INFECTION AND IMMUNITY, Dec. 1985, p. 734–737
0019-9567/85/120734-04$02.00/0

Vol. 50, No. 3

Prevention and Therapy of Experimental *Escherichia coli* Infection with Monoclonal Antibody

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Received 23 April 1985/Accepted 27 August 1985

Mouse hybridoma antibody of immunoglobulin class M prepared with live group B meningococci was evaluated for its ability to protect against and treat *Escherichia coli* infections in a newborn-rat model. In these studies, antibody was administered intraperitoneally and bacteria were administered subcutaneously to avoid introducing the antibody and bacteria to the same site. The activity of this hybridoma antibody was specific; the antibody provided protection against the K-1 strain, but not against the K-92 strain. In addition, the amount of the antibody required for protection was dependent upon the size of bacterial challenge. With an increase of the bacterial inocula from the 100% lethal dose to 10 times the 100% lethal dose there was a threefold increase in the amount of the antibody required for 50% protection. Similarly, therapeutic efficacy of the antibody was also dependent upon the magnitude of bacteremia before therapy. The antibody successfully cleared the bacteremia only when the pretherapy bacterial counts in blood were $<10^4$ CFU/ml. These findings suggest that the monoclonal immunoglobulin M antibody against the capsular polysaccharide of the group B meningococcus may be useful in the prevention and treatment of K-1 *E. coli* infections.

The morbidity and mortality associated with neonatal gram-negative meningitis have remained significant despite continuing introduction of newer antimicrobial agents (13). The most common gram-negative organism causing meningitis in the neonatal period is *Escherichia coli* (6, 18). Given the plethora of *E. coli* serotypes (164 O, 56 H, and 103 K antigens) (15), it is striking that *E. coli* strains possessing the K-1 capsular polysaccharide have been the predominant serotype isolated from neonatal *E. coli* meningitis patients (17). In addition, meningitis caused by K-1 *E. coli* strains appears to be somewhat more virulent than that caused by non-K-1 strains with respect to mortality and long-term sequelae (12).

Recently, with the introduction of human immunoglobulin modified for intravenous preparation, immunotherapy in conjunction with antimicrobial chemotherapy has been suggested as a possible treatment of some serious infections for the neonate (e.g., of group B streptococcus) (19). These modified preparations, however, have low levels of antibody to the K-1 capsule of *E. coli* (4). Previous attempts to raise a high-titered antibody against K-1 *E. coli* have also been unsuccessful because of the relatively low immunogenicity of the K-1 polysaccharide (8). Since the K-1 capsular polysaccharide of *E. coli* and the capsular polysaccharide of the group B meningococcus are immunochemically identical (8), Robbins et al. were able to demonstrate in a mouse model a protective effect of goat meningococcal group B polysaccharide antibody against lethal *E. coli* K-1 infection (17). Recently, Cross et al. and Moreno et al. have reported that functionally active murine immunoglobulin M (IgM) monoclonal antibody to the K-1 polysaccharide has been prepared by using live group B meningococci (4, 14). These investigators have shown that this monoclonal antibody is capable of protecting mice from lethal challenge by a K-1 *E. coli* strain (4, 14). However, in these studies, the antibody was given simultaneously with the bacteria and at the same site (4, 14), unlike the situation which would occur clinically.

The present study was performed therefore to evaluate further the in vivo activity of this same monoclonal antibody against K-1 and non-K-1 strains of *E. coli* in an experimental animal model which resembles very closely *E. coli* infections in human infants.

MATERIALS AND METHODS

Bacterial strains. Serum-resistant *E. coli* C5 (O18ac:K1:H7) and A40 (O13:K92:H4), isolated from the cerebrospinal fluid of newborn infants with meningitis (1,2), were kindly provided by R. Bortolussi of Dalhousie University, Nova Scotia, Canada, and M. Achtman of Max-Planck-Institut, Munich, Federal Republic of Germany, respectively. *E. coli* strains grown in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) to late logarithmic phase were stored at −70°C in aliquots until used.

Hybridoma antibody. Procedures for preparation of the hybridoma clone 2-2-B, which secretes IgM antibody specific for the K-1 capsular polysaccharide, were previously described (4). Briefly, mice were immunized with live group B type 15 meningococci. Fusion of a P3-derived nonproducer mouse myeloma cell line (X63-Ag8.653) and spleen cells from the immunized BALB/cJ mice was done by the method of Kennett (9). Cells from clones that secreted antibody specific for the capsular polysaccharide were injected into pristane-primed mice. This resulted in ascitic fluid that contained IgM antibody specific for the two cross-reactive capsular polysaccharides (*E. coli* K-1 and group B meningococcus). Ascitic fluid contained 3 mg of specific antibody per ml as determined by a quantitative solid-phase radioimmunoassay (20).

Animal model for *E. coli* infection. Outbred pathogen-free

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TABLE 1. Protective effect of intraperitoneally administered group B meningococcal hybridoma antibody against subcutaneous challenge with the LD_{100} and 10× LD_{100} of K-1 and LD_{100} of non-K-1 strains of *E. coli*

Challenging strain	Amt of antibody injected (μg/rat)	No. surviving/ no. challenged with LD_{100} (%)	No. surviving/ no. challenged with 10× LD_{100} (%)
C5 (O18ac:K1:H7)	15	5/5 (100)	4/6 (67)
	1.5	13/18 (72)	7/12 (58)
	1.5×10^{-1}	10/18 (56)	6/14 (43)
	1.5×10^{-2}	2/10 (20)	0/11
	1.5×10^{-3}	0/10	0/6
	1.5×10^{-4}	0/6	
A40 (O13:K92:H4)	150	0/5	
	15	0/5	
	Control	0/5	

Sprague-Dawley pregnant rats with timed conception were purchased from Charles River Breeding Laboratories, Wilmington, Mass., and gave birth in our vivarium 5 to 7 days after arrival. Each adult rat and her pups were housed in a separate solid polypropylene opaque cage with a filter hood. *E. coli* infection was induced in 5-day-old rats by the method detailed previously (10, 11). Although our previous studies have used the intraperitoneal route for infection, *E. coli* were injected subcutaneously in this study to avoid introducing the infecting organism and antibody to the same site.

In vivo studies. We performed three sets of experiments to study the in vivo activity of the group B meningococcal hybridoma antibody.

The first study was to examine the protective efficacy of the group B meningococcal hybridoma antibody against K-1 (strain C5) and non-K-1 (strain A40) strains of *E. coli*. At 5 days of age, all members of each litter were divided into designed study groups to receive intraperitoneally the ascitic fluid containing the group B memingococcal hybridoma antibody (3 mg/ml) diluted 10-fold (to 10^{-6}) in sterile distilled water in a dose of 0.05 ml/10 g of body weight. Control animals received ascitic fluid containing a murine IgM hybridoma antibody (4.5 mg of antibody per ml) to the lipopolysaccharide of *Pseudomonas aeruginosa* (Fisher-Devlin immunotype 5). Immediately thereafter (≤0.5 h), animals were inoculated subcutaneously with 10^2 CFU of strain C5 or 2×10^4 CFU of strain A40. As determined previously (10, 11), these inocula of C5 and A40 strains produce bacteremia within 18 h and subsequent death within 5 days of inoculation in 100% of animals (LD_{100}). Mortality was recorded for 5 days, and postmortem blood cultures were obtained to confirm the *E. coli* infection. A dose of the group B meningococcal antibody to protect 50% of animals from death was calculated by the method of Reed and Muench (16).

The second study was to evaluate the relationship between the size of bacterial challenge and the amount of antibody required for protection. Experimental design was the same as for the first study except that animals were challenged with 10^3 CFU of strain C5 (10 × LD_{100}). Results were analyzed as described in the first study.

The third study was to determine whether the K-1 antibody would be useful in treatment of established *E. coli* infection. At 5 days of age, all members of each litter were inoculated subcutaneously with 10^2 CFU of strain C5. As described previously, this inoculum produces nonlethal bacteremia in 100% of animals within 18 h of inoculation. At 18 h after inoculation and daily thereafter for 4 days, 0.1 ml of blood was obtained as described previously (10, 11) for quantitative cultures. Immediately after the first blood specimens were obtained, each litter was randomly divided into two groups to receive intraperitoneally the group B meningococcal antibody or the antibody to the lipopolysaccharide *P. aeruginosa* in a dose of 0.05 ml/10 g of body weight for each. Therapeutic efficacy was determined by comparing rates of bacterial clearance from blood and of mortality between the two treatment groups.

RESULTS

The protective efficacy of the ascitic fluid containing 3 mg of the group B meninogococcal antibody per ml against the K-1 and non-K-1 strains of *E. coli* is summarized in Table 1. When newborn rats were given antibody intraperitoneally and then challenged subcutaneously with the LD_{100} of *E. coli* strains (10^2 CFU for strain C5 and 10^4 CFU for strain A40), the protection by the group B meningococcal antibody was demonstrated only against the K-1-possessing C5 strain. A concentration of 15 μg of this antibody per rat provided complete protection against strain C5, and a 50% protection was achieved with 0.17 μg of this antibody per rat. In contrast, death could not be prevented by even 150 μg of the group B meningococcal antibody per rat against the K-92-possessing strain A40, and all postmortem blood cultures were positive for *E. coli*. These findings suggest that the in vivo protective activity of the group B meningococcal hybridoma antibody is specific to *E. coli* strains possessing the K-1 capsular polysaccharide.

However, the protective effect of the group B meningococcal antibody depended upon the size of the bacterial challenge. When the inoculum size of strain C5 was increased to 10 × LD_{100} (10^3 CFU), even 15 μg of the antibody per rat provided protection in only 67% of animals (Table 1).

Table 2 summarizes the therapeutic effect of the group B meningococcal hybridoma antibody against the K-1 *E. coli* strain. As shown previously (10, 11), 18 h after receiving subcutaneous injection of 10^2 CFU of the strain C5, all 24 (100%) animals were bacteremic. At this time, the bacterial counts (mean ± standard deviation) in blood were not significantly different between the two treatment groups (4.30 ± 1.62 and 4.49 ± 1.75 $\log_{10}$ CFU/ml of blood for animals receiving the group B meningococcal antibody or the lipopolysaccharide antibody to *P. aeruginosa* [control], respectively). All control animals died within 12 h of therapy, whereas at this time, 57% (8/14) of the animals receiving

TABLE 2. Therapeutic effect of the group B meningococcal hybridoma antibody or the antibody to the lipopolysaccharide of *P. aeruginosa* on survival of newborn rats with *E. coli* bacteremia

Antibody	No. of animals	No. surviving at h after beginning therapy:					
		0	12	24	48	72	96
Group B meningococcus	14	14	8[a]	4	4	4	4 (29%)
Lipopolysaccharide of *P. aeruginosa*	10	10	0	0	0	0	0

[a] Significantly more animals ($\chi^2 = 6.19$; $P < 0.02$) receiving the group B meningococcal antibody survived than did those receiving the antibody to the lipopolysaccharide of *P. aeruginosa*.

the group B meningococcal antibody survived ($\chi^2 = 6.19$; $P < 0.02$) (Table 2). Overall, 29% (4/14) of the animals receiving the group B meningococcal antibody completely eradicated the K-1 *E. coli* from blood in contrast to none of the control group. Of note, these four survivors had bacterial counts before therapy of $<10^4$ CFU/ml of blood and the mean bacterial counts (± standard deviation) were significantly lower than those of animals that died (2.36 ± 1.21 versus 5.08 ± 0.98 $\log_{10}$ CFU/ml of blood; $P < 0.01$). These findings suggest that the therapeutic efficacy of the group B meningococcal antibody depends upon the size of the bacterial population in the blood. In comparison, four animals in the control group also had the bacterial counts before therapy of $<10^4$ CFU/ml of blood, but all these animals subsequently died, and there was no resolution of *E. coli* bacteremia.

DISCUSSION

Several investigators have recently reported that an IgM hybridoma antibody prepared with live group B meningococcus is functionally active against K-1 *E. coli* strains in vitro and in vivo (4, 14), suggesting that this antibody may be useful in treatment of *E. coli* infection. The present study was performed therefore to evaluate further the protective and therapeutic efficacy of this hybridoma antibody against *E. coli* in the newborn-rat model. As shown previously (3, 10, 11), this model bears several important similarities to human *E. coli* infection, which are age dependency, susceptibility to *E. coli* strains possessing the K-1 polysaccharide, and high mortality. In this study, we also established the infection by subcutaneous injections of *E. coli*. This, in contrast to other studies where antibody and bacteria were introduced to the same site (i.e., peritoneum) (4, 14), gave us the opportunity to examine the in vivo activity of the antibody without a direct contact with bacteria at the site of injection.

Our studies have confirmed in the different-animal model that the IgM hybridoma antibody prepared with group B meningococcus was highly protective against a K-1 *E. coli* strain but not against a K-92 strain containing the capsular polysaccharide structurally similar to the K-1 polysaccharide. The K-1 polysaccharide is an α-2,8-linked homopolymer of sialic acid, while the K-92 polysaccharide is linked by α-2,8 alternating with α-2,9-ketosic bonds (7). With as little as 0.17 μg of this hybridoma antibody, 50% of newborn rats were protected from lethal challenge by the K-1 *E. coli* strain, although even 150 μg of this antibody was not protective against the K-92 strain. These findings corroborate those of Cross et al. (4), who also demonstrated that the opsonophagocytic activity of this antibody was specific for *E. coli* strains possessing the K-1 capsular polysaccharide. As expected, the amount of the group B meningococcal antibody needed to protect the animals from lethal *E. coli* infection was dependent upon the size of the bacterial inoculum. With the increase of an inoculum from LD_{100} to $10\times$ LD_{100}, the amount of the antibody required for 50% protection increased approximately threefold.

Similarly, therapeutic efficacy of this hybridoma antibody appeared to depend upon the magnitude of bacteremia. When the pretherapy bacterial counts in blood were $<10^4$ CFU/ml, the group B meningococcal antibody successfully cleared the *E. coli* from blood. Conversely, when the initial bacterial counts were $\geq 10^4$ CFU/ml of blood, this antibody failed to clear the bacteremia, and the animals subsequently died. Since bacterial counts in blood of most (69%) newborn infants with *E. coli* septicemia are reported as $<10^3$ CFU/ml (5), the potential therapeutic effect of the group B meningococcal antibody for neonatal *E. coli* infection cannot be underestimated.

ACKNOWLEDGMENTS

This work was supported in part by Basic Research Grant 1-976 from March of Dimes Birth Defects Foundation and research grant RO1-A1 19454 from the National Institutes of Health.

We thank Daisy Green for her technical assistance and Joy Heiner for typing the manuscript.

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