INFECTION AND IMMUNITY, Apr. 1977, p. 213-217

Vol. 16, No. 1
Printed in U.S.A.

Protective Role of Complement in Experimental *Escherichia coli* Endocarditis

DAVID T. DURACK* AND PAUL B. BEESON
Department of Medicine, University of Washington, Seattle, Washington 98195

Received for publication 1 November 1976

Fourteen strains of *Escherichia coli* were tested for ability to cause infective endocarditis in rabbits prepared by prior placement of an intracardiac catheter. Strains that were resistant to the bactericidal action of serum caused *E. coli* endocarditis in 91.4% of rabbits, whereas serum-sensitive strains usually failed to cause persisting infection (11.3% infected, $P < 0.001$). Although serum-sensitive *E. coli* lodged on heart valves within 1 h after intravenous injection, they survived less than 24 h in most normal rabbits. In contrast to normals, all five C6-deficient rabbits injected with a serum-sensitive strain of *E. coli* developed infective endocarditis ($P < 0.005$). No correlation was found between the presence of K_1 antigen and the incidence of experimental *E. coli* endocarditis. Thus, the ability of strains of *E. coli* to establish persisting endocardial infection in rabbits appears to be directly associated with resistance to the complement-mediated serum bactericidal system. These findings may explain in part the rarity of gram-negative bacillary endocarditis in patients; they also indicate that in certain special circumstances the serum bactericidal system can play a decisive role in host defense.

Although *Escherichia coli* is one of the most common organisms encountered in clinical bacteremias, it rarely causes infective endocarditis. Hansing et al. (10) could find only nine well-documented cases of *E. coli* endocarditis in the literature before 1967. Recent reviews have indicated some increase in incidence of gram-negative bacillary endocarditis (6, 18) often due to *Pseudomonas* (19) or *Serratia* (15) in drug addicts or hospitalized patients, but overall this group of bacteria accounts for only a small proportion of cases (28).

It has been suggested that the remarkable contrast between the frequency of gram-negative bacteremia and the rarity of gram-negative endocarditis may be due to the bactericidal action of serum (16). To test this hypothesis, we compared the ability of serum-sensitive and serum-resistant strains of *E. coli* to cause experimental endocarditis in normal rabbits as well as in rabbits genetically deficient in the sixth component of complement (C6).

Under suitable conditions, fresh human serum lyses many strains of gram-negative bacilli. About one-third of *E. coli* strains from clinical sources are ultrasensitive to the bactericidal action of serum (>99% killed), while another one-third of strains are lysed to some degree (27). This property of serum is mediated by antibody and complement (9). Both classical and alternate pathways are required for maximal bactericidal effect, but bacteriolysis can proceed via the alternate pathway alone in sera deficient in C4 or C2 (8, 22). All the later components of complement (C5 through C9) are required for bacteriolysis to occur (9).

Although complement-mediated bacteriolysis is a powerful antimicrobial system in vitro, surprisingly little evidence has been forthcoming showing that it plays an important role in host defense. Several studies have found a higher incidence of serum resistance among gram-negative organisms isolated from blood cultures than from fecal and urinary cultures; this has been interpreted as evidence that the serum bactericidal system might be a significant factor limiting spread of organisms into the bloodstream from local sites (5, 21, 27). On the other hand, genetically determined defects in the complement system do not necessarily result in frequent infections. In man, early-component complement deficiencies are associated with collagen-vascular disease syndromes rather than with an increased incidence of infection (7). Patients with inherited defects of C3 and C5 do suffer recurrent infections, probably because of deficient opsonization and chemotaxis rather than defective bacteriolysis (1, 7, 23). Patients with late-component deficiencies (C6 and C8) may be more susceptible than normal patients to *Neisseria*, but apparently not to most other pathogens (12, 13, 17). The bacteri-

cidal activity of sera from guinea pigs genetically deficient in C4 is somewhat reduced, but the animals are not more susceptible to spontaneous or experimental infection than normal animals (22). C6-deficient rabbits, whose sera can opsonize bacteria normally but have no bacteriolytic activity (11, 24), do not suffer from a striking increase in incidence of infections (25). Mice resist infection satisfactorily even though normal murine serum lacks bactericidal activity (14).

The evidence to date has therefore been consistent with the conclusion reached by Roantree and Pappas: "that the bactericidal properties of serum play, at most, a minor role in freeing the blood stream of bacteria, but that, nevertheless, this role may be of some importance" (20).

MATERIALS AND METHODS

Preparation and infection of rabbits. The experimental animals used were random-bred New Zealand White (NZW) rabbits weighing 1.5 to 2.5 kg. NZW rabbits homozygous for C6 deficiency were kindly supplied by the Small Animal Resources Section, National Institutes of Health, Bethesda, Md. A polyethylene catheter was fixed in the left heart of each rabbit under pentobarbital anesthesia as previously described (23). Twenty-four to 48 h later, each rabbit was injected via an ear vein with *E. coli* grown for 18 h in Trypticase soy yeast broth (TSY), washed once in Hanks' balanced salt solution (HBSS), and diluted 1:10 in HBSS; this inoculum contained 8.1 ± 2.1 ($\pm$ standard deviation, $n = 15$) $\times$ 10^7 colony-forming units (CFU) of *E. coli*.

Quantitative culture of endocardial vegetations. Rabbits were killed by intravenous (i.v.) injection of pentobarbital at intervals from 1 h to 7 days after inoculation with *E. coli*, and the hearts were removed and opened using antiseptic precautions. Vegetations were excised, weighed, and homogenized in TSY; the number of CFU of *E. coli* per gram of vegetation was determined by incorporating portions of serial 10-fold dilutions in TSY in MacConkey agar pour plates and counting colonies after incubation at 37°C for 24 to 36 h.

Test for bactericidal action of serum. Fresh normal rabbit serum (NRS) from five healthy NZW rabbits was pooled and stored at −70°C. Samples were heated to 56°C for 30 min to inactivate complement. Sera from two C6-deficient NZW rabbits (C6DS) were also pooled and stored at −70°C. Strains of *E. coli* were grown overnight in TSY, washed once in HBSS, and resuspended and diluted in HBSS. Tubes containing approximately 10^3 CFU of *E. coli* per ml and 10% serum in HBSS were incubated in a shaking water bath at 37°C for 2 h. Duplicate 0.1-ml samples were withdrawn at 0, 1, and 2 h, and CFU of *E. coli* per milliliter determined by counting colonies in MacConkey agar pour plates.

Selection of *E. coli* strains. A total of 20 strains of *E. coli* were tested for sensitivity to NRS. Thirteen were obtained from routine urine cultures, five were supplied by G. M. Kalmanson (Wadsworth VA Hospital, Los Angeles), and two K_1 antigen-positive strains were supplied by R. Bortolussi (University of Minnesota, Minneapolis). Six strains (A through F) resistant to the bactericidal action of serum were selected; their numbers rose slightly in tubes containing NRS, heated NRS, and C6DS due to multiplication during the 2-h incubation, and fell slightly in HBSS (Fig. 1A). Another six strains (H through M) were selected because they were killed by NRS (≥99% in 2 h); they survived or multiplied in heated NRS or C6DS (Fig. 1B). These 12, together with 2 further strains G and N, not shown in Fig. 1, were used in rabbit experiments. Eleven of the 14 strains were tested for presence of K_1 capsular polysaccharide antigen by the method of Sarff et al. (26), using equine antiserum to meningococcus B prepared by J. B. Robbins (Food and Drug Administration, Division of Bacterial Products, Bethesda, Md.) and supplied by G. W. Counts (University of Washington, Seattle).

RESULTS

Progress of experimental *E. coli* endocarditis. Quantitative cultures of endocardial vegetations were made at intervals from 1 h to 7 days after i.v. injection of a serum-resistant strain (A) or a serum-sensitive strain (H) of *E. coli* (Fig. 2). One hour after injection, all eight vegetations from rabbits that received strain A were infected. Five of six vegetations from rabbits given strain H were also infected, but the mean number of *E. coli* per gram of vegetation was significantly lower in rabbits that received this serum-sensitive strain. After the first hour, the number of serum-sensitive organisms recovered fell sharply; all five vegetations cultured at 24 h were sterile, as were vegetations from all but 1 of 21 rabbits killed between 2 and 7 days after injection of strain H. This single animal accounts for the slight rise in mean CFU per gram on day 5. These results differed from those obtained when the serum-resistant

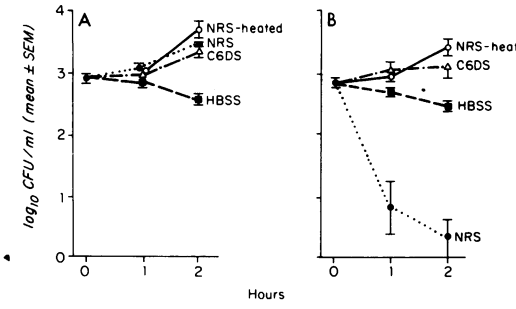

FIG. 1. *Mean $\log_{10}$ CFU per milliliter of six serum-resistant strains of E. coli (A) and six serum-sensitive strains (B) incubated at 37°C for 2 h in 10% NRS, 10% heated NRS, 10% C6DS and HBSS.*

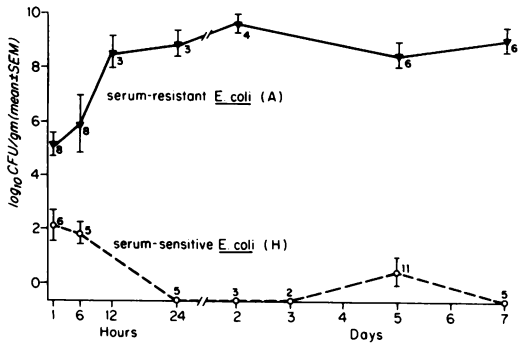

FIG. 2. *Progress of E. coli endocarditis over 7 days. Number of E. coli in vegetations from rabbits inoculated with a serum-resistant strain (A) contrasted with counts from rabbits receiving a serum-sensitive strain (H). The number of rabbits is indicated beside each point.*

strain A was injected; after the first hour the number of bacteria in the vegetations increased exponentially for 12 h and then remained above 10^8 CFU/g throughout the 7-day period. This experiment demonstrated a major difference between the ability of the two strains to persist in vegetations.

Comparison of 14 strains of *E. coli*. Fifty-three of 58 rabbits (91.4%) had *E. coli* endocarditis at necropsy 1 to 7 days (mean 4.4 days) after injection of serum-resistant strains A through G (Table 1). In contrast, only 7 of 62 rabbits (11.3%) injected with serum-sensitive strains H through N had *E. coli* endocarditis 1 to 7 days later (mean, 4.6 days). This difference is highly significant ($\chi^2 = 73.72$, $P << 0.001$). The mean number of *E. coli* present in the seven vegetations that contained serum-sensitive organisms (6.34 ± 1.01, mean ± standard error of the mean $\log_{10}$ CFU per g) was lower than the number in 45 vegetations infected with serum-resistant strains (8.63 ± 0.21; $P < 0.001$ by Student's t test).

***E. coli* endocarditis in C6-deficient rabbits.** Fresh serum from C6-deficient rabbits failed to kill strains of *E. coli* that were killed by NRS (Fig. 1B). To test whether C6 deficiency influences susceptibility to *E. coli* endocarditis in vivo, five C6-deficient rabbits were catheterized and injected with a serum-sensitive strain that caused endocarditis in only one of eleven normal rabbits at 5 days (strain H; Table 1 and Fig. 2). All five C6-deficient rabbits had *E. coli* endocarditis 5 days after injection ($P < 0.005$; $\chi^2 = 8.55$).

Investigation of influence of K_1 antigen. There is an association between the presence of K_1 capsular polysaccharide antigen and the ability of *E. coli* to cause neonatal meningitis (26). To test whether this antigen might also be a virulence factor for endocarditis, 11 of the 14 strains were tested for presence of K_1 antigen, and the results of the injection of these 11 strains into rabbits were reanalyzed. There was no significant difference in infection rate between animals receiving K_1-positive and K_1-negative strains (Table 2). However, the striking association between serum resistance and ability to cause endocarditis, previously observed, was maintained within the K_1 groupings.

DISCUSSION

The work described here provides evidence that under certain circumstances complement-mediated bacteriolysis can be a decisive factor in host defense. Although serum-sensitive strains lodged upon the area of endocardial injury in most rabbits during the first hour after i.v. injection, significantly fewer were recovered than from rabbits given serum-resist-

TABLE 1. *Incidence of E. coli endocarditis in rabbits 1 to 7 days after i.v. injection of serum-resistant strains, contrasted with incidence in rabbits receiving serum-sensitive strains*

Serum-resistant strains injected	No. of rabbits infected/total	Serum-sensitive strains injected	No. of rabbits infected/total
A	21/21	H	1/26
B	7/8	I	0/5
C	3/3	J	2/8
D	6/8	K	0/8
E	7/9	L	2/5
F	3/3	M	1/4
G	6/6	N	1/6
Total 7	53/58 (91.4%)	7	7/62 (11.3%) $P << 0.001$ ($\chi^2 = 73.72$)

TABLE 2. *Incidence of E. coli endocarditis in rabbits 1 to 7 days after i.v. injection of E. coli, analyzed according to presence or absence of K_1 capsular antigen and serum sensitivity of the strains injected*

E. coli strains	No. of rabbits infected/total
E, G, N (K_1 positive)	14/21 Not significant
A, B, D, H, I, J, K, M (K_1 negative)	38/88 ($\chi^2 = 2.87$)
E, G, (K_1 positive, serum resistant)	13/15 $P < 0.02$
N (K_1 positive, serum sensitive)	1/6 ($\chi^2 = 6.56$)
A, B, D (K_1 negative, serum resistant)	34/37 $P < 0.001$
H, I, J, K, M (K_1 negative, serum sensitive)	4/51 ($\chi^2 = 58.36$)

ant *E. coli*. Thereafter, the number of surviving organisms fell rapidly, so that infection was eradicated within 24 h in most animals (Fig. 2). This indicates that the bactericidal action of serum is not limited to circulating organisms, but can kill bacteria in special anatomic locations.

It is possible that one or more bacterial virulence factors (for example, ability to adhere to endocardium) might be associated with resistance to serum bactericidal activity. If this were the case, the association reported here between serum resistance of *E. coli* and propensity to cause experimental endocarditis might not be due to complement-mediated bacterial lysis per se. However, the observation that a serum-sensitive strain (H) regularly caused endocarditis in C6-deficient NZW rabbits but not in normal NZW rabbits supports our thesis that the complement system is responsible, unless another unknown host resistance factor genetically linked to C6 deficiency is involved.

Archer and Fekety have recently reported an observation consistent with these results. While establishing a model for *Pseudomonas* endocarditis, they noted a higher mortality in rabbits receiving one serum-resistant strain of *Pseudomonas aeruginosa* than in others that were injected with a serum-sensitive strain (2).

In most situations, phagocytes serve as the major defense against invasion by gram-negative bacilli. However, phagocytes cannot act effectively in the vegetations of infective endocarditis, because colonies of bacteria lie protected by layers of fibrin and platelets (3). We suggest that the vitiation of phagocytic defense mechanisms in this pathological setting is the circumstance that makes it possible to demonstrate a protective effect of the complement-mediated serum bactericidal system.

These experimental findings provide evidence that the bacteriolytic action of serum plays an important protective role in pathogenesis of *E. coli* endocarditis and presumably of endocarditis due to other gram-negative bacilli. If this is correct, the majority of gram-negative bacilli that cause endocarditis in patients are likely to be resistant to lysis by human serum.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant HL 17751 from the National Heart and Lung Institute.

LITERATURE CITED

1. **Alper, C. A., H. R. Colten, F. S. Rosen, A. R. Rabson, G. M. MacNab, and J. S. S. Gear.** 1972. Homozygous deficiency of C3 in a patient with repeated infections. Lancet ii:1179–1181.
2. **Archer, G., and F. R. Fekety.** 1976. Experimental endocarditis due to *Pseudomonas aeruginosa*. I. Description of a model. J. Infect. Dis. 134:1–7.
3. **Durack, D. T.** 1975. Experimental bacterial endocarditis. IV. Structure and evolution of very early lesions. J. Pathol. 115:81–89.
4. **Durack, D. T., P. B. Beeson, and R. G. Petersdorf.** 1973. Experimental bacterial endocarditis. III. Production and progress of the disease in rabbits. Br. J. Exp. Pathol. 54:142–151.
5. **Fierer, J., F. Finley, and A. I. Braude.** 1972. A plaque assay on agar for detection of gram-negative bacilli sensitive to complement. J. Immunol. 109:1156–1158.
6. **Finland, M., and M. W. Barnes.** 1970. Changing etiology of bacterial endocarditis in the antibacterial era. Ann. Intern. Med. 72:341–348.
7. **Frank, M. M., and J. P. Atkinson.** 1975. Complement in clinical medicine. Disease-a-Month, January.
8. **Gewurz, H., R. J. Pickering, L. H. Muschel, S. E. Mergenhagen, and R. A. Good.** 1966. Complement-dependent biological functions in complement deficiency in man. Lancet ii:356–360.
9. **Goldman, J. N., S. Ruddy, K. F. Austen, and D. S. Feingold.** 1969. The serum bactericidal reaction. III. Antibody and complement requirements for killing a rough *Escherichia coli*. J. Immunol. 102:1379–1387.
10. **Hansing, C. E., V. D. Allen, and J. D. Cherry.** 1967. *Escherichia coli* endocarditis. Arch. Intern. Med. 120:472–477.
11. **Johnston, R. B., Jr., M. R. Klemperer, C. A. Alper, and F. S. Rosen.** 1969. The enhancement of bacterial phagocytosis by serum. The role of complement components and two cofactors. J. Exp. Med. 129:1275–1290.
12. **Leddy, J. P., M. M. Frank, T. Gaither, J. Baum, and M. R. Klemperer.** 1974. Hereditary deficiency of the sixth component of complement in man. I. Immunochemical, biologic, and family studies. J. Clin. Invest. 53:544–553.
13. **Lim, D., A. Gewurz, T. F. Lint, M. Ghaze, B. Sepheri, and H. Gewurz.** 1976. Absence of the sixth component of complement in a patient with repeated episodes of meningococcal meningitis. J. Pediat. 89:42–47.
14. **Marcus, S., D. W. Esplin, and D. M. Donaldson.** 1954. Lack of bactericidal effect of mouse serum on a number of common microorganisms. Science 119:877.
15. **Mills, J., and D. Drew.** 1976. *Serratia marscescens* endocarditis: a regional illness associated with intravenous drug abuse. Ann. Intern. Med. 84:29–35.
16. **Paterson, P. Y.** 1963. The pathogenesis of bacterial endocarditis, p. 5–11. Conference on Infectious Diseases of the Heart and Circulation. New York Heart Association, New York.
17. **Petersen, B. H., J. A. Graham, and G. F. Brooks.** 1976. Human deficiency of the eighth component of complement. The requirement of C8 for serum *Neisseria gonorrhoeae* bacterial activity. J. Clin. Invest. 57:283–290.
18. **Quinn, E. L., K. H. Burch, F. Cox, E. Fisher, and T. Madhavan.** 1975. The changing character of infective endocarditis. Am. Fam. Physician 11:117–124.
19. **Reyes, M. P., W. A. Palutke, R. F. Wylin, and A. M. Lerner.** 1973. Pseudomonas endocarditis in the Detroit Medical Center 1969–1972. Medicine 52:173–194.
20. **Roantree, R. J., and N. C. Pappas.** 1960. The survival of strains of enteric bacilli in the blood stream as related to their sensitivity to the bactericidal effect of serum. J. Clin. Invest. 39:82–88.
21. **Roantree, R. J., and L. A. Rantz.** 1960. A study of the relationship of the normal bactericidal activity of human serum to bacterial infection. J. Clin. Invest. 39:72–81.
22. **Root, R. K., L. Ellman, and M. M. Frank.** 1972. Bactericidal and opsonic properties of C4-deficient guinea pig serum. J. Immunol. 109:477–486.

23. **Rosenfeld, S. I., M. E. Kelly, and J. P. Leddy.** 1976. Hereditary deficiency of the fifth component of complement in man. J. Clin. Invest. 57:1626–1634.
24. **Rother, K., U. Rother, K. F. Petersen, D. Gemsa, and F. Mitze.** 1964. Immune bactericidal activity of complement. Separation and description of intermediate steps. J. Immunol. 93:319–330.
25. **Ruddy, S., I. Gigli, and K. F. Austen.** 1972. The complement system of man. New Engl. J. Med. 287:489–495, 545–549, 592–596, 642–646.
26. **Sarff, L. D., G. H. McCracken, Jr., M. S. Schiffer, M. P. Glode, J. B. Robbins, I. Orskov, and F. Orskov.** 1975. Epidemiology of *Escherichia coli* K1 in healthy and diseased newborns. Lancet i:1099–1104.
27. **Vosti, K. L., and E. Randall.** 1970. Sensitivity of serologically classified strains of *Escherichia coli* of human origin to the serum bactericidal system. Am. J. Med. Sci. 259:114–119.
28. **Weinstein, L., and R. H. Rubin.** 1973. Infective endocarditis—1973. Prog. Cardiovasc. Dis. 16:239–274.