig. 1. The bacteremia patients with gram-negative organisms were further subdivided according to the severity of their underlying diseases, 93 with nonfatal underlying diseases and 108 with ultimately fatal underlying diseases, as described previously. Since no significant differences could be detected between titers of antibody to CA in the two groups of control patients, these were combined for analysis. Titers of antibody to CA fell within a relatively narrow range in the 141 controls, with 95% of these patients having CA titers ranging from 1:160 to 1:2,560. Titers of antibody to CA did not appear to be influenced by age in these controls. There was a wider range of antibody levels to CA among patients with bacteremia caused by gram-negative organisms. The distribution of antibody titers to CA did not differ materially, however, between the 98 bacteremic patients with nonfatal underlying diseases and the 141 controls. In contrast, antibody titers to CA among the 108 patients with ultimately fatal underlying diseases had an almost bimodal distribution, with a significantly greater proportion ($X^2 = 12$; $P < 0.001$) having low titers of CA antibody ($\leq$1:80) than controls or patients with nonfatal underlying diseases.

The exact nature of the patient's underlying disease was examined in an attempt to determine its influence on antibody titers to CA. Patients with preexisting renal infection, pyelonephritis, did not have significantly higher titers of antibody

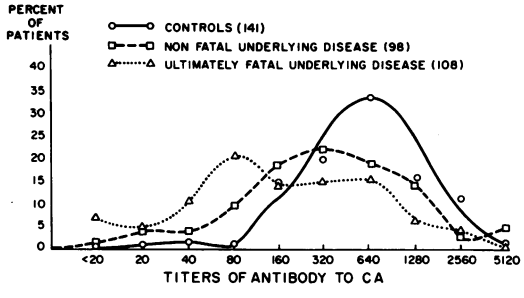

FIG. 1. *Frequency distribution of antibody titers to common enterobacterial antigen in controls and in acute serum specimens from patient with bacteremia, caused by gram-negative bacilli, who have either nonfatal or ultimately fatal underlying diseases.*

to CA than patients who did not have renal infection.

Among patients with ultimately fatal underlying diseases, the exact nature of the patient's underlying disease appeared to exert a significant influence on titers of antibody to CA. The arithmetic mean titer of antibody to CA was 1:448 ± 30 in acute serum specimens from patients with ultimately fatal underlying diseases of types not kown to be associated with immunological abnormalities. Mean CA titers in patients with ultimately fatal underlying diseases with chronic uremia were 1:200 ± 38. In contrast, mean CA titers were only 1:122 ± 20 among patients with metastatic neoplastic diseases, myeloproliferative, or lymphoproliferative disorders. Among the latter patients, low titers of antibody to CA appeared to relate more closely to recent chemotherapy or radiation than the type of neoplastic disease. Thus, the first peak of biphasic distribution of titers of antibody to CA (Fig. 1) among patients with ultimately fatal underlying diseases appeared to reflect patients with neoplastic diseases.

Mercaptoethanol treatment. Antibody to CA in these patients appeared to be predominantly of the 19S mercaptoethanol-sensitive variety. Treatment with 2-mercaptoethanol almost completely destroyed antibody activity against CA-coated erythrocytes. Of 90 acute serum specimens studied, with and without 2-mercaptoethanol treatment, such treatment either produced an eightfold or greater decrease or reduced CA titers to less than 1:20 in 83 of the specimens. The highest titer of antibody to CA persisting after 2-mercaptoethanol treatment was 1:320 in one patient whose pre-2-mercaptoethanol treatment titer was 1:1,280. Similar 2-mercaptoethanol treatment of convalescent serum specimens showing a rise in CA antibody titers demonstrated that this new antibody was also predominantly of the 2-mercaptoethanol-sensitive variety.

Relation of CA titers to outcome of bacteremia. Table 1 relates to height of antibody titers to CA in acute serum specimens from bacteremia patients with gram-negative organisms to the frequency of occurrence of shock or a fatal outcome among patients with nonfatal or ultimately fatal underlying diseases. Eight patients with nonfatal and 11 patients with ultimately fatal underlying diseases are excluded from these tabulations because they had bacteremia caused by gram-negative bacilli not proven to contain CA (*Pseudomonas* and *Bacteroides*), and antibody to CA might not be expected to exert any protective effect in such infections. A greater proportion, 40 of 97 (41%), of patients with ultimately fatal underlying diseases experienced either shock or a fatal outcome from bacteremia than patients, 18 of 90 (20%), with nonfatal underlying diseases, as has been reported previously. The frequency with which these complications occurred bore no relation to the height of titers of antibody to CA at the onset of bacteremia. Although it appeared that shock and death occurred less frequently in patients with nonfatal underlying diseases with CA titers of ≥1:640, analysis by point biserial correlation failed to demonstrate a significant correlation between the height of antibody to CA and the frequency of shock or a fatal outcome in either patients with nonfatal underlying diseases, ultimately fatal underlying diseases, or both groups of patients combined.

Serial daily measurements of antibody to CA were obtained from several patients to determine whether consumption of antibody or combination

TABLE 1. *Relation of antibody titers to CA in acute serum specimens to the frequency of occurrence of shock or a fatal outcome in bacteremia caused by gram-negative bacilli*

Antibody titers to CA	Nonfatal underlying disease	Ultimately fatal underlying disease	Both groups combined
<1:20	2/02[a] (100%)	3/08 (38%)	5/10 (50%)
1:20	1/04 (25%)	1/04 (25%)	2/08 (25%)
1:40	1/04 (25%)	6/11 (55%)	7/15 (47%)
1:80	3/09 (33%)	7/20 (35%)	10/29 (34%)
1:160	2/15 (13%)	4/13 (31%)	6/28 (21%)
1:320	7/21 (33%)	6/14 (44%)	13/35 (37%)
1:640	0/14 (0%)	7/15 (47%)	7/29 (24%)
1:1,280	2/13 (15%)	4/07 (57%)	6/20 (30%)
1:2,560	0/03 (0%)	2/04 (50%)	2/07 (19%)
>1:2,560	1/05 (20%)	0/01 (0%)	1/06 (17%)

[a] Number of patients in whom shock or a fatal outcome occurred/number of patients with this titer of antibody to CA (% of patients with shock or fatal outcome).

TABLE 2. *Antibody response by etiologic agent of bacteremia*

Genera or species of bacteria	Patients (no.)	Increase in CA titer (mean fold increase)
Escherichia coli	12/41 (29%)[a]	2.5
Klebsiella-Enterobacter-Serratia	16/54 (30%)	2.7
Proteus sp	5/11 (45%)	3.6
Salmonella sp	1/3 (33%)	2.0

[a] Number of patients with ≥ fourfold change in CA antibody titers/number of patients studied; (% with fourfold or greater change).

with circulating antigen might influence these observations. No significant change in titers of antibody to CA were observed as the infection was eradicated. Similarly, convalescent serum was available from six patients who had experienced an earlier episode of bacteremia. Again, no significant decrease in the titer of antibody to CA was apparent when acute serum specimens from the second episode of bacteremia were compared with the earlier serum specimens.

Antibody response to CA in bacteremia caused by gram-negative organisms. Follow-up specimens were obtained from 108 patients 10 or more days after the onset of bacteremia with CA containing bacilli. Significant changes (fourfold or greater) in titers of antibody to CA were observed in approximately one-third of these patients after bacteremia. Among 43 patients with nonfatal underlying diseases, a fourfold or greater change occurred in 15 (36%) patients. Nineteen (29%) of 65 patients with ultimately fatal underlying diseases had a fourfold or greater change in CA titers. The maximum change in titer of antibody to CA observed was a 128-fold increase from 1:40 to 1:5,120. Overall, a mean increase of 2.7-fold in CA titer occurred after bacteremia in these 108 patients.

Changes in antibody titer to CA after bacteremia caused by different species of gram-negative bacilli were also examined to determine whether different genera or species prompted antibody responses of varying magnitude. As shown in Table 2, both the frequency and the magnitude of change in titers of antibody to CA were similar irrespective of whether *E. coli*, *Klebsiella*, *Enterobacter*, *Serratia*, or species of *Proteus* or *Salmonella* were the cause of bacteremia.

DISCUSSION

The progressively increasing importance of gram-negative bacilli as a cause of nosocomial infections and the relative dearth of knowledge concerning immunity to such infections prompted this series of investigations of the protective effect and other features of antibodies to O-specific and cross-reactive antigens in man and experimental animals (8, 8a, 11). These studies also afforded the opportunity of delineating the basal levels of antibody and antibody response to one of the antigens, CA, shared by most gram-negative bacilli. Prior studies by Kunin demonstrated that titers of antibody to CA tended to increase with increasing age during childhood, but only a limited number, 15, of adult patients were studied (4). These same investigations also demonstrated that titers of antibody to CA were similar in children with recurrent urinary tract infections and age-matched controls (4). Similar results were obtained in the present studies, indicating that urinary tract infection does not influence titers of antibody to CA in adults. Diaz and Neter also did not find significant increases in antibody titers to CA after urinary tract infections in children (1). In contrast, 29% of children with salmonellosis and 56% withh sigellosis demonstrated significant rises in CA antibody titers (1). Since immunization with whole bacteria containing CA, except for *E. coli* 0:14, 0:56, 0:124, and 0:144 (4–6), failed to induce an antibody response to CA, it seemed desirable to confirm these results in another type of systemic infection with gram-negative bacilli. Significant changes in titers of antibody to CA were observed in 31% of 108 patients with bacteremia caused by CA containing gram-negative bacilli. There was no evidence to suggest that the genera or species of the infecting organism influenced antibody response to CA. Although the present investigation and prior studies indicate that an immune response to CA may result from systemic infections, it is still not clear why a majority of patients fail to mount an antibody response to this antigen. Studies of mercaptoethanol sensitivity of antibody to CA indicate that it is similar to antibody to O antigens of gram-negative bacilli and is of the IgM variety.

The present studies coupled with previous reports indicate that circulating antibody to CA exerts no protective activity in gram-negative infections in animals or man (2, 11). It might be questioned whether consumption or binding of antibody to circulating antigen might influence the titers of antibody in acute serum specimens. Comparison of prior serum specimens and serial daily antibody determinations suggest that this did not affect antibody titers. In addition, the lack of correlation between height of antibody titers to CA and the frequency of shock and death is unlikely to have resulted from binding or con-

sumption of antibody. If consumption of antibody occurred to an equal degree among all patients, equivalent reductions in antibody titers should result. This would still allow identification of any protective effect of high titers of antibody. Since shock and death occurred with similar frequency in patients with all titers of antibody, a preferential decrease in antibody content would have had to occur in patients with the highest titers to mask any protective effect of high titers of antibody to CA. Such a disproportionate decrease in antibody titers would require that antibody in patients with the highest titers either had a greater affinity for binding antigen or that bacteremia in these patients was associated with a larger quantity of circulating bacteria or antigen than in patients with lower titers of antibody to CA.

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